Tripartite Motif-Containing 3 (TRIM3) Enhances ER Signaling and Confers Tamoxifen Resistance in Breast Cancer

Run-yi Ye  
Sun Yat-sen University First Affiliated Hospital

Xia-ying Kuang  
Sun Yat-sen University First Affiliated Hospital

Hui-juan Zeng  
Sun Yat-sen University First Affiliated Hospital

Nan Shao  
Sun Yat-sen University First Affiliated Hospital

ying lin  
Sun Yat-sen University First Affiliated Hospital

pian liu  
Wuhan Union Hospital

shenming wang  (wshenm@163.com)  
Sun Yat-sen University First Affiliated Hospital

Research

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Abstract

**Background:** Tamoxifen resistance remains a tricky clinical problem in estrogen receptor (ER)-positive breast cancer. SUMOylation of ERα enhances ERα-induced transcription activity. TRIM proteins are a new class of SUMO E3 ligases, regulating SUMOylation of proteins. However, the precise molecular mechanism and function of TRIM3 in SUMOylation and response to tamoxifen remains unclear.

**Methods:** The expression of TRIM3 was assessed in 48 cases with breast cancer which received tamoxifen therapy. The protein and RNA levels of TRIM3 in 12 ER+ breast cancer cell lines were assessed by Western blotting and qRT-PCR assays. Effects of TRIM3 in regulating tamoxifen resistance were evaluated both in vitro and in vivo via Cell viability, Colony formation, Anchorage-independent growth ability assays and Tumor xenografts. Molecular mechanism of TRIM3 in regulating SUMOylation of ERα was analyzed by Western blotting, Immunoprecipitation, Far Western blotting and Luciferase activity assays.

**Results:** We reported that TRIM3 was dramatically overexpressed in breast cancer, which correlated with tamoxifen resistance. Furthermore, TRIM3 overexpression significantly correlated with poor survival of ER+ breast cancer treated with tamoxifen. TRIM3 overexpression conferred cell survival and tumorigenesis, whereas knocking down TRIM3 reduced these capabilities. Moreover, TRIM3, as a UBC9 binding protein, promoted SUMO modification of ESR1 and activated ER pathway. Silencing UBC9 abolished the function of TRIM3 in regulating tamoxifen resistance.

**Conclusions:** These results suggest a novel biomarker for breast cancer therapy, indicating that inhibiting TRIM3 combined with tamoxifen may provide a potential treatment for breast cancer.

**Background**

With almost 25% cancer cases among women, Breast cancer remains a global challenge among females in worldwide. Estrogen receptor alpha (ERα) is the main molecular target for endocrine therapies, which antagonize ER and suppress estrogen synthesis to inhibit tumor growth. Tamoxifen as one of adjuvant endocrine therapy, is widely used as ER antagonists in breast cancer, which blocked the binding of estrogen and ER and suppressed ER-α target genes. Unfortunately, approximately 30%-40% patients of ER+ breast cancer fail to response (de novo resistance) or become resistance (acquired resistance), and with a deadly outcome, which presents a huge clinical challenge for breast cancer. Therefore, to unveil the underlying molecular mechanism of endocrine resistance and potential therapies for breast cancer are of great importance.

Emerging evidences have proved that several mechanisms contribute to tamoxifen resistance, such as activation of RTKs signal transduction pathways, activation of oncogenic signaling pathways (PI3K/Akt/mTOR, NF-κB) and modulation of ER signaling. ER is widely expressed in breast cancer (almost 70%), which is a strong predictor for tamoxifen therapy. Notably, loss of ESR1 expression...
confers tamoxifen resistance, which is reported in ~15%-20% of breast cancer\(^9,10\). However, the expression of ER in most of tamoxifen-resistance cases, remains to be expressed and active\(^9\), suggesting that there might other mechanism regulate ESR1 to confer tamoxifen resistance. Emerging evidence shows that \textit{ESR1} expression is regulated by diverse aspect, including histone modification\(^11\), DNA methylation\(^12\), somatic mutation\(^9\), \textit{ESR1} fusion genes\(^13\) and post-translational modifications (PTMs)\(^14\). SUMOylation, as important PTM, effects subcellular localization, protein-protein interaction, protein stability and transcriptional activity, which regulated by three important enzymes, including activating enzyme (E1), conjugating enzyme (E2) and ligases (E3). Stephanie Sentis and his colleagues proved that small ubiquitin-like modifier (SUMO)-1 modifies ER\(\alpha\) SUMOylation, which enhances ER\(\alpha\)-induced transcription activity via improving ER\(\alpha\)' DNA binding property\(^15\). However, the mechanism and biology function of ESR1 SUMOylation in breast cancer remains unclear.

