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Original Article

Synergistic therapeutic effects of pertuzumab and pyrrolitinib in HER-2-positive breast cancer: modulation of the PI3K/AKT pathway

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Abstract: Objective: To investigate the underlying mechanism of pertuzumab combined with pyrrolitinib in the treatment of breast cancer. Methods: Real-time PCR and Western blot (WB) were used to detect the expression of HER-2 in breast cancer cells (MCF-10A, BT-474 and SK-BR-3). Subsequently, BT-474 cells were treated with different concentrations of pertuzumab (0, 0.1, 0.5, 1, 5, 10 μg/mL), pyrrolizinib (0, 2, 4, 6, 8, 10 nMol/L) or a synergistic mixture of pertuzumab and pyrrolizinib. The cell viability, migration, invasion and programmed cell death were detected by CCK-8, cell colony formation, wound healing, transwell migration, TUNEL apoptosis assay and WB. KEGG pathway analysis was used to identify key pathways with HER-2 involvement. String database was used to analyze the relationship between HER-2 and PI3K/AKT signaling pathway related proteins. Subsequently, the effects of HER-2 knockdown on PI3K/AKT signaling pathway and cell function were investigated. Results: Elevated HER-2 expression was observed in breast cancer tissues and cells. The combination of pertuzumab and pyrrolitinib effectively reduced HER-2 levels, inhibited cell viability, proliferation, migration and infiltration, and promoted apoptosis. Knockdown of HER-2 inhibited the viability, proliferation, migration and invasion, downregulated the expression of PI3K and AKT, and increased the apoptosis of BT-474 cells, with these effects restored by IGF-1. Conclusion: Pertuzumab and pyrrolizinib target HER-2 to downregulate the PI3K/AKT signaling pathway, thereby inhibiting breast cancer cells.

Keywords: Pertuzumab, pyrrolitinib, breast cancer, HER-2, PI3K/AKT signaling pathway

Introduction

Breast cancer [1] is one of the most common malignant tumors, seriously affecting women's health globally. It ranks first in the incidence of malignant tumors in women [2]. In China, breast cancer has become one of the most prevalent cancers in women, accounting for 12% of the global incidence and ranking sixth in cancer-related deaths among females [3]. Currently, breast cancer is primarily treated through a comprehensive approach, with surgery as the primary modality supplemented by other treatments such as chemotherapy, radiotherapy, endocrine therapy, and targeted therapy [4]. These adjuvant therapies have proven effective in preventing recurrence and metastasis. In recent years, the advent of personalized and molecular-targeted treatments have substantially improved treatment effectiveness and the quality of life for breast cancer patients [5].

Around 25% of breast cancer patients have an elevated HER-2 expression [6], which is associated with poor tumor cell differentiation, rapid proliferation, and high invasiveness, indicating a high malignancy and a poor prognosis [7]. The standard treatments for early-stage HER-2-positive breast cancer include a year-long administration of trastuzumab, accompanied by sequential chemotherapy [8]. Clinical implementation of trastuzumab has proven effective in reducing the incidence and mortality rates of HER-2-positive breast cancer [9]. However, some patients still experience recurrence or death [10]. Therefore, new anti-HER-2 drugs have become a research focus [11]. Currently,

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novel anti-HER-2 drugs in use include trastuzumab, pertuzumab, lapatinib, neratinib, pyrrolitinib, and pembrolizumab [12, 13].

Pertuzumab is a prominent monoclonal antibody that specifically targets the extracellular domain I of HER-2. It exerts its anti-tumor effects by preventing the formation of HER-2 homodimers and HER-2/HER-3 heterodimers [14]. In 2013, the FDA approved pertuzumab for neoadjuvant treatment of breast cancer [15]. Currently, the combination of pertuzumab, trastuzumab, and taxane is the first-line treatment for advanced HER-2-positive breast cancer patients. Notably, the Swain study demonstrated that the incorporation of pertuzumab and trastuzumab, along with chemotherapy, provides a striking advancement over chemotherapy plus trastuzumab alone, not only enhancing the pathological complete response (pCR) rate but also significantly improving progression-free survival (PFS) [16].

Pyrrolitinib, a tyrosine kinase inhibitor (TKI) developed in China [17], covalently binds to the ATP-binding site of the intracellular tyrosine kinase domains of HER-1, HER-2, and HER-4. By doing so, it prevents the transmission of mitotic signals into the cell, inhibiting HER receptor phosphorylation and blocking the activation of downstream signaling pathways, ultimately exerting anti-tumor effects [18]. In China, Pyrrolitinib is approved for the treatment of HER-2-positive advanced breast cancer patients who have previously undergone anthracycline or taxane chemotherapy, as a combination therapy alongside capecitabine [19]. Phase II clinical studies have shown that compared to lapatinib, pyrrolitinib significantly improves the overall response rate (ORR) [20]. In the subgroup analysis of patients previously treated with trastuzumab, the pyrrolitinib group demonstrated significantly better median PFS than the lapatinib group. Similarly, in the subgroup of patients who had not received previous trastuzumab treatment, pyrrolitinib also showed significantly better median PFS than the lapatinib group [21].

