Clinical and Translational Research
High expression of autophagy-related gene EIF4EBP1 could promote tamoxifen resistance and predict poor prognosis in breast cancer

Yang S et al. EIF4EBP1 promote TAM resistance
Abstract

BACKGROUND
Breast cancer (BC) remains a public health problem. Tamoxifen (TAM) resistance has caused great difficulties for treatment of BC patients. Eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) plays critical roles in the tumorigenesis and progression of BC. However, the expression and mechanism of EIF4EBP1 in determining the efficacy of TAM therapy in BC patients are still unclear.

AIM
To investigate the expression and functions of EIF4EBP1 in determining the efficacy of TAM therapy in BC patients.

METHODS
High-throughput sequencing data of breast tumours were downloaded from the Gene Expression Omnibus database. Differential gene expression analysis identified EIF4EBP1 to be significantly upregulated in cancer tissues. Its prognostic value was analyzed. The biological function and related pathways of EIF4EBP1 was analyzed. Subsequently, the expression of EIF4EBP1 was determined by real-time reverse transcription polymerase chain reaction and western blotting. Cell Counting Kit-8 assays, colony formation and wound healing assay were used to understand the phenotypes of function of EIF4EBP1.

RESULTS
EIF4EBP1 was up-regulated in the TAM-resistant cells, and EIF4EBP1 related to the prognosis of BC patients. Gene Set Enrichment Analysis showed that EIF4EBP1 might be involved in Hedgehog signaling pathways. Decreased in the expression of EIF4EBP1 could reversed TAM resistance, whereas overexpression of EIF4EBP1 promoted TAM resistance.
CONCLUSION
This study indicated that EIF4EBP1 was overexpressed in the BC and TAM-resistant cell line, which increased cell proliferation, invasion, migration and TAM resistance in BC cells.

**Key Words:** Breast cancer; Eukaryotic translation initiation factor 4E binding protein 1; Tamoxifen; Resistance; Prognosis; Bioinformatics


**Core Tip:** Eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) was overexpressed in the breast cancer tamoxifen (TAM)-resistant cell line, and high expression of EIF4EBP1 is associated with poor prognosis in TAM-treated patients.

**INTRODUCTION**
Breast cancer (BC) is the most frequently diagnosed cancer in women and the leading cause of female deaths from cancer in females worldwide. BC is the most prevalent cancer worldwide. An estimated 3.9 million women have been diagnosed with BC in the past 5 years, and more than 350000 annual deaths related to BC in the world\[^1,2\]. Estrogen receptor alpha (ER\(\alpha\)) is expressed in more than 70% of all BC cases, playing key roles in the gene transcription of genes related to the development of BC cells\[^3,4\]. Endocrine therapy is the major treatment strategy for both premenopausal and postmenopausal ER positive patients. It functions by blocking the ERs or inhibiting estrogen production\[^5,8\]. Tamoxifen (TAM), a selective estrogen modulator, is the most frequently prescribed antiestrogenic medication in the BC setting\[^7\]. The introduction of TAM has significantly prolonged the overall survival (OS) and disease-free survival of BC patients\[^8,9\]. However, approximately half of BC patients have intrinsic resistance to
TAM, or develop acquired drug resistance to the medication during treatment. TAM resistance remains one of the major causes of BC mortality today\[^{10}\]. Therefore, it is necessary to identify biomarkers, therapeutic targets and to understand the molecular mechanisms of TAM resistance to improve patient survival.

