YAP/TAZ Pathway Promoted the Trastuzumab Resistance in HER2-Positive Breast Cancer

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ABSTRACT

The developed resistance of trastuzumab remained a problem for clinical therapy of HER2-positive breast cancer. However, effects of YAP/TAZ pathway on resistance of trastuzumab have not been explored. Tumor tissues were collected from 40 breast cancer patients for clinical studies. For in vitro studies, human breast cancer cell lines SK-BR-3-TS was obtained, and trastuzumab resistant model SK-BR-3-TR was constructed. Cell viability was determined using MTT assay. Cell apoptosis was analyzed by flow cytometry. Protein and mRNA expression was measured using western blotting and RT-qPCR, respectively. The mRNA and protein level of YAP was significantly increased in the tumor tissues of HER2-positive breast cancer patients. Consistently, the expression of YAP and TAZ were both dramatically upregulated in SK-BR-3-TR cells. The cell viability was increased, while cell apoptosis was inhibited in SK-BR-3-TS cells compared with SK-BR-3-TS. The depletion of YAP by si-YAP reversed the YAP/TAZ expression, cell viability and cell apoptosis in SK-BR-3-TR cells. YAP/TAZ pathway might induce the trastuzumab resistance in HER2-positive breast cancer and targeting YAP would be an alternative way for the clinical therapeutic methods of HER2 positive breast cancer.

INTRODUCTION

Breast cancer has been regarded as one of the cancers that caused significant morbidity and mortality in female (Akram et al., 2017). It was estimated that the 5-year survival rate was 80% in developed countries, whereas that was only 40% for developing countries (Coleman et al., 2008). Human epidermal growth factor receptor 2 (HER2) belonged to HER family, whose expression was demonstrated to be increased in around 20% breast cancer and associated with aggressive cancers and disease recurrence (Kunte et al., 2020). Trastuzumab has been identified as a monoclonal antibody, which could target against the extracellular domain of the HER2 receptor, and chemotherapy combined with trastuzumab was the standard therapeutic methods for the early-stage therapy of breast cancer in the past few decades (Zhang, 2021). However, it was reported that only a small part of metastatic patients was responded to trastuzumab, and around 60% of them developed resistance (Han et al., 2020). Therefore, investigations on the mechanisms regarding resistance occurrence and approaches to reducing resistance are of vital importance.

YAP was identified as a 65 kDa protein that contained transcriptional activation domain and TEAD binding domain, and the sequence identity between TAZ and YAP was 50% (Reuven et al., 2019). The domains of YAP/TAZ played various biological roles in the cellular processes of tumorigenesis. The transcriptional co-activators YAP/
TAZ could be regulated by multiple upstream signals, of which Hippo pathway accounted for a main part (Zhao et al., 2010). Previous studies also revealed that YAP/TAZ pathway participated in the proliferation and adhesion of breast cancer (Zhao et al., 2021). Recent studies indicated that YAP was able to induce cisplatin resistance through cellular autophagy and could mediate paclitaxel resistance in ovarian cancer cells (He et al., 2019; Xiao et al., 2016). However, the relations of YAP and trastuzumab resistance has not been comprehensively explored in breast cancer.

In the present study, we hypothesized that the YAP/TAZ pathway could induce the trastuzumab resistance in HER2-positive breast cancer. The clinical tissues were collected, and the expression of YAP were detected. Moreover, the drug-resistant cell model was established for in vitro verifications. With the deepening understanding of the biological behavior of breast cancer and the transformation and updating of the treatment concept, this study is expected to provide new ideas for the mechanism of acquired drug resistance in breast cancer.

**MATERIALS AND METHODS**

**Clinical studies**

The frozen tumor tissues and the adjacent tissues were collected from 40 breast cancer patients. The experiments obtained the informed consent of all the patients. The study was performed as per the guidelines of the committee and the declaration of Helsinki. All the clinical experiments were approved by the Ethics Committee of the Hainan General Hospital, Hainan Medical University.

**Cell culture**

The human breast cancer cell lines SK-BR-3-TS was obtained from American Type Culture Collection (ATCC, USA). The cells were cultured in cultured in Roswell Park Memorial Institute (RPMI, Gibco, USA) media containing 10% fetal bovine serum and 1% penicillin/streptomycin. For construction of trastuzumab resistant models, cells at logarithmic growth phase were collected, after which 0.5 μg/ml (10 times of 50% inhibition concentration) trastuzumab (Roche, Shanghai, China) was added into complete medium. Subsequently, the concentration of trastuzumab was added (0.5, 1, 2, 4, 6 to 8 μg/ml), and the SK-BR-3-TR cells were collected when cells stably grew in medium containing 8 μg/ml trastuzumab. For further experiments, cells were maintained in medium containing 4 μg/ml trastuzumab. All the cells were incubated in a 5% CO₂ humidified incubator at 37°C.

**Cell transfection**

For constructing the knockdown model of YAP, si-YAP was synthesized by GenePharma Co., Ltd. (Shanghai, China). Cell transfection was performed when the confluence of cells reached 90% using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) as per the protocol of manufacturer.

**Cell viability**

MTT assay was employed for measurement of cell viability. Briefly, cells were seeded into 96-well plates at a density of 5×10³ cells/well and then treated under various conditions for 48 h. After three PBS washes, the cells were treated with MTT solution (0.1 mg/ml, Solarbio, Beijing, China) as per the protocol of manufacturer and incubated for 4 h at 37°C. The absorbance was detected at 570 nm by a microplate reader (Bio-Tek, Norcross, GA, USA).