Recent studies have shown that changes in PI3K/AKT signaling pathway play a crucial role in the resistance mechanism of HER2-overexpressing breast cancer [22]. For instance, Wuerkenbieke D et al. found that AKT activation contributes to ovarian cancer cell resistance to pertuzumab [23]. The PI3K/Akt pathway plays a multifaceted role in breast cancer, influencing tumor growth, progression, metastasis, therapy resistance, and patient outcomes. Understanding the intricate signaling networks involving the PI3K/Akt pathway is essential for developing targeted therapies and improving the management of breast cancer [24].

Both pertuzumab and pyrrolizinib have shown good effects in anti-HER-2 targeted drugs and combination therapy, though their mechanisms of action differ. However, no relevant studies have yet explored the combined treatment effect of pertuzumab and pyrrolizinib. Therefore, we speculate that the combination of pertuzumab and pyrrolitinib may alleviate breast cancer progression by inhibiting HER-2 and the PI3K/AKT signaling pathway.

Material and methods

Cell culture and treatment

The MCF-10A, BT-474 and SK-BR-3 cell lines were procured from the American Type Culture Collection (ATCC) and cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS; Merck, Germany) and 1% penicillin-streptomycin (Merck, Germany). All cells were maintained at 37°C in a 5% CO_2 incubator.

BT-474 cells were treated with varying concentrations of pertuzumab (0, 0.1, 0.5, 1, 5, 10 μg/mL) and pyrrolitinib (0, 2, 4, 6, 8, 10 nMol/L), either alone or in combination. Dose-response curves of pertuzumab and pyrrolitinib were first determined individually in BT-474 cells to calculate their respective IC50 values. Notably, when used in combination, the ratio of their IC50 values was approximately 1:5. Based on this, a combined dose of pertuzumab (1 μg/mL) and pyrotinib (6 nM) was selected for further experiments to study their potential synergistic effect at concentrations near their respective IC50 values.

Lipofectamine 2000 (Hyclone, USA) was used for the transfection of BT-474 cells with si-NC, si-HER-2, with or without IGF-1 (an activator of the PI3K/AKT signaling pathway). Following diverse treatments administered for 24 hours, the cells were collected for functional analysis.

Gene expression analysis

RNA sequencing expression data from 8,587 normal samples and 9,736 tumor samples, sourced from Genotype-Tissue Expression (GTEx) project and TCGA, were stored in the Gene Expression Profiling Interactive Analysis (GEPIA) database. To ascertain the expression levels of HER-2 in both breast cancer patients and healthy individuals, the GEPIA database was employed, encompassing 1,085 breast cancer samples and 291 healthy controls. The GEPIA database, accessible at http://gepia.cancer-pku.cn/detail.php, served as a valuable resource for this investigation. Statistical significance was set at a cutoff value of $P < 0.05$.

Cell viability

The cells were cultured in 96-well plates at 37°C with 5% CO_2 for 24 hours. To assess cell proliferation, the CCK-8 kit (C0037, Beyotime, Beijing, China) was utilized, whereby the optical density (OD) at 450 nm was quantified using a microplate reader. The experiments were performed in eight parallel sets, with three replicates per set.

Clone formation assay

Upon completion of different treatments, cell quantification was performed following a 48-hour incubation. Roughly 800 cells from each group were seeded into a Petri dish and cultured in a CO_2 incubator. Media were replaced every 2-3 days, while monitoring cell progression. After approximately 14 days of culture, the dishes were removed and rinsed twice with PBS buffer. Subsequently, a solution of 4% paraformaldehyde (Beyotime, Beijing, China) was added to fix the cells for 0.5 hours. The paraformaldehyde was then aspirated, and the cells were washed twice with PBS buffer. Following this, 4 mL of staining solution containing crystal violet (Beyotime, Beijing, China) was added, and the cells were incubated overnight at room temperature. After staining, the dishes were washed 3-5 times with PBS, and photographs of the spot formation were captured once the dishes had dried. A clone was defined as a cell cluster containing ≥ 50 cells. The experiment was conducted in triplicate.

Wound healing assay

At a concentration of 1×10^6 mL/well, the cells were seeded into 6-well plates. After 24 hours, when the cell density reached approximately 90%, serum deprivation was carried out for 24 hours using serum-free medium. Once the cell monolayer was confluent, a straight-line scratch was created using a 100 μL pipette tip. The detached cells were removed by washing with PBS (GIBCO) three times. The cells were then cultured in drug-enriched serum-free DMEM/F12 medium for 48 hours to allow wound healing. Images were captured at 0 and 48 hours at the same location where the scratch was made. The migration length was measured using Image J software (V1.8.0, National Institutes of Health, Bethesda, Maryland, USA) based on changes in wound size.