Autophagy is a lysosomal degradation process that plays critical roles in cell survival and maintenance through the degradation of cytoplasmic organelles, proteins and macromolecules as well as the recovery of metabolites\[^{11,12}\]. Studies have shown that defects in autophagy pathways can promote or inhibit drug resistance in many cancer types\[^{13,14}\]. Eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) encodes one member of a family of translational repressor proteins, that directly interacts with eIF4E. The Human Autophagy Database (http://www.autophagy.lu/) provides a complete list of human genes and proteins that are directly or indirectly involved in autophagy. EIF4EBP1 can be searched in this database. Rutkovsky \textit{et al}\[^{15}\] found that EIF4EBP1 was overexpressed in BC cells and that knockdown of EIF4EBP1 led to dramatic reductions in cell growth. Du \textit{et al}\[^{16}\] showed that EIF4EBP1 has significant prognostic value for BC. It has been reported that EIF4EBP1 is an independent prognostic factor for progesterone receptor (PgR) positive BC, and that high expression of EIF4EBP1 is associated with drug resistance to endocrine treatment in the ER/PgR positive patients\[^{17}\]. Moreover, Hsieh \textit{et al}\[^{18}\] illustrated that EIF4EBP1 could enhance drug resistance in prostate cancer cells. However, the expression and molecular mechanism of EIF4EBP1 in TAM resistance in BC remains unknown.

In this study, we aimed to investigate the effects of EIF4EBP1 on TAM resistance and establish it as a novel biomarker for TAM resistance. Bioinformatics analysis indicated that EIF4EBP1 was overexpressed in TAM-resistant BC cells. Moreover, high expression of EIF4EBP1 was associated with poor prognosis in TAM-resistant BC patients with TAM resistance and with a higher probability of metastasis and endocrine therapy resistance. \textit{In vitro} experiments indicated that the expression level of EIF4EBP1 was positively correlated with TAM resistance in TAM-resistant BC cells.
MATERIALS AND METHODS

Datasets of TAM-resistant BC tissues
In this study, high-throughput sequencing data of TAM-resistant BC were downloaded from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database. The GSE21648 contains 1 TAM-sensitive sample and 6 TAM-resistant samples, and GSE26459 contains 3 TAM-sensitive samples and 3 TAM-resistant samples. Then, the data in these datasets were normalized and summarized by the R software packages “limma” and “affy”. The R software package “limma” was used to identify differentially expressed genes (DEGs) in these datasets. The Benjamini-Hochberg method was used to adjust the fold change (FC) and P values. For DEGs, the cut-off criteria were $|\log FC| > 1$ and $P$ value < 0.05.

Bioinformatics analysis of EIF4EBP1
Gene Expression Profiling Interactive Analysis (GEPIA, http://geopia.cancer-pku.cn/) is a web-based tool that can be used to conduct patient survival analysis based on the The Cancer Genome Atlas (TCGA) database[20]. KM plotter (https://kmplot.com/analysis/) is a web-based tool that can be used to conduct patient survival analysis based on the GEO, EGA, and TCGA databases[21]. In this study, survival was compared between the high and low EIF4EBP1 expression groups based on GEPIA and Kaplan-Meier-plotter. The cut-off criterion was $P < 0.05$. GSEA is a powerful method to explore biological insights and potential pathways related to a gene list by determining the genes in a set that of the list with those in various previously identified gene sets[19]. In this study, GSEA was used to explore the potential functions and molecular mechanisms of EIF4EBP1 in TAM-resistant BC cells. The 6 TAM-resistant BC samples in GSE21648 were classified into two groups according to the median expression level of EIF4EBP1. Then, GSEA was conducted by the R package “clusterProfiler”. The reference gene set used for GSEA was h.all.v6.2.syntmbols.gmt obtained from the Molecular Signatures Database.
**BC samples and cell culture**

There were 71 BC tissue specimens acquired from patients at the Fourth Hospital of Hebei Medical University (Shijiazhuang, China). The diagnosis of BC was made by 2 pathologists, and none of the patients had received any treatment (radiotherapy or endocrine therapy). This study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University. All individuals were informed of the purpose of the study and had given written informed consent.

The BC cell line T47D (ER positive) was purchased from the American Type Culture Collection (Manassas, Virginia, United States). 4-hydroxytamoxifen was purchased from Sigma-Aldrich (Shanghai, China). The TAM-resistant T47D-R cell line was obtained by continuous exposure to 4-hydroxytamoxifen (6 μM). Cells were maintained in a humidified incubator with 5% CO₂ at 37 °C.