**Cell apoptosis**

The cell apoptosis was determined by flow cytometer analysis. Briefly, cells were seeded into 6-well plates at a density of 2.5×10⁵ cells/well and then treated under various conditions for 48 h. After three PBS washes, the cells were treated with Annexin V-FITC/PI apoptosis detection kit (BD Bioscience, USA) as per the manufacturer’s instructions. Briefly, cells were suspended with 100 μL 1×Binding Buffer and 5 μL Annexin V solutions and incubated for 5 min at room temperature, after which cells were centrifugated at 1000 rpm for 5 min and resuspended with 10 μL propidium iodide and 190 μL buffer. The double-stained cells were analyzed by a flow cytometer (Thermo Scientific, Waltham, MA, USA).

**RT-qPCR**

Total RNA was extracted from cells by TRizol reagent (Life Technologies, Carlsbad, CA, USA). Then the cDNA was synthesized using Prime Script reverse transcription reagent kit (TaKaRa, Japan). RT-qPCR were performed with quantitative fluorescent PCR kit (Cwbiotech, Beijing, China) and performed on ABI Prism system (Applied Biosystems, USA). The relative expressions of mRNA were measured using 2−ΔΔCt method via normalization to GAPDH. The primers were list as follows:

- YAP-F: 5'- CCTTCTTCAAGGCGGAGGAG -3'
  5'- CAGTGTCCCAGGAGAAACAGC -3'
- TAZ-F: 5'- TATCCCAGCCAAATCTCGTG -3'
  5'- TTCTGCTGGCTCAGGGTACT -3'
- GAPDH-F: 5'- TCAAGAAGGTGGTGAAGCAGG - 3'
  5'- TCAAAGGTGGAGGAGTGGGT -3'

**Western blotting**

Total protein was extracted from cells using using RIPA buffer (Sigma-Aldrich, USA) that supplemented with protease (1:100) and phosphatase (1:100) inhibitors.
The concentration was determined using the BCA Assay Kit (Beyotime, Shanghai, China). The samples were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Massachusetts, USA). The nonfat milk was obtained for blocking at room temperature for 1 h. Then the membrane was incubated with primary antibodies that included anti-YAP (Abcam, ab52771, 1:1000), anti-TAZ (Abcam, ab110239, 1:1000) and anti-GAPDH (Abcam, ab8245, 1:1000). Then the samples were incubated with horseradish peroxidase-conjugated secondary antibody (Abcam, ab6721 1:1000) for 2 h at room temperature.

Statistical analysis

All data were expressed as means ± standard derivation (SD). GraphPad Prism 8 was used for statistical analysis. Student’s t tests were used for comparisons between two groups. One-way ANOVA analysis was performed for comparisons among multiple groups. P-values < 0.05 were considered to indicate statistically significant results.

RESULTS

The analysis on clinical tissues revealed that the mRNA (Fig. 1A) and protein (Fig. 1B) level of YAP was dramatically upregulated in the tumor tissues compared with adjacent tissues in HER2-positive breast cancer patients. Consistently, the in vitro experiments showed that the mRNA (Fig. 2A) and protein (Fig. 2B) level of YAP and TAZ were dramatically upregulated in SK-BR-3-TR cells compared with SK-BR-3-TS. Moreover, MTT assay indicated that the cell viability was increased (Fig. 3A), while flow cytometry results (Fig. 3B) proved that cell apoptosis was inhibited in SK-BR-3-TR cells compared with SK-BR-3-TS. For elucidating the role of YAP, the depletion of YAP was achieved by transfection of si-YAP. The protein level of YAP and TAZ was downregulated after transfection of si-YAP (Fig. 4A), and the cell viability was decreased in si-YAP group (Fig. 4C). The above results suggested that inhibition of YAP might alleviate the tumor processes of trastuzumab resistant HER2-positive breast cancer cells.

DISCUSSION

HER2 was reported to be overexpressed in around 20% of breast cancers, which contributed to increased clinical behavior (Guarneri et al., 2010; Zimmer et al., 2022). The poor prognosis has always been an important problem for patients with breast cancer till the availability of HER2-directed monoclonal antibodies (Hudis, 2007). The diagnosis and treatment of HER2 positive breast cancer was considerably changed in the past few decades, and the
Fig. 3. Cell viability was increased while cell apoptosis was inhibited in SK-BR-3-TR cells. (A) Cell viability detected by MTT assay. (B) Cell apoptosis analyzed by flow cytometry. ** P<0.01 vs SK-BR-3-TS group (Student’s t tests).

Fig. 4. Depletion of YAP reversed the induced trastuzumab resistance in SK-BR-3-TR cells. (A) Protein expression of YAP and TAZ detected by western blotting. (B) Cell apoptosis analyzed by flow cytometry. (C) Cell viability detected by MTT assay. ** P<0.01 vs SK-BR-3-TS group. ** P<0.01 vs SK-BR-3-TR group (One way ANOVA).
breast cancer cells to trastuzumab. The depletion of YAP significantly increased apoptosis rate and decreased cell viability of SK-BR-3-TR cells.

In conclusion, the present study elucidated that YAP/TAZ pathway might induce the trastuzumab resistance in HER2-positive breast cancer and targeting YAP would be an alternative way for the clinical therapeutic methods of HER2 positive breast cancer. However, further in vivo investigations and clinical studies are needed to comprehensively understand and verify the role of YAP/TAZ signaling in trastuzumab resistance in HER2-positive breast cancer. The down streaming pathways of YAP/TAZ signaling in trastuzumab resistance also needs further exploration.

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DECLARATIONS

Ethics approval and consent to participate

The ethic approval was obtained from the Ethic Committee of Hainan General Hospital, Hainan Medical University and written informed consent was obtained from all patients.

Consent to publish

All of the authors have Consented to publish this research.

Availability of data and materials

The data are free access to available upon request.

Competing interests

All authors declare no conflict of interest.

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