Transwell experiment

Migration analysis was performed using transwell chambers (Corning, USA) with 8-μm pore size. Initially, 8×10^5 cells were seeded in the upper compartment of the chamber in serum-free medium. Medium containing 10% FBS was added into the lower compartment as an attractant. Incubation was conducted at 37°C for 24 hours. Following that, the permeable inserts were removed, and the migrated cells were fixed, stained, and quantified (at a magnification of ×200). The experiment was repeated at least thrice. Cell images were analyzed using ImageJ software (V1.8.0, National Institutes of Health, Bethesda, Maryland, USA). For invasion analysis, the same conditions were applied using BD Biocoat Matrigel Invasion Chambers (BD Biosciences), with negative controls and transfected cells subjected to the assay in 24-well plates.

TUNEL assay

To assess programmed cell death in BT-474 cells, the TUNEL method (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) was carried out using the apoptosis detection kit (Millipore, Billerica, MA, USA). Cells were cultured and plated on chamber slides (Becton Dickinson, Franklin Lakes, NJ). The DNA fragments at free 3'-OH ends and DNA strand breaks in apoptotic cells were labeled. The incorporated fluorescein was detected using alkaline phosphatase-conjugated anti-fluorescein antibody. Stained cells were subsequently examined under a light microscope (Cx43, Olympus, Hachioji-shi, Tokyo, Japan) after the substrate reaction.

Detection of cell apoptosis by flow cytometry

Cells were harvested and washed twice with cold PBS. They were then resuspended in binding buffer at a concentration of approximately 1 × 10^6 cells/mL. Subsequently, 5 μL of Annexin V-FITC and 5 μL of propidium iodide (PI) were added to 100 μL of the cell suspension. After mixing, the samples were incubated in the dark at room temperature for 15 minutes. Finally, 400 μL of binding buffer was added to each sample, and apoptotic cells were analyzed immediately using a flow cytometer.

KEGG signaling pathway enrichment analysis

To evaluate the expression of HER-2 in breast cancer tissues compared to normal tissues, the KEGG database was utilized. Signaling pathways with a false discovery rate (FDR) of < 0.05 were considered notably enriched. Subsequently, the results of this enrichment analysis were visualized using the GOplot R package (https://cran.r-project.org/web/packages/GOplot/index.html).

Databases to analyze HER-2-targeted genes related to PI3K/AKT signaling pathway

To investigate the interactions between HER-2 and proteins associated with the PI3K/AKT signaling pathway, String database (https://string-db.org/) was utilized. The key interaction nodes were subsequently imported into STRING, with the minimum interaction threshold set at > 0.7.

qRT-PCR

RNA extraction from cells was carried out using Trizol reagent (Boyotime, Beijing, China) according to the manufacturer's instructions. The extracted RNA was used to synthesize cDNA (Beyotime, Beijing, China). PCR was performed on an ABI 7900 fluorescence quantitative PCR instrument (ABI, USA). Primers were as follows: HER-2 (human): F-5'-GAGGCTCCTCTGTGAGGAA-3' and R-5'-GGTGGAGGAGATGAGCAGAG-3', PI3K (human): F-5'-AGCTGCTTGGAGATGAGAGG-3' and R-5'-TGTGAGGACAGGAGGAGGAG-3', AKT (human): F-5'-AGGATGAGAAGTTTCAGGAGG-3' and R-5'-GGAGGAGGAGGAGGAGGAGT-3', GAPDH (human): F-5'-GTCTCCTATGACTTCAACAGCG-3' and R-5'-ACCACCCTGTTGGTGTAGCCAA-3'. To standardize mRNA expression, GAPDH expression was used as an internal control, and relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Western blot

The obtained protein was transfected onto polyvinylidene fluoride (PVDF) film and sealed with 5% skim milk. Then, the membranes were incubated with primary antibodies at 4°C overnight, including HER-2 (2242S, 1:2000), Bax (2772S, 1:2000), Bcl-2 (3498S, 1:2000), p-PI3K (4249S, 1:2000), PI3K (4249S, 1:1000), AKT (9272S, 1:2000), p-AKT (4060S, 1:1000), and GAPDH (2118S, 1:2000). All the primary antibodies were obtained from Beyotime (Beijing, China). Subsequently, the membranes were incubated with a secondary antibody (A0201, 1:2000, Beyotime, Beijing, China). Enhanced chemiluminescence was employed to visualize the protein bands, with GAPDH serving as an internal reference. The quantification of protein levels mentioned above was conducted densitometrically using Image-J (V1.8.0, National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis

Data were analyzed using SPSS Statistics (Version 20, Chicago, IL, USA). Continuous data were expressed as means ± standard deviation (SD). For comparisons between two groups, a t-test was performed. For comparisons involving three or more groups, a one-way ANOVA and LSD test were utilized. A P value of < 0.05 was considered statistically significant.

Results

Expression of HER-2 in human breast cancer and in vitro

As illustrated in **Figure 1A**, breast cancer patients exhibited significantly higher HER-2 expression compared to the control group (P < 0.05). To further investigate HER-2 expression, real-time PCR and Western blot analyses were conducted on MCF-10A, BT-474, and SK-BR-3 cells. Compared to MCF-10A cells, both BT-474 and SK-BR-3 cells displayed a remarkable increase in HER-2 expression (P < 0.001). Among the three cell types, BT-474 cells exhibited the highest level of HER-2 expression (**Figure 1B**, **1C**).