**Quantitative reverse transcription-polymerase chain reaction assay**

The expression of EIF4EBP1 in BC samples and cell culture was measured by real-time reverse transcription polymerase chain reaction (RT-qPCR). RNAsin Plus (TaKaRa, Otsu, Japan) was used to extract total RNA from BC cells. The PrimeScript™ RT Reagent Kit (TaKaRa) was used to generate complementary DNA (cDNA). RT-qPCR was performed using the TB Green Premix Ex Taq™ II kit on a MasterCycler5333 instrument (Eppendorf, Hamburg, Germany) according to the manufacturer's instructions. The 2^ΔΔCt method was used to calculate the relative expression of genes. GAPDH was used as an internal control.

**Immunohistochemistry assay**

In this study, the expression of EIF4EBP1 in BC tissues from our hospital was determined by immunohistochemistry assay. Immunohistochemical analysis of BC samples were performed according to standard protocols. In brief, paraffin sections were dewaxed using xylene and rehydrated in a graded ethanol series. Hydrogen peroxide (0.3%) was used to block endogenous peroxidase activity. The Ventana
Discovery XT automated stainer was used for immunohistochemistry, and ImageJ software was used for visualization.

**Cell transfection**

Short interference RNAs (siRNAs) for EIF4EBP1 and corresponding scrambled siRNA negative controls were synthesized by GenePharma (Shanghai, China). The EIF4EBP1 plasmid and negative control vector were purchased from GenePharma (Shanghai, China). Lipofectamine 3000 transfection reagent (Thermo Fisher Scientific) was used to transiently transfect cells according to the manufacturer’s instructions. The transfection efficiency was evaluated by RT-qPCR after 24 h. Then, the slides with tissue samples were heated in sodium citrate buffer (95 °C, 10 min) and sealed in normal goat serum (37 °C, 1 h). The tissue samples were incubated with an anti-EIF4EBP1 antibody (Abcam, Shanghai, China) for 1 h at 37 °C. Then, the tissue samples were incubated with a secondary antibody conjugated with horseradish peroxidase (Abcam). The experimental results were independently assessed by three blinded pathologists and analysed using ImageProPlus software (version 6; MediaCybernetics, Rockville, MD, United States).

**Cell Counting Kit-8 assay**

A Cell Counting Kit-8 (CCK-8) assay was performed according to the assay kit manufacturer's instructions to evaluate the effect of EIF4EBP1 knockdown or overexpression of EIF4EBP1 on the sensitivity of T47D-R cells to TAM treatment according to manufacturer instructions. T47D-R cells were seeded into 96-well plates (5000 cells/well) and incubated with TAM at different concentrations for 48 h. Subsequently, 10 µL of CCK-8 solution (Abcam) was added to each well. The OD450 values were measured with a microplate reader. Experiments were performed in triplicate.

**Colony formation assay**
To evaluate the effect of EIF4EBP1 knockdown or overexpression of EIF4EBP1 on the proliferation of the T47D-R cells after TAM treatment, a colony formation assay was performed. Cells in the logarithmic growth phase were seeded in 6-well plates at a density of 400 cells/well. T47D-R cells were cultured in a medium supplemented with 5 μM TAM. After 2 wk, cell colonies were fixed with 4% paraformaldehyde and visualized by staining with 0.1% crystal violet. Then cell colonies were counted and photographed. Experiments were performed in triplicate.

**Transwell assay**

The invasion and migration abilities of T47D-R cells with knockdown or overexpression of EIF4EBP1 were analysed via a transwell wound healing assay. T47D-R cells were seeded into six-well plates with at $1 \times 10^3$ cells/well for 24 h. Then T47D-R cells (200 mL/well) were seeded in a transwell chamber with 10% TBS and culture medium. The cells were cultured at 37 °C with 5% CO$_2$ for 24 h. Then the liquid in the transwell chamber was removed. Cells in the lower chamber were fixed with 100% methanol, stained with 0.1% crystal violet and observed under a microscope. Experiments were performed in triplicate.

