Deubiquitinase USP16 stabilizes CYBA expression and promotes progression and chemoresistance in triple-negative breast cancer

Yuxin Huang, Senmiao Zhang, Yang Qin, Guanwen Wang, Fanli Qu, Sisi Li, Ling Wang, Ping Wen, Dongping Jiang, Qing Shao, Ningning Zhang*, Xiaohua Zeng*.

Department of Breast Center, Chongqing Key Laboratory for Intelligent Oncology in Breast Cancer (iCQBC), Chongqing University Cancer Hospital, Chongqing 400030, China.

BACKGROUND

Triple-Negative Breast Cancer (TNBC) has become a challenge in global breast cancer prevention and treatment due to its aggressive nature, poor prognosis, and lack of molecular markers for targeted therapy. It is urgent to explore its progression mechanisms and find effective therapeutic targets. The aim of this study was to explore the role and mechanism of tumor promoter CYBA (Cytochrome B-245 Alpha Chain) in the progression of TNBC.

METHODS

- WB, qRT-PCR and IHC were used to evaluate the expression of CYBA in tumor and adjacent normal tissues.
- CCK-8, EDU assay and subcutaneous xenograft model were used to assess its effect on proliferation.
- Scratch assay, Transwell assay and tail vein injection model were used to assess its effect on metastasis.
- LC-MS/MS, RNA-seq, IF and CoIP were used to explore the mechanisms by which CYBA exerts its biological effects.

RESULTS

CYBA is highly expressed in TNBC and associated with poor OS

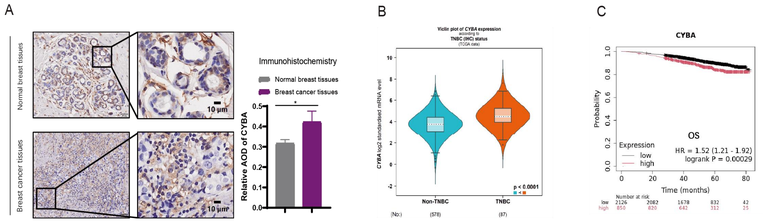


Fig.1 A. IHC staining of CYBA in breast cancer tissues and normal breast tissues. **B.** Differential expression of CYBA in non-TNBC and TNBC. **C.** Kaplan–Meier curve illustrating overall survival by CYBA expression level.

RESULTS

CYBA promotes the migration and invasion of TNBC

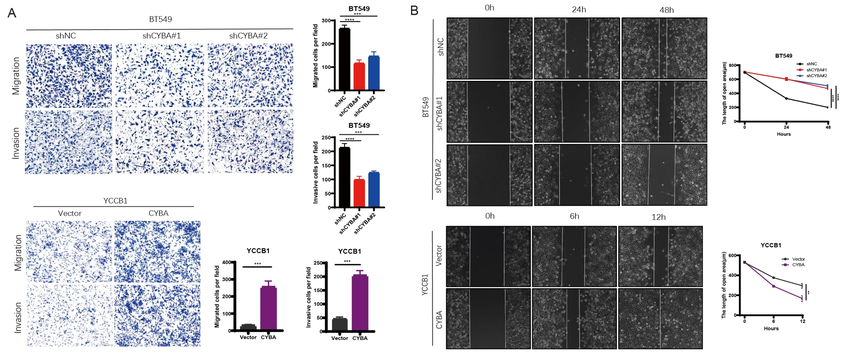


Fig.2 A. The effect of CYBA silencing or overexpression on the migratory and invasive capabilities of TNBC cells were assessed by migration and Matrigel invasion assays, and the corresponding quantification is shown in histograms. **B.** The effect of CYBA silencing or overexpression on TNBC cell migration was evaluated using a wound healing assay, with quantitative measurements displayed in histograms.