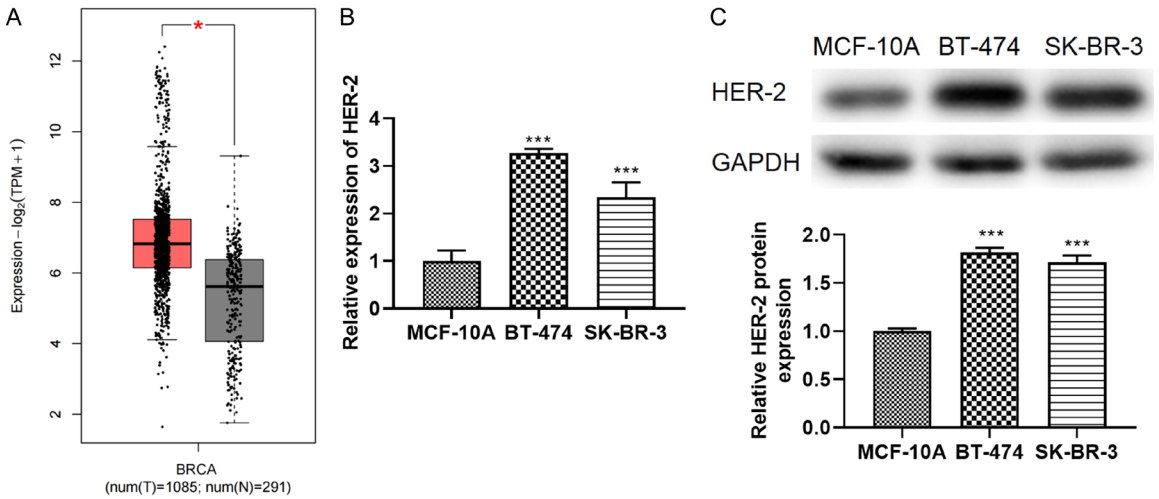

Figure 1. The expression of HER-2 in breast cancer tissues and cells. A. The expression of HER-2 in breast cancer tissues and healthy controls obtained from GEPIA database. B. The mRNA expression of HER-2 in MCF-10A, BT-474, and SK-BR-3 cells detected by Real-time PCR. C. The protein expression of HER-2 in MCF-10A, BT-474, and SK-BR-3 cells detected by Western blot. $^{*}P < 0.05$, $^{***}P < 0.001$.

Toxicity of pertuzumab combined with pyrrolitinib on breast cancer cells

Both pertuzumab and pyrrolitinib demonstrated dose-dependent inhibition of BT-474 cell growth. The IC50 values for pertuzumab, pyrrolizinib, and their combination were determined. Notably, the combination of pertuzumab and pyrrolizinib exhibited significantly greater efficacy in inhibiting cell proliferation in BT-474 cells compared to either agent alone ($P < 0.001$, **Figure 2A**).

Following that, a comprehensive evaluation was performed to assess the effects of pertuzumab, pyrrolitinib, and their combination on the viability, proliferation, migration, invasion, and apoptosis of BT-474 cells. As depicted in **Figure 2B-E**, both the pertuzumab group ($P < 0.01$) and pyrrolitinib group ($P < 0.05$) suppressed cell viability, proliferation, migration, and invasion, and their combination demonstrated a more potent inhibition of these cellular functions (all $P < 0.001$).

Additionally, both pertuzumab and pyrrolitinib treatment alone promoted cell apoptosis (all $P < 0.05$), with the combination treatment showing even higher levels of apoptosis ($P < 0.001$, **Figure 2F**, **2G**). Furthermore, a noticeable elevation in Bax expression and a significant reduction in Bcl-2 levels were observed in the pertuzumab group and the pyrrolitinib group ($P < 0.05$), and their combination demonstrated a striking enhancement of Bax induction and Bcl-2 reduction ($P < 0.001$, **Figure 2H**).

Effects of the combination of pertuzumab and pyrrolidinyl on HER-2 expression in vitro

The level of HER-2 in BT-474 cells under different treatment conditions was assessed using Real-time PCR and Western blot analyses. As depicted in **Figure 3A** and **3B**, both single treatments with pertuzumab and pyrrolitinib significantly decreased HER-2 expression ($P < 0.001$), and their combination led to a more pronounced reduction in HER-2 levels in BT-474 cells ($P < 0.001$).

Effects of knocking down HER-2 on the PI3K/AKT signaling pathway

KEGG pathway enrichment analysis revealed the PI3K/AKT signaling pathway as a key pathway associated with HER-2 (**Figure 4A**). String database showed that HER-2 (ERBB2) interacted with AKT1 and PIK3CA (**Figure 4B**). Subsequent transfection with si-HER-2 resulted in a reduction in HER-2 expression ($P < 0.05$, **Figure 4C**, **4D**). Furthermore, si-HER-2 significantly decreased the levels of PI3K and AKT ($P < 0.001$, **Figure 4E**, **4F**). However, these effects were reversed upon treatment with IGF-1, an activator of the PI3K/AKT signaling pathway ($P < 0.05$).