**Wound healing assay**

The invasion and migration abilities of T47D-R cells with EIF4EBP1 knockdown or overexpression of EIF4EBP1 were analysed with wound healing assay. T47D-R cells were seeded in 6-well culture plates at $1 \times 10^3$ cells/well. After overnight incubation with 5 μM TAM, a wound was created with a sterile pipette tip. Then, the cells were imaged at 0 and 48 h after the wound was created. Experiments were performed in triplicate.

**Statistical analysis**

In this study, all statistical analyses were performed by R software (version 3.5.3), SPSS 22.0 (SPSS Inc., Chicago, United States) and GraphPad Prism 7.0. The R software
package "limma" was used to identify DEGs. The OS of patients was analysed by the R software package "survival ROC". The experimental data are presented as the mean ± SD values. The differences between the low- and high-EIF4EBP1 expression groups were compared by Pearson's chi-square test. The Kaplan-Meier method was employed to analyse the OS of BC patients, and the log-rank test was used to estimate the differences between groups. \( P \) values less than 0.05 were considered to be statistically significant.

RESULTS

Differential expression and bioinformatics analysis of EIF4EBP1

In this study, high-throughput sequencing data of TAM-resistant BC cells were downloaded and re-analysed. As shown in Figures 1A and 1B, EIF4EBP1 was upregulated in TAM-resistant BC cells in GSE21618 and GSE26459. Survival analysis based on the GEPIA database suggested that high expression of EIF4EBP1 was significantly associated with worse OS (\( P = 0.023 \), Figure 1C) and disease-free survival (\( P = 0.01 \), Figure 1D). Moreover, survival analysis based on the Kaplan-Meier-plotter database showed that high expression of EIF4EBP1 was significantly associated with worse OS (\( P = 6.5e-08 \), Figure 1E), disease-free survival (\( P < 1E-16 \), Figure 1F) and post progression survival (\( P = 6.5e-08 \), Figure 1G). These results suggested that EIF4EBP1 could be a prognostic marker for BC patients.

The potential functions and molecular mechanisms of EIF4EBP1 in TAM-resistant BC cells were explored by GSEA. The TAM-resistant BC samples in GSE21618 were divided into two groups according to the median expression level of EIF4EBP1. Then, the potential functions and molecular mechanisms were explored by GSEA. Gene Ontology analysis based on the GSEA results suggested that cyclic nucleotide biosynthetic processes, negative regulation of cell substrate adhesion, and nucleobase biosynthetic processes were upregulated, whereas antigen processing and presentation of endogenous peptide antigen, autophagosome maturation, and intrinsic component of endoplasmic reticulum membrane were downregulated (Figure 1H). Kyoto
Encyclopedia of Genes and Genomes analysis indicated that aminocyl tRNA biosynthesis, the Hedgehog (Hh) signaling pathway, and the peroxisome proliferator-activated receptor (PPAR) signaling pathway were upregulated, while the cell adhesion molecules cam, cytosolic DNA sensing pathways and ERBB signaling pathways were downregulated (Figure 1H).

*The expression of EIF4EBP1 in TAM-resistant cells and clinical BC samples*

In this study, RT-qPCR and immunohistochemistry were used to explore the expression of EIF4EBP1. As shown in Figures 1I, 1J and 2, EIF4EBP1 was significantly upregulated in BC tissues and TAM-resistant T47D cells. Moreover, the expression of EIF4EBP1 in BC tissues obtained at our hospital was determined, and the correlations of its expression with clinicopathological data (age, lymph node metastasis, radiotherapy status, endocrine therapy status, tumour stage, histological grade, and metastasis stage) were calculated (Table 1). As shown in Table 1, the expression of EIF4EBP1 was significantly associated with lymph node metastasis ($P < 0.0001$), endocrine therapy status ($P = 0.0005$) and metastasis stage ($P < 0.0001$).