Tripartite motif-containing (TRIM) proteins belong to RING type E3 ubiquitin ligase, which involved cellular signaling, cell progression and tumorigenesis\(^16\). Furthermore, it has reported that TRIM proteins are a new class of SUMO E3 ligases, transferring small ubiquitin-related modifier (SUMO) to substrates\(^16,17\). TRIM27, TRIM32 and TRIM36 are well-known SUMO E3 ligases, which modify SUMOylation via binding to ubiquitin-conjugating enzyme 9 (UBC9)\(^16,17\). TRIM3, as one of TRIM proteins, is located at chromosome 11p15, which is thought to harbor tumor suppressor genes\(^18–20\). However, the precise molecular mechanism and function of TRIM3 in SUMOylation and response to tamoxifen remains unclear.

Herein, we found that TRIM3 was significantly upregulated in tamoxifen-resistant breast cancer, and associated with poor survival in breast cancer during tamoxifen therapy. Overexpressed of TRIM3 conferred estrogen-independent growth and contributed tamoxifen resistance. Experiments revealed that TRIM3 upregulated ER SUMO modification and activated ER signaling pathway via binding to UBC9, which could be abolished by deSUMOylation enzyme SENP1. Taken together, our results unveil the crucial role of TRIM3 in SUMOylation of ESR1 and modulation of tamoxifen response, which identify a potential target to improve clinical outcomes of breast cancer.

**Methods**

**Cell culture**

The human breast cancer cell lines BT-474, BT-483, CAMA-1, HCC1428, HCC1500, MCF7, MDA-MB-134-VI, MDA-MB-175-VII, MDA-MB-361, T-47D, ZR-75-1 and ZR-75-30 were cultured according to the manufacturer’s instruction. Short tandem repeat (STR) profiling were used and authenticated in all cell lines. Mycoplasma eradication was evaluated by PCR.

**Chemical reagents**
β-estradiol (E2) and 4-hydroxytamoxifen (TAM) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Tissue specimens and immunohistochemistry**

A cohort of paraffin-embedded breast cancer (48 cases) which received tamoxifen therapy was used to detect the expression of TRIM3 via Anti-TRIM3 (ab111840). The specimens were obtained from the First Affiliated Hospital of Sun Yat-sen University between 2010 and 2015. 10 freshly breast cancer tissues with tamoxifen sensitive and tamoxifen resistant were collected. Prior patient consent and approval were obtained.

**Constructs and transfection**

Genomic DNA of TRIM3 was PCR-amplified and cloned into a pBABE-puro retroviral vector. The pSUPER-puro shRNA of TRIM3 and UBC9 were purchased from Transheep Bio. All clone primer and siRNA obigonucleotides are listed as Supplemental Table 1. Estrogen Response Element (ERE) was cloned into a pGL3 basic vector (Progema). The Renilla luciferase TK was used as transfection control. All cells overexpressing TRIM3 or silencing TRIM3 were selected with 0.5 µg/ml puromycine.

**Immunoprecipitation analysis**

Immunoprecipitation assay was performed according to described previously. Lysates were incubated with Flag or His affinity beads (Sigma-Aldrich). The agarose beads were washed with wash buffer. Then the elutions were detected using appropriate antibodies.

**Far Western blotting**

Far immunoblotting were performed by using the proteins immunoprecipitated by anti-His antibody. The proteins were detected using western blotting. Empty vector or Flag-ESR1 cell lysate was added to the PVDF membrane and incubated overnight. Then the membrane was subjected to immunoblotting analysis by indicated antibody.

**Western blotting analysis**

Western blotting was performed using antibodies against TRIM3 (ab111840), ESR1 (ab108398), UBC9 (ab75854), SENP1 (ab108981) and SAE1 (ab185552). The membranes were re-probed with an anti-GAPDH (BOSTER, BM3876) as the loading control.

**Quantitative real-time reverse transcription PCR (qPCR)**

Total RNA was isolated with TRIzol reagent (Invitrogen) according to manufacturer's instructions. RNA was reverse-transcribed into cDNA and carried out via Real-time PCR with SYBR Green Master (Roche). The data were assessed base on the threshold cycle (Ct), and calculated as $2^{-[(C_t \text{ of gene}) - (C_t \text{ of GAPDH})]}$, which was normalized to GAPDH expression. All primers are listed as Supplemental Table 1.

**Cell viability assay**
Indicated cells were treated with Estrogen and/or tamoxifen for 48 h. The cells were added MTT dye (Sigma-Aldrich), and then the cells were resuspended with dimethyl sulfoxide (Sigma-Aldrich) and measured with automatic microplate reader.

**Colony formation assay**

Indicated cells were incubated at a level of 5% CO\textsubscript{2} at a temperature of 37 °C for 2 weeks. Then the cells were fixed, stained with crystal violet stain, and counted.

**Luciferase activity assay**

Luciferase reporter plasmid and pRL-TK Renilla plasmid were transfected into indicated cells. After 48 h, the cells were lysis and measured using a Dual Luciferase Reporter Assay (Promega) according to the manufacturer's instructions.

**Anchorage-independent growth ability assay**

Indicated cells were suspended with medium plus 0.33% agar, and then plated on top of 0.66% agar medium mix. After 10 days, colonies > 0.1 mm in diameter were counted. The expriments was performed in triplicates.

**Tumor xenografts**

Under the guideline of National Institutes of Health Guide for Care and Use of Laboratory Animals, xenografts were performed using athymic nude female mice (4–5 weeks of age, 18–20 g). 5 × 10\textsuperscript{6} of indicated cells were injected into the left and right dorcal flank of mice implantd with E2 pellets (0.72 mg/pellet; 60-day release). After one week, a subcutaneous injection with or without tamoxifen pellet (5 mg/pellet; 60-day release). Tumor were examined twice a week, and tumor volum was calculated as (L × W\textsuperscript{2})/2.

**Data processing and visualization**

The datasets is available in The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/tcga/). Gene set enrichment analysis (GSEA) was performed on GSEA 2.0. 9 (http://www.broadinstitute.org/gsea/).

The relationship between the expression of TRIM3 and ESR1 was determined by correlation coefficient. For the relationship between TRIM3 and the OS, RFS and DMFS of breast cancers, Kaplan-Meier Plotter ((http://kmplot.com/analysis) was used.

**Statistics**

Unpaired Student's test was used to evaluate the statistical significance of the differences between two groups. Spearman's correlation analysis and Chi-square were used to evaluate the correlations. All \( P \) values were two-siled, and a value of \( P < 0.05 \) was considered statistically significant. All data analyze were performed with SPSS19.0 software and presented with GraphPad Prism 8.0.

**Results**
TRIM3 contributes tamoxifen-resistant in breast cancer

To determine the molecular mechanism which contributes to breast cancer tamoxifen resistance, we analyze the genes expression in breast cancer patients from TCGA database. As shown in Fig. 1a, the expression of TRIM3 is significantly higher in the patients without response to tamoxifen therapy [stable disease (SD), progressive disease (PD)] than those response to tamoxifen treatment [complete remission (CR), partial remission (PR)]. The mRNA of TRIM3 was higher expressed in ER+ breast cancer than ER− breast cancer (Supplemental Fig. 1a, p = 1.365E-68). Furthermore, 48 cases clinical breast cancer specimens with ER+ after tamoxifen treatment were used to examine the expression of TRIM3(Supplemental table 2). As expect, TRIM3 expression was significantly higher in tamoxifen-resistant ER+ breast cancer than that in tamoxifen-sensitive ER+ breast cancer (Fig. 1b). Statistical analysis revealed that the expression of TRIM3 was closely correlated with recurrence of patients with tamoxifen treatment (Fig. 1c). By analyzing published expression profiles obtained from breast cancer (TCGA) data, we found that levels of TRIM3 mRNA is positively correlated with levels of TRIM3 protein, and TRIM3 mRNA expression was upregulated in ER+ breast cancer tissues compared to ER− cancer tissues and normal breast tissue (Supplemental Fig. 1b and 2a). Consistently, Kaplan-Meier plotter showed that the higher expression of TRIM3 was positively correlated with poorer overall survival (OS), relapse-free survival (RFS) and distant metastasis-free survival (DMSF) in cases of ER+ breast cancer with tamoxifen treatment and/or chemotherapy, but not in ER− breast cancer (Fig. 1c and Supplemental Fig. 1c). Herein, these results reveal that TRIM3 might correlate with the tamoxifen resistance of ER+ breast cancer.

TRIM3 promoted tamoxifen resistance in ER+ breast cancer in vitro

To further determine the role of TRIM3 in regulating tamoxifen resistance, we detected the expression levels of TRIM3 in 12 ER+ breast cancer cell lines. As shown in Fig. 2a, TRIM3 was high expressed in MDA-MB-134-VI, MDA-MB-175-VII and MDA-MB-361, and low expressed in BT-483, CAMA-1 and MCF7. Consistent with the clinical evidence, higher expression of TRIM3 in MDA-MB-134-VI, MDA-MB-175-VII and MDA-MB-361 resistance to tamoxifen than lower expression of TRIM3 in BT-483, CAMA-1 and MCF7 (Fig. 2b-2c), suggesting that TRIM3 might contribute to tamoxifen resistance in breast cancer. Furthermore, we established BT-483 and MCF7 stably overexpressed TRIM3, MDA-MB-134-VI and MDA-MB-361 stably silencing TRIM3 (Fig. 3a). As expected, overexpressed TRIM3 induced tamoxifen resistance and formed more colony formation in BT-483 and MCF7, whereas silencing TRIM3 expression promoted tamoxifen sensitivity and formed less colony formation in MDA-MB-134-VI and MDA-MB-361 (Fig. 3b-3c). Therefore, these results revealed that TRIM3 induced tamoxifen resistance in ER+ breast cancer in vitro.

TRIM3 conferred tamoxifen resistance in ER+ breast cancer in vivo
To further investigate the function of TRIM3 in regulating tamoxifen resistance *in vivo*, we first examined the anchor-independent growth ability in manipulation of TRIM3 expression cells compared with control cells. As shown in Fig. 4a, TRIM3 overexpression promoted cell growth and formed more and larger colonies compared with vector cells, whereas silencing TRIM3 expression inhibited cell growth and formed less and smaller colonies compared with vector cells. Furthermore, we subcutaneously inoculated TRIM3-deregulated cells into NOD/SCID mice. Consistently, TRIM3 overexpressed cells formed larger tumors compared with control vector cells (Fig. 4a). Taken together, these results revealed that TRIM3 response to tamoxifen resistance and promotes tumorigenicity of breast cancer *in vivo*.

**Trim3 Promoted Sumoylation Of Esr1 Via Binding To Ubc9**

To further investigate the mechanism by which TRIM3 response to tamoxifen resistance, gene set enrichment analysis was analyzed in published ER+ breast cancer expression profiles from TCGA data. We found that TRIM3 levels were positively correlated with estrogen receptor 1 (ESR1) expression, suggesting that TRIM3 might be involved in regulating ESR signaling pathway (Fig. 5a). As expect, we found that overexpressed of TRIM3 increased estrogen response element (ERE) luciferase reporter activity and promoted ER-regulated genes upon tamoxifen treatment, whereas silencing of TRIM3 expression decreased (Fig. 5b-5c). However, analysis of breast cancer in TCGA showed that levels of TRIM3 mRNA and protein were not correlated with levels of ESR1 mRNA and protein (Supplemental Fig. 2a and 3a). Furthermore, we found that E2 treatment did not induce ESR1 and TRIM3 expression in breast cancer (Supplemental Fig. 2b). Overexpressed of ESR1 did not affect the expression of TRIM3 (Supplemental Fig. 2c). Collectively, TRIM3 induced ESR1 transcription activity, but not induced ESR1 expression.

The E3 ubiquitin-protein ligase TRIM proteins are identified as a new class of SUMO ligases (E3s), which regulate SUMOylation specificity. It has been reported that transcription activity of ERα is upregulated by SUMOylation via binding to UBC9, the unique SUMO E2-conjugating enzyme, and repressed by tamoxifen treatment. Here, we found that TRIM3 overexpression increased SUMO modification of ESR1, whereas silencing TRIM3 decreased (Fig. 6a). Overexpressed SENP1, a deSUMOylase enzymes, decreases SUMOylation of ESR1 and ERE luciferase reporter activity in TRIM3-transduced cells compared with control cells upon tamoxifen treatment (Fig. 6b-6c). Furthermore, immunoprecipitation and western blot assay showed that TRIM3 formed complex with ESR1 and UBC9, suggesting that TRIM3 might regulate SUMOylation of ESR1 via binding to ESR1, UBC9 and SAE1 (Fig. 6d). Silencing UBC9 in TRIM3-transduced cells reduced the SUMOylation of ESR1 and ERE luciferase reporter activity (Supplemental Fig. 3b-3c). To further investigate the binding of TRIM3 and ESR1, three truncated ESR1 fragments were constructed (Fig. 6e). Next, immunoprecipitation and far-western blot assay showed that TRIM3 interacted with DNA binding fragment of ESR1 (ER2 fragment) and directly interacted with Flag-tagged ESR1 cell lysate (Fig. 6f-6 g). Taken together, these data reveal that TRIM3 promoted SUMOylation and activated transcription activity of ESR1 via interacting with ESR1 and UBC9.
Ubc9 Is Required For Tamoxifen Resistance Effect Of Trim3

To further investigate whether SUMOylation of ESR1 is required for promoting tamoxifen resistance effect of TRIM3, the effect of silencing of UBC9 and overexpressed SENP1 in TRIM3-induced tamoxifen resistance were examine. As shown in Fig. 7a-7b, silencing UBC9 and overexpressed SENP1 in TRIM3-transduced cells formed less colony formation, which promoted tamoxifen sensitivity. Furthermore, silencing UBC9 in TRIM3-transduced cells reversed the ability of TRIM3-induced promoted tamoxifen sensitivity and decreased tumortigenicity in vivo (Fig. 7c). Taken together, these results suggest that UBC9 is required for the effect of TRIM3 regulating ESR1 SUMOylation and promoting tamoxifen resistance in breast cancer.

TRIM3 corrlated with ESR1 SUMOylation and tamoxifen resistance in berast cancer

To further investigate the correlation of TRIM3 and ESR1 in regulating tamoxifen resistance, 10 freshly collected clinical breast cancer samples were examined. As shown in Figure in 8a, TRIM3 expression was upregulated in tamoxifen resistant breast cancer than tamoxifen sensitive breast cancer, and strongly associated with ESR1 SUMOylation. Consistently, TRIM3 promoted the transcription levels of ER-targeted genes in tamoxifen resistant ER\(^+\) breast cancer (Fig. 8b). Collectively, these results support that TRIM3 upregulation promotes ESR1 SUMO modification and ER signaling pathway, leading to tamoxifen resistance in ER\(^+\) breast cancer.

Discussion

Tamoxifen is a widely used treatment for ER-positive breast cancer patients, but de novo resistance or acquired resistance results in failure for tamoxifen therapy, which is a clinical challenge \(^4\). In the present study, we found that TRIM3 was upregulated in tamoxifen resistant breast cancer tissues compared to tamoxifen sensitive breast cancer tissues, and was closely correlated with poorer survival in ER\(^+\) breast cancer. Overexpression of TRIM3 contributed to cell survival upon tamoxifen treatment both in vivo and in vitro. Moreover, TRIM3 promoted ESR1 SUMO modification and activated transcription of ER target genes via binding with UBC9, which could be abolished with overexpression of SENP1. Taken together, our results demonstrated that TRIM3 modify SUMOylation and confer tamoxifen resistance, which provided a novel therapeutic target for breast cancer therapy.

TRIM3, a member of RING-type E3 ubiquitin ligases, plays diverse roles in regulating neoplastic processes\(^20,25-27\). TRIM3 is identified as a novel RING finger protein expressed in the rat brain, which involved in regulating neuronal outgrowth\(^18,28\). TRIM3 is a candidate brain tumor suppressor gene, which suppresses brain tumorigenesis via attenuating Notch signaling and suppressing \(^c\)-MYC expression\(^29,30\). Furthermore, TRIM3 is downregulated in various types of cancer, including liver cancer\(^19\), esophageal squamous cell carcinoma\(^27\) and colon cancer\(^31\), and inhibited cell proliferations, invasion and
metastasis. However, the correlation of TRIM3 and tamoxifen resistance in breast cancer remains unclear. In current study, we found that TRIM3 was significantly higher expression in recurrence ER+ breast cancer than that in non-recurrence ER+ breast cancer, which consistent to the analysis of TRIM3 expression response to tamoxifen treatment in ER-positive breast cancer data obtained from TCGA (Fig. 1a-1b, Fig. 8a). Furthermore, the expression of TRIM3 correlated with poorer OS, RFS and DMSF in ER+ breast cancer upon tamoxifen treatment, but not in ER- breast cancer (Fig. 1c and Supplemental Fig. 1b), suggesting that TRIM3 might be a potential predictive marker for tamoxifen response in ER+ breast cancer.

ERα has been recognized as a favorable prognostic biomarker and a determinative role for breast cancer therapy. ERα belongs to nuclear receptor superfamily, and activates the transcription of targeted genes to conquer tamoxifen treatment. SUMOylation as a post-translational modification (PTMs), is hyperactivated in breast cancer, and correlated with ERα signaling pathways. Though SUMO modification is widely known to repress transcription via binding with co-repressors, including HDACs and DAXX, a growing number of evidence has been showed that SUMOylation involved in promoting transcription activity. For example, the transcription activity of ERα is upregulated in breast cancer via binding with SUMOylation proteins, such as CLOCK and ZFP282. Furthermore, ERα is proved to be SUMOylated via binding with UBC9, and blocking its SUMOylation impaired the transcription activity of ERα through decreased DNA binding without influencing ERα cellular localization. Therefore, the precise molecular mechanism by which SUMO modification enhances transcription activity of ERα in breast cancer needs further investigation.

In current study, we identified that TRIM3 played an important role in regulating ER signaling pathway and conferred tamoxifen resistance in breast cancer. Overexpression of TRIM3 enhanced ERE luciferase reporter activity and upregulated ER-regulated genes upon tamoxifen treatment, whereas silencing of TRIM3 expression decreased (Fig. 5b-5c). Furthermore, TRIM proteins are proved to be a new class of SUMO E3 ligases, regulating SUMO modification via transferring SUMO1 from UBC9 to substrate. Consistently, the SUMOylation and transcription activity of ESR1 was upregulated in TRIM3-transduced cells upon tamoxifen treatment, which abolished by overexpression SENP1 (Fig. 6a-6c). Furthermore, immunoprecipitation analysis demonstrated that TRIM3 interacted with ESR1, UBC9 and SAE1, suggesting that TRIM3 acted as SUMO E3 ligase via transferring SUMO from SAE1 (E1) and UBC9 (E2) to ESR1 (substrate). Importantly, TRIM3 interacted with ESR1 directly binding to its DNA-binding domain (Fig. 6e-6g), which might induce ESR1 transcription. Overexpression of UBC9 promotes tumorigenesis of breast cancer, while a dominant negative UBC9 decreases. Similarly, blocking UBC9 expression also decreased cell survival and tumorigenesis in TRIM3-transduced cells upon E2 and tamoxifen treatment in vitro and in vivo (Fig. 7). Taken together, our results confirm that TRIM3 acted as a SUMO E3 ligase in regulating ESR1 SUMO modification and transcription activity, and conferred tamoxifen resistance via the TRIM3/UBC9/ESR1 axis.

Conclusions
In conclusion, we demonstrated that TRIM3, as a novel SUMO E3 ligase, promoted SUMO modification and transcription activity of ESR1 via binding to UBC9, which contributed to tamoxifen resistance in ER$^+$ breast cancer. Silencing UBC9 in TRIM3-transduced cells conferred tamoxifen sensitive in breast cancer. Importantly, overexpression of TRIM3 was correlated with poor survival of ER$^+$ breast cancer upon tamoxifen resistance, which present a potential biomarker for treatment of ER$^+$ breast cancer.

**Abbreviations**

ER$^+$
Estrogen receptor -positive
ER$\alpha$
Estrogen receptor alpha
SUMO
small ubiquitin-like modifier
TRIM3
Tripartite motif-containing 3
UBC9
ubiquitin-conjugating enzyme 9
SD
stable disease
PD
progressive disease
CR
complete remission
PR
partial remission
OS
overall survival
RFS
relapse-free survival
DMSF
distant metastasis-free survival

**Declarations**

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Not applicable.

**Ethics approval and consent to participate:**
All animal experiments in this study were under the guideline of National Institutes of Health Guide for Care and Use of Laboratory Animals. Prior patient consent and approval from the Institutional Research Ethics Committee were obtained.

**Consent for publication:**

Not applicable.

**Availability of data and materials:**

All data generated or analyzed during this study are included in this published article and its supplementary information files.

**Competing interests:**

The authors declare that they have no competing interests.

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**Authors’ contributions:** RY and XK designed this study, analyzed data and wrote the manuscript. HZ and NS performed the vitro experiments. RY and YL performed the vivo experiments. RY and XK contributed to collection of clinical samples. PL and SW provided important comments for the manuscript. All authors read and approved the final manuscript.

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