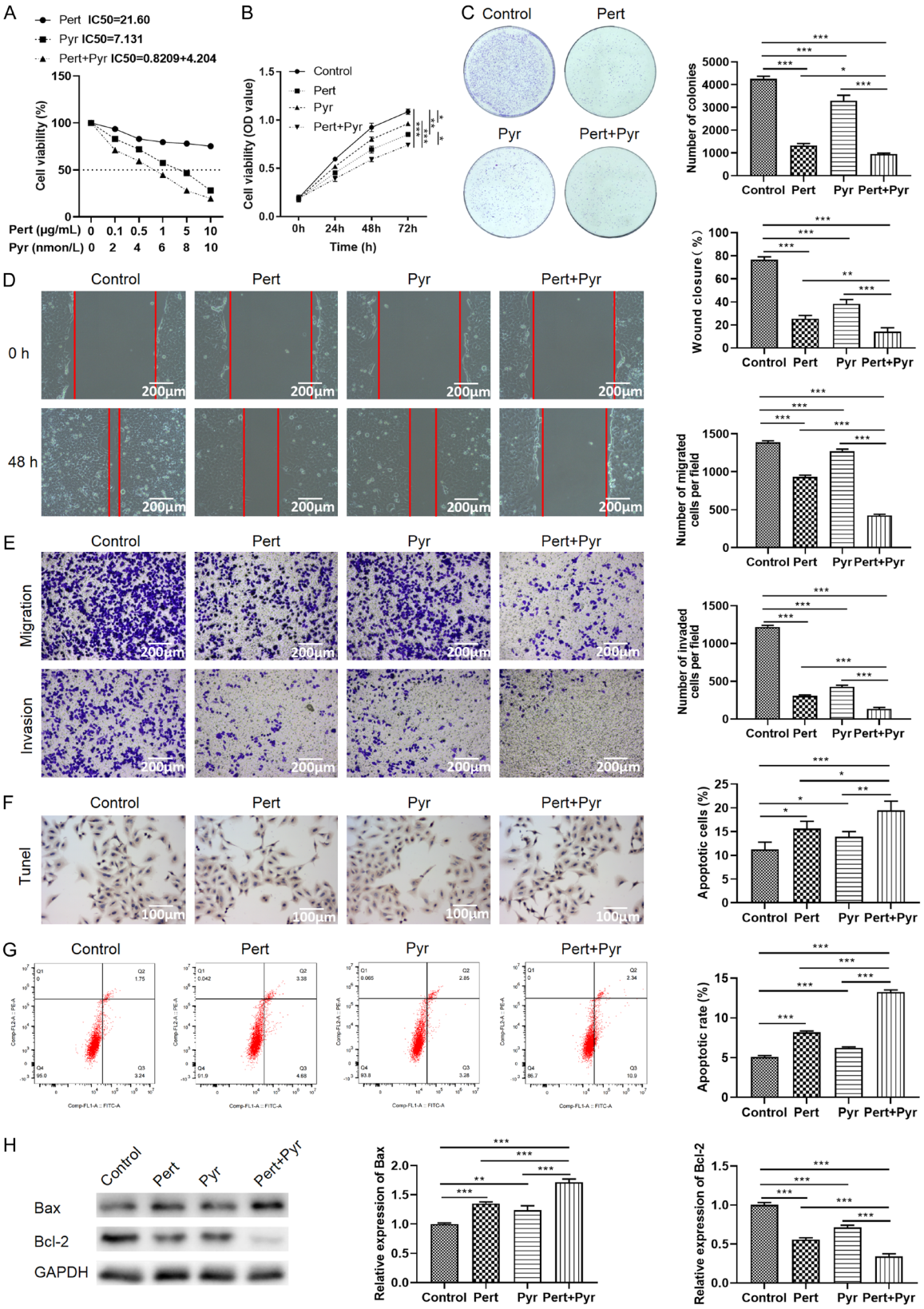

Figure 2. Toxicity of pertuzumab, pyrrolitinib, and their combination on breast cancer cells. A. IC50 detected by CCK-8 assay. B. Cell viability detected by CCK-8 assay. C. Cell proliferation detected by clone formation assay. D. Cell migration detected by wound healing assay. E. Cell migration and invasion detected by transwell assay. F. Cell apoptosis detected by TUNEL assay. G. Cell apoptosis detected by Flow Cytometry. H. The expression of Bax and Bcl-2 detected by Western blot. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