*Knockdown of EIF4EBP1 in TAM-resistant cells*

To explore the functions of EIF4EBP1 in TAM-resistant cells, the expression of EIF4EBP1 was reduced by transfecting siRNAs targeting EIF4EBP1. RT-qPCR showed that transient transfection of siRNA significantly decreased the expression of EIF4EBP1 (Figure 3A). The results of a CCK-8 assay suggested that downregulation of EIF4EBP1 significantly decreased the degree of resensitization to TAM in T47D-R cells (Figure 3B). Colony formation experiments indicated that the number of colonies consisting of T47D-R cells was significantly decreased after siRNA transfection (Figure 3C). The results of transwell and wound healing assays indicated that EIF4EBP1 knockdown could reduce the invasion and migration of T47D-R cells treated with TAM. These results indicate that knockdown of EIF4EBP1 caused T47D-R cells to be resensitized to TAM.
**Overexpression of EIF4EBP1 in TAM-resistant cells**

To further understand the functions of EIF4EBP1 in TAM-resistant cells, the expression of EIF4EBP1 was upregulated by the transient transfection of a plasmid expressing EIF4EBP1. The overexpression of EIF4EBP1 was confirmed by RT-qPCR (Figure 4A). The cell viability of T47D-R cells treated with TAM was assessed by CCK-8 and colony formation assays. The results indicate that the resistance of T47D-R cells was further enhanced after EIF4EBP1 plasmid transfection (Figures 4B and 4C). The invasion and migration of T47D-R cells treated with TAM were explored by transwell and wound healing assays. The results suggested that cell invasion and migration were increased by EIF4EBP1 expression. These results indicate that the overexpression of EIF4EBP1 could increase the resistance of T47D-R cells to TAM.

**DISCUSSION**

BC is one of the most common cancers in the world, and ER-positive is the most common subtype of BC. Endocrine therapy, which target the ER directly and/or suppress estrogen production, is the main treatment strategy for ER + BC\[^{10}\]. TAM is a nonsteroidal antioestrogen drug and has historically been the most widely used antioestrogen drug for the treatment of ER-positive BC patients\[^{20,21}\]. Although TAM has greatly reduced the recurrence and mortality of BC, the emerging and acquired resistance to TAM has been a major obstacle for the successful treatment of patients\[^{22,23}\]. Many studies have been conducted on the potential mechanism of TAM resistance, and several mechanisms have been shown to be related to TAM resistance. These mechanisms include autophagy, mutations of the ER and endoplasmic reticulum stress\[^{10,22,24,25}\].

Autophagy is a “self-degradative” process in which cellular materials are sent to lysosomes for degradation. Autophagy plays a critical role in the turnover of cell components and provides energy and macromolecules\[^{26,27}\]. Studies have shown that autophagy plays an dual, context-dependent roles in drug resistance: It can kill drug-
resistant cancer cells with inactive apoptotic pathways, but it can also participate in the development of drug resistance and protect cancer cells from endocrine therapy drugs\textsuperscript{[13,14]}. EIF4EBP1 directly interacts with a limiting component of the multisubunit complex that recruits 40S ribosomal subunits to the 5′ end of mRNAs. Further studies showed that EIF4EBP1 was upregulated in many cancer types including BC, hepatocellular carcinoma, lung squamous cell carcinoma, and glioblastoma\textsuperscript{[16,28-30]}, suggesting that EIF4EBP1 plays an important role in tumorigenesis. Ito et al\textsuperscript{[31]} showed that EIF4EBP1 was overexpressed and phosphorylated in renal cell carcinoma (RCC) and was involved in the clinical chemoresistance of RCC cells to mechanistic target of rapamycin complex 1 inhibitors. Tsai et al\textsuperscript{[32]} found that EIF4EBP1 could induce glioma stem-like cells through the epidermal growth factor receptor/protein kinase B cascade, making a major contribution to drug resistance. However, the expression and molecular mechanisms of EIF4EBP1 in TAM resistance in BC remained unrevealed. In this study, we investigated the role of EIF4EBP1 in TAM resistance. Scholars have performed studies on the role of EIF4EBP1 in TAM resistance, and their conclusions of these studies were similar with no controversy. Du et al\textsuperscript{[16]} pointed out in a 2020 study that EIF4EBP1 showed significant prognostic value as a prognostic indicator in BC, specifically indicating poor prognosis\textsuperscript{[33]}. A 2019 study reported that EIF4EBP1 is located within the 8p11-p12 genomic locus, frequently highly amplified in BC and predicts poor prognosis and resistance to endocrine therapy\textsuperscript{[34]}. Another study, from 2022, indicated that the addition of EIF4EBP1 to cultures significantly reduced the proliferation and metastasis of TNBC cells\textsuperscript{[35]}. These studies demonstrate that EIF4EBP1 plays an oncogenic role in BC, and offer the latest and most important findings in the field. However, most of these conclusions are based on bioinformatics analysis. The role of EIF4EBP1 in TAM resistance has not been experimentally demonstrated. This study indicated that EIF4EBP1 enhanced increase the resistance of T47D-R cells to TAM. EIF4EBP1 is an autophagy-related gene, some studies have demonstrated a role for EIF4EBP1 in autophagy. For example, it has been reported that in CACO-2 cells exposed to cetuximab, EIF4EBP1 expression and autophagosome formation increased,
and autophagy increased the efficacy of cetuximab in colorectal cancer\textsuperscript{[36]}. Moreover, Lai et al.\textsuperscript{[35]} indicated that Lamictal ketoneses and YXM110 are new synthetic drugs that exhibit excellent anti-tumour activity in many cancer cells by mediating EIF4EBP1 depletion and regulating autophagy\textsuperscript{[37]}. In this study, we suggest that EIF4EBP1 may increase the resistance of T47D-R cells to TAM by regulating autophagy.