CYBA promotes the proliferation and chemoresistance of TNBC

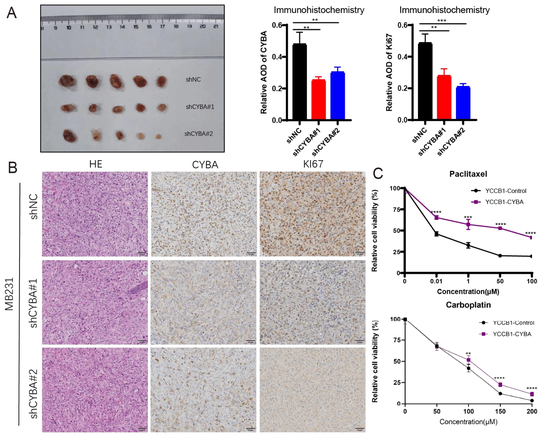


Fig.3 A. Tumor size in mice from the control (sh-NC) and CYBA-knockdown groups (shCYBA#1 and shCYBA#2). **B.** Representative H&E staining, IHC staining of CYBA and Ki67, and quantitative analysis of positive staining in tumor tissues from the control and CYBA-knockdown groups. **C.** Cell viability assays showing the sensitivity of control and CYBA-overexpressing TNBC cells to paclitaxel and carboplatin treatment.

USP16 physically interacts with CYBA and mediates its deubiquitination

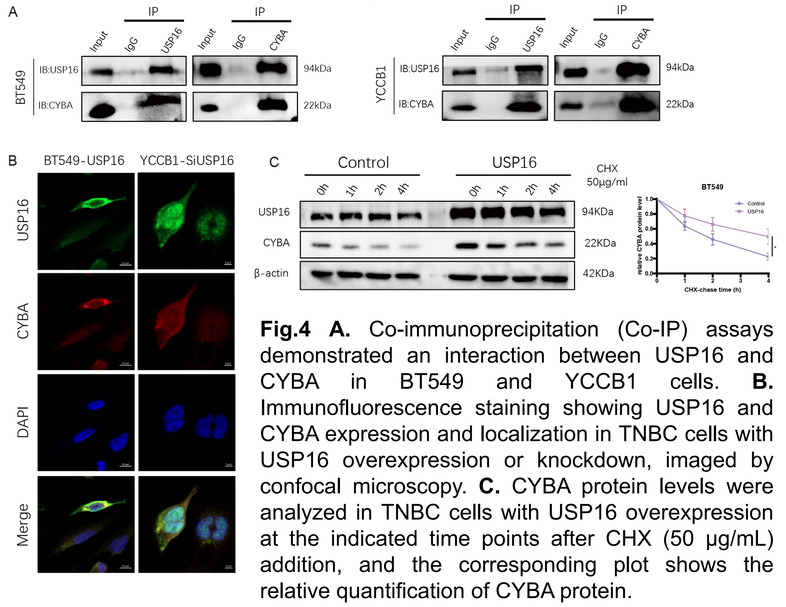


Fig.4 A. Co-immunoprecipitation (Co-IP) assays demonstrated an interaction between USP16 and CYBA in BT549 and YCCB1 cells. **B.** Immunofluorescence staining showing USP16 and CYBA expression and localization in TNBC cells with USP16 overexpression or knockdown, imaged by confocal microscopy. **C.** CYBA protein levels were analyzed in TNBC cells with USP16 overexpression at the indicated time points after CHX (50 μg/mL) addition, and the corresponding plot shows the relative quantification of CYBA protein.

CONCLUSION

- The high expression of CYBA in TNBC correlates with tumor progression, poor prognosis, and resistance to chemotherapy.
- USP16 stabilizes the expression of tumor promotor CYBA through deubiquitination.
- Targeting the USP16/CYBA axis or inhibiting the expression of CYBA may be a potential strategy for the treatment of TNBC.

Corresponding author:
Ningning Zhang, ning1abc@126.com;
Xiaohua Zeng, zxiaohuacqu@126.com.