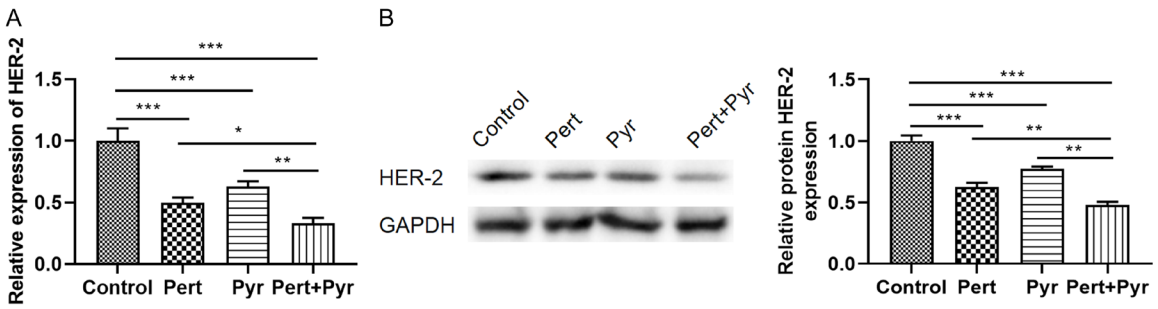

Figure 3. Effects of pertuzumab, pyrrolitinib, and their combination on the expression of HER-2 in BT-474 cells. A. The expression of HER-2 detected by Real-time PCR. B. The expression of HER-2 in BT-474 cells detected by Western blot. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

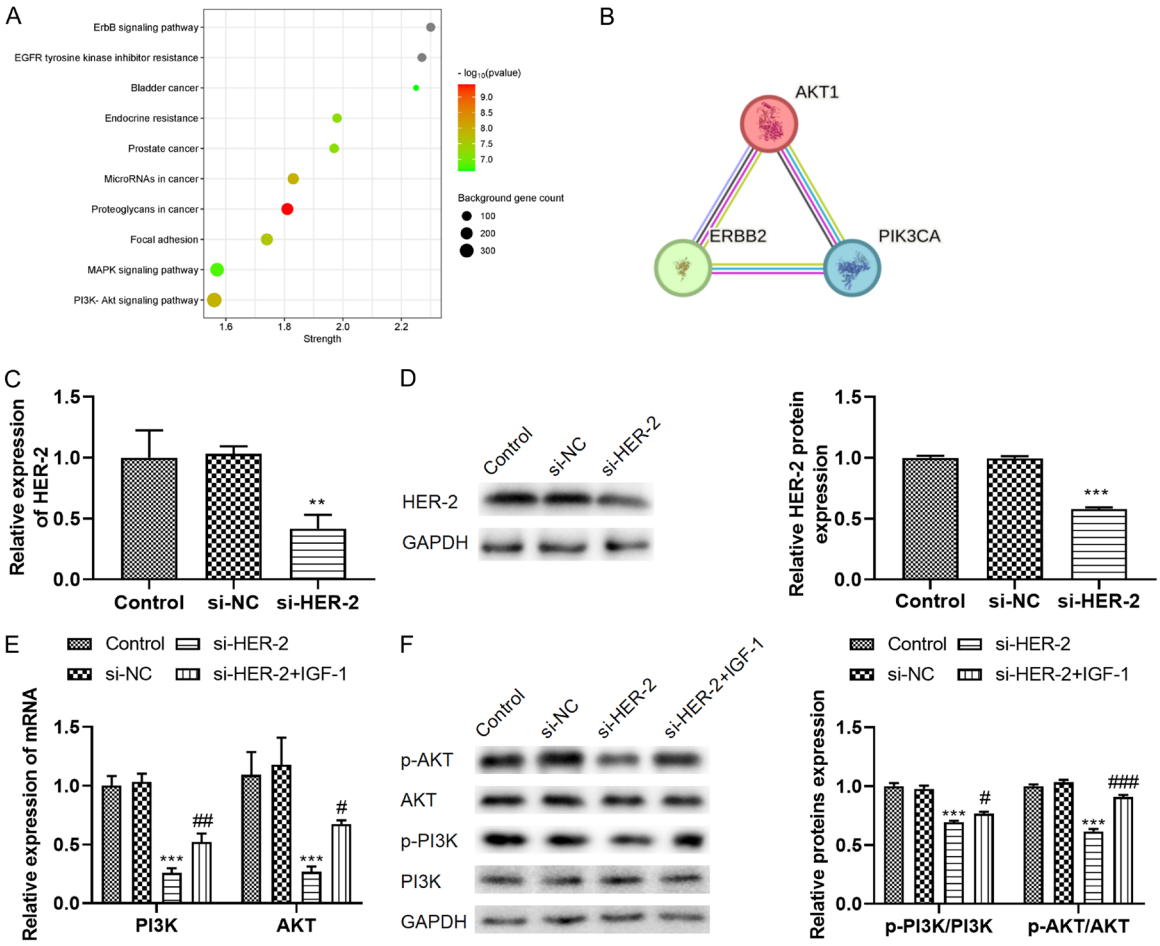

Figure 4. Effects of knocking down HER-2 on the PI3K/AKT signaling pathway. A. KEGG pathway enrichment analysis. B. String database analysis of the targeted protein of HER-2. C. The mRNA level of HER-2 in BT-474 cells transfected with si-HER-2 detected by Real-time PCR. D. The protein level of HER-2 in BT-474 cells transfected with si-HER-2 detected by Western blot. E. The expression of PI3K and AKT in BT-474 cells transfected with si-HER-2 detected by Real-time PCR. F. The protein level of PI3K and AKT in BT-474 cells transfected with si-HER-2 detected by Western blot. $^{**}P < 0.01$, $^{***}P < 0.001$ comparing with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ comparing with the si-HER-2 group.

Effects of knockdown of HER-2 on cell proliferation, migration, invasion, and apoptosis of breast cancer cells via the PI3K/AKT signaling pathway

As illustrated in **Figure 5A-D**, the transfection of si-HER-2 led to a remarkable reduction in cell viability, proliferation, migration, and invasion of BT-474 cells ($P < 0.05$). However, the addition of IGF-1 effectively reversed these effects ($P < 0.05$). In contrast, a notable elevation in cell apoptosis was observed in the si-HER-2 group ($P < 0.001$), and such response was ameliorated by IGF-1 ($P < 0.01$, **Figure 5E**, **5F**). Furthermore, a discernible increase in Bax expression, accompanied by a notable decrease in Bcl-2 levels, was observed in the si-HER-2 group ($P < 0.001$). Nevertheless, these alterations in protein expression were subsequently reversed upon IGF-1 treatment ($P < 0.05$, **Figure 5G**).

Discussion

Breast cancer is a complex and heterogeneous disease [25], making it crucial to understand the molecular mechanisms underlying its development and progression in order to identify effective therapeutic approaches. Previous studies have demonstrated the therapeutic potential of pertuzumab in HER2-positive breast cancer [26, 27], but the effects of its combination with pyrrolitinib and the underlying mechanism remain unclear. In this study, we provide the first evidence of the combined impact of pertuzumab and pyrrolitinib on breast cancer cells, while also exploring the role of HER-2 in the PI3K/AKT signaling pathway and its subsequent effects on cell proliferation, migration, invasion, and apoptosis.

Our results demonstrated that the level of HER-2 was higher in breast cancer patients than that in healthy controls, which aligns with previous studies reporting HER-2 amplification and overexpression in a subset of breast cancer cases [28, 29]. HER-2 overexpression is linked to an aggressive tumor phenotype, unfavorable prognosis, and resistance to conventional treatments [12, 30]. Additionally, in our in vitro breast cancer model, including MCF-10A, BT-474, and SK-BR-3 cells, we observed similar results to those found in human samples. Specifically, we witnessed a noteworthy surge in HER-2 expression in BT-474 and SK-BR-3 cells compared to MCF-10A cells, with BT-474 cells showing the highest HER-2 levels. Therefore, BT-474 cells were selected for subsequent studies. The treatment targeting HER2 in breast cancer with HER-2 overexpression has introduced a novel therapeutic approach. Notably, survival rates for women with HER2-positive breast cancer have reached nearly 5 years, with 75% of patients achieving a complete pathological response. Over the past decade, treatment advancements have significantly improved the prognosis for patients with HER-2-positive breast cancer. Nevertheless, the persistently high mortality rate associated with HER2-positive breast cancer underscores the need for continued clinical research into novel therapies and their combinations [31].

Furthermore, we observed a more pronounced reduction in HER-2 levels in BT-474 cells following combination treatment with pertuzumab and pyrrolitinib. This discovery aligns with prior investigations documenting a decline in HER-2 expression after treatments specifically targeting HER-2 [32]. The downregulation of HER-2 is believed to be a result of receptor internalization, degradation, and/or decreased gene transcription [33]. The combination of pertuzumab and pyrrolitinib may enhance these mechanisms, leading to a more pronounced reduction in HER-2 levels. Then, we investigated the effects of the combination treatment on various cellular functions, including cell viability, proliferation, migration, invasion, and apoptosis. Our findings exemplified that the combination therapy exhibited a more potent suppressive effect on these cellular functions compared to either drug alone, which is in line with previous studies showing enhanced anti-tumor activity and reduced metastasis when pertuzumab is combined with other HER-2-targeted therapies [27].

Furthermore, our study examined the involvement of HER-2 in the PI3K/AKT signaling cascade. Pathway enrichment revealed a fundamental association between the PI3K/AKT signaling pathway and HER-2. The PI3K/AKT pathway plays a critical role in cell viability, proliferation, and migration, and its dysregulation is commonly observed in various malignancies, including breast cancer [34]. Then, we focused on the role of HER-2 in the PI3K/AKT signaling pathway. Silencing HER-2 utilizing siRNA led to a decline in HER-2 expression, followed by a

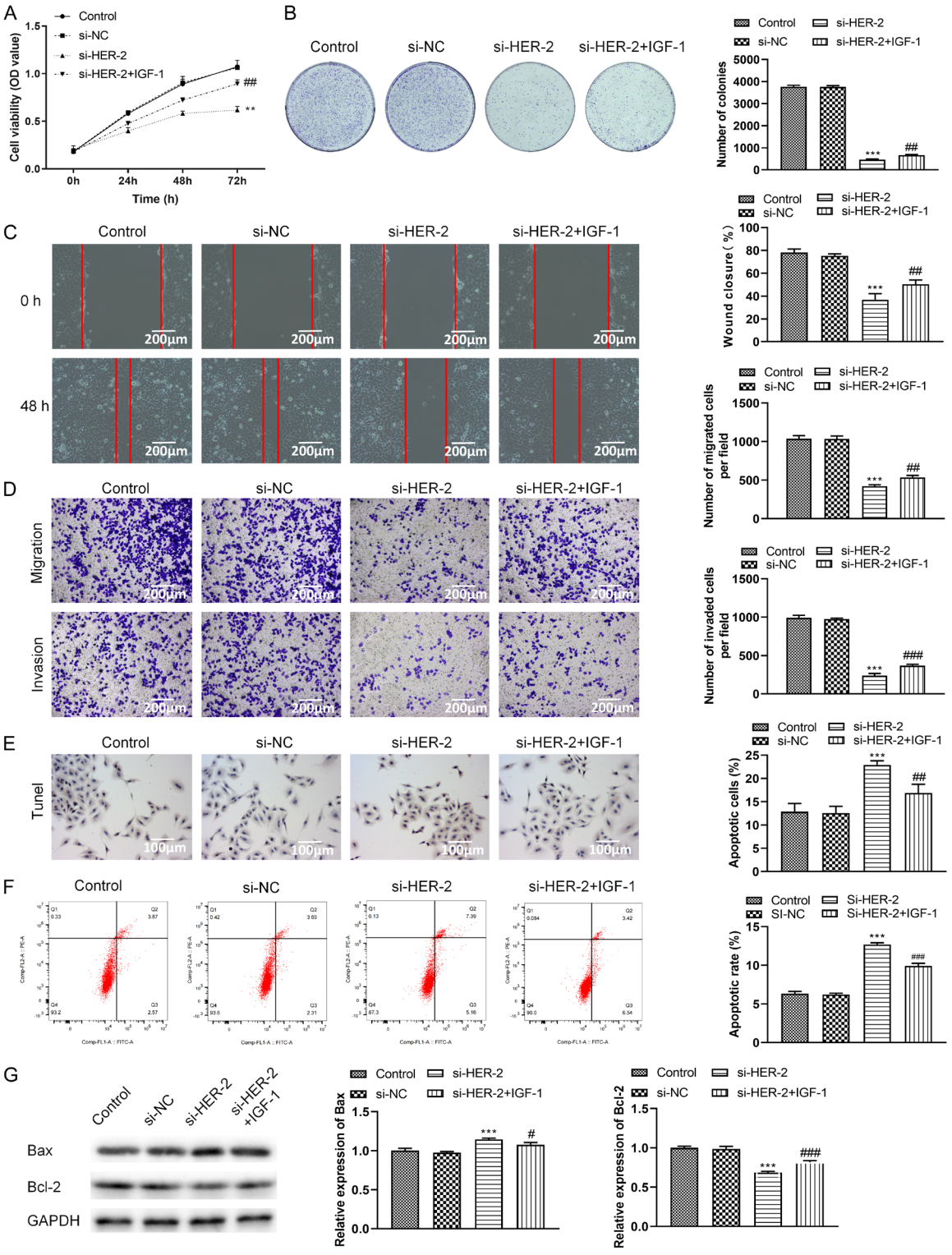

Figure 5. Effects of HER-2 knockdown on cell functions of BT-474 cells via the PI3K/AKT signaling pathway. A. Cell viability detected by CCK-8 assay. B. Cell proliferation detected by clone formation assay. C. Cell migration detected by wound healing assay. D. Cell migration and invasion detected by transwell assay. E. Cell apoptosis detected by TUNEL assay. F. Cell apoptosis detected by Flow Cytometry. G. The expression of Bax and Bcl-2 detected by Western blot. $^{***}P < 0.001$ comparing with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ comparing with the si-HER-2 group.

decrease in the expression of PI3K and AKT. These findings indicate that HER-2 is upstream of the PI3K/AKT pathway and regulates its activation [35]. Moreover, the impacts of suppressing HER-2 on cell survival, proliferation, migration, invasion, and apoptosis were consistent with the inhibitory effects observed with the combination therapy of pertuzumab and pyrrolitinib. These discoveries imply that the anti-cancer effects of the combined therapy may, in part, be mediated through the inhibition of the PI3K/AKT pathway.

Conclusion

In conclusion, HER-2 is highly expressed in breast cancer tissues and cell lines. Our study demonstrates that the combination of pertuzumab and pyrrolitinib exerts a synergistic effect, effectively inhibiting cell proliferation and reducing HER-2 expression. Additionally, both HER-2 knockdown and the combination treatment lead to the inhibition of the PI3K/AKT pathway and the suppression of cell viability, proliferation, migration, invasion, and promotion of apoptosis. These findings highlight the potential of combining pertuzumab and pyrrolitinib as an enhanced therapeutic strategy for HER-2-positive breast cancer. However, it is important to note the limitations of our study, such as the lack of further investigations in the animal models of breast cancer, which should be explored in future research.

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Disclosure of conflict of interest

None.

Abbreviations

HER-2, human epidermal growth factor receptor-2; GEPIA, Gene Expression Profiling Interactive Analysis.

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