Given the critical role played by EIF4EBP1 in the development of drug resistance and BC, we explored whether EIF4EBP1 is involved in TAM resistance. In this study, the gene expression profiles of TAM-resistant or TAM-sensitive BC cells were reanalyzed. EIF4EBP1 was overexpressed in TAM-resistant cells, and high expression of EIF4EBP1 is associated with poor prognosis in BC patients. Based on the clinical specimens from our hospital, we found that high expression of EIF4EBP1 was associated with metastasis and endocrine therapy in BC patients. Moreover, cell experiments suggested that EIF4EBP1 deficiency could reverse TAM resistance, whereas overexpression of EIF4EBP1 increased TAM resistance.

In this study, the potential functions, and molecular mechanisms of EIF4EBP1 in TAM-resistant BC cells were identified by GESA. Notably, the Hh signaling pathway was significantly enriched in the high EIF4EBP1 expression group. It has been reported that Hh signaling pathway is involved in developmental processes in vertebrates, and that abnormal activation of this pathway plays an important role in tumorigenesis and maintenance of multiple cancers\textsuperscript{[38]}. Ren et al.\textsuperscript{[37]} indicates that tumor suppressor candidate 3 may improve the expression of CD133 and ABCC1 by activating the Hh signaling pathway, and inhibitor of Hh signaling pathway could reduce drug resistance of colorectal cancer cells\textsuperscript{[39]}. Zeng et al.\textsuperscript{[36]} demonstrated that the inhibition of Hh signaling pathway could induce autophagy in chronic myeloid leukaemia cells and inhibiting autophagy and Hh signaling pathway could reduce cell viability and induce apoptosis of imatinib-resistant chronic myeloid leukemia cells. Thus, we hypothesized that EIF4EBP1 could induce TAM resistance through Hh signaling pathway and autophagy. However, the results need to be examined by further investigations. We also found that components of the PPAR signaling pathway were significantly enriched.
in the EIF4EBPI high-expression group. The PPAR signaling pathway has been linked to glucose and lipid metabolic disorders, endothelial function, and inflammation. It has been reported that PPAR-γ is upregulated in glioma cells, which could regulate genes associated with apoptosis-related and multidrug resistance related gene and increase intracellular accumulation of drugs[40]. Moreover, Karen Bräutigam et al[39] showed that the death ligand TRAIL (TNF superfamily member 10) could sensitize tumor cells to cytostatic drugs without affecting normal tissues. The combinatorial treatment with PPARγ ligands and TRAIL has been shown to synergistically induce apoptosis in ovarian cancer cell lines[41]. Thus, we hypothesize that the PPARγ agonists may be a promising drugs for targeting drug-resistant cells. Moreover, proteins in the ERBB signaling pathway were significantly enriched in the EIF4EBPI low-expression group. Studies have shown that the abnormal activation of ERBB family members is involved in tumorigenesis and in the escape from anti-tumour immunity in many types of cancers[42]. Song et al[41] indicated that the host genes of the identified circular RNAs in platinum-based drug-resistant NSCLC cells were involved in ErbB signaling pathway[43]. Moreover, Kenneth Macleod et al[42] indicated that ErbB receptor signaling was altered in cisplatin-resistant ovarian cancer cells, suggesting that downregulation of the ErbB signaling pathway could play an important role in the development of drug resistance[44]. Thus, we hypothesized that EIF4EBPI could induce TAM resistance by regulating the ErbB signaling pathway.

