



Research Article

# Tripartite motif-containing protein 28 promotes drug resistance to bortezomib in gastric cancer through proteasome activity regulation

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## ABSTRACT

**Objective:** Gastric cancer (GC) persists as a leading global cause of cancer-related mortality. Although bortezomib (BTZ), a proteasome inhibitor, has demonstrated efficacy in treating various cancers, its therapeutic potential is hindered by drug resistance in GC. This study aims to explore the regulatory role of tripartite motif-containing protein 28 (TRIM28) in BTZ resistance in GC cells and to evaluate the antitumor effect of targeting TRIM28 in combination with BTZ.

**Material and Methods:** We established control groups (including Lenti-control and short hairpin non-targeting control groups), TRIM28-overexpressing (OE), and TRIM28-knockdown models using the MGC-803 gastric cancer cell line to investigate TRIM28-mediated BTZ resistance. A series of assays was performed, including cell counting kit-8 analysis to assess cell viability, flow cytometry for apoptosis analysis, colony formation assays to evaluate cell proliferation, western blot to measure the protein expression of 20S proteasome subunits ( $\alpha 1/4$  and  $\beta 1/2/5$ ), proteasome activity assays, and immunohistochemistry to assess TRIM28 expression in clinical samples. Bioinformatic tools were also used to analyze the clinical correlation of TRIM28 expression with cancer stage and grade.

**Results:** Our results demonstrate that TRIM28 markedly enhanced BTZ resistance in GC cells. TRIM28 OE increased cell viability, inhibited apoptosis, enhanced colony-forming ability, upregulated the expression of proteasome subunits, and increased proteasome activity, contributing to a protective effect against BTZ-induced cytotoxicity. For the clinical GC samples, TRIM28 was highly expressed in tumor tissues, and its expression was correlated with advanced cancer stages and high tumor grades.

**Conclusion:** TRIM28 is critical in promoting BTZ resistance in GC cells. Targeting TRIM28 could potentiate BTZ treatment outcomes and offer a promising therapeutic strategy for overcoming drug resistance in GC treatment.

**Keywords:** Bortezomib, Drug resistance, Gastric cancer, Proteasome activity, Tripartite motif-containing protein 28

## INTRODUCTION

Gastric cancer (GC) ranks among the most prevalent and deadly malignancies worldwide and is characterized by frequent diagnosis and dismal survival rates.<sup>[1-3]</sup> Despite advances in early detection methods, such as endoscopy and imaging, and the advent of novel therapeutic strategies such as chemotherapy, immunotherapy, and targeted therapy, GC continues to

present a substantial challenge in oncology.<sup>[4,5]</sup> Patients with advanced GC demonstrate persistently poor 5-year survival outcomes, highlighting the critical demand for improved therapeutic strategies.<sup>[6,7]</sup> Among the therapeutic agents used in GC treatment, bortezomib (BTZ), a proteasome-targeting therapeutic agent, demonstrates significant clinical activity across several hematologic cancers such as multiple myeloma and specific lymphoma subtypes.<sup>[8,9]</sup> BTZ induces apoptosis by inhibiting the proteasome and impairing proteostatic balance in tumor cells.<sup>[10]</sup> Although clinically effective against several malignancies, BTZ demonstrates restricted therapeutic potential in GC due to acquired resistance mechanisms, leading to treatment failure, disease recurrence, and metastasis, all of which further complicate patient outcomes.<sup>[11,12]</sup>

Drug resistance is a multifaceted problem that remains a major obstacle in cancer therapy. In BTZ resistance, tumor cells often employ various molecular mechanisms to evade the therapeutic effects of the drug.<sup>[13,14]</sup> These mechanisms include alterations in proteasome composition, apoptotic pathway dysregulation, and changes in the tumor microenvironment.<sup>[15,16]</sup> Deciphering these fundamental mechanisms is essential for designing novel approaches to circumvent therapeutic resistance and enhance BTZ's clinical effectiveness against GC.

Emerging evidence identifies tripartite motif-containing protein 28 (TRIM28), KRAB-associated protein 1, transcriptional intermediary factor 1-beta (TIF1 $\beta$ ), a multifunctional nuclear cofactor, as a key mediator of BTZ resistance mechanisms in GC.<sup>[17]</sup> As a member of the tripartite motif (TRIM) family, TRIM28 exhibits diverse functional roles spanning transcriptional modulation, genomic stability maintenance, cellular stress adaptation, and proliferation regulation.<sup>[18]</sup> It also mediates genomic regulation through its molecular interactions with diverse partners, including transcriptional regulators and epigenetic modifiers.<sup>[19,20]</sup> Recent studies highlighted its critical contributions to oncogenesis, including facilitating malignant transformation, cancer dissemination, and resistance to chemotherapy.<sup>[18,20-22]</sup>

Emerging evidence supports the role of TRIM28 in regulating proteasomal activity and its implications for cancer progression and drug resistance. By acting as an E3 small ubiquitin-like modifier (SUMO) ligase, TRIM28 binds to NLR family pyrin domain containing 3 (NLRP3) and promotes its SUMOylation, thereby preventing the latter's ubiquitination and proteasomal degradation.<sup>[23]</sup> This regulatory mechanism enhances NLRP3 expression and inflammasome