This study also has some limitations. First, as with many previous studies before[45,46], only T47D cells were used to establish TAM-resistant cell lines. This may make our conclusions less generalizable. Moreover, gene set enrichment analysis was used to explore the potential functions and molecular mechanisms of EIF4EBPI in TAM-resistant BC cells. However, these pathways have not been verified by in-vitro and in-vivo experiments.

CONCLUSION
In conclusion, our study showed that the overexpression of EIF4EBP1 was significantly associated with poor prognosis and metastasis in BC patients. Moreover, EIF4EBP1 plays important roles in the development of TAM resistance. EIF4EBP1 knockdown could reversed TAM resistance, whereas overexpression of EIF4EBP1 increased TAM resistance in BC cells. In addition, our GSEA results may provide new insights into the molecular mechanism of TAM resistance. In brief, EIF4EBP1 could be a marker for the early diagnosis and a therapeutic target for the therapy of TAM resistance.

ARTICLE HIGHLIGHTS

Research background
Tamoxifen (TAM) resistance is a major obstacle in the treatment of breast cancer (BC) patients. It has been reported that eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) plays critical roles in the tumorigenesis and development of BC.

Research motivation
TAM resistance remains one of the major causes of BC mortality today. Therefore, it is necessary to identify biomarkers, therapeutic targets and understand molecular mechanisms of TAM resistance to help patients.

Research objectives
The objectives was to investigate the expression and functions of EIF4EBP1 in determining the efficacy of TAM therapy in BC patients.

Research methods
Gene Set Enrichment Analysis (GSEA) was performed to explore the biological function and related pathways of EIF4EBP1. Real-time reverse transcription polymerase chain reaction were employed to explore the expression of EIF4EBP1 in TAM-resistant and the TAM-sensitive BC cell lines. Cell count kit-8 assay, colony formation experiments
and wound healing assay were used to understand the phenotypes of loss- and gain-of-function of EIF4EBP1 in a TAM-resistant cell line.

**Research results**

EIF4EBP1 was upregulated in TAM resistant cells, and EIF4EBP1 was associated with the prognosis of BC patients. GSEA suggested that EIF4EBP1 may be involved in the Hedgehog signaling pathway. Reducing the expression of EIF4EBP1 can reverse TAM resistance, while overexpression of EIF4EBP2 can promote TAM resistance.

**Research conclusions**

In this study, we investigated the role of EIF4EBP1 in TAM resistance. Scholars have performed studies on the role of EIF4EBP1 in TAM resistance, and their conclusions of these studies were similar with no controversy. However, most of these conclusions are based on bioinformatics analysis. The role of EIF4EBP1 in TAM resistance has not been experimentally demonstrated. This study indicated that EIF4EBP1 enhanced increase the resistance of T47D-R cells to TAM. In addition, our GSEA results may provide new insights into the molecular mechanism of TAM resistance. In brief, EIF4EBP1 could be a marker for the early diagnosis and a therapeutic target for the therapy of TAM resistance.

**Research perspectives**

To explore the potential function and molecular mechanism of EIF4EBP1 in TAM resistant BC cells through *in vitro* and *in vivo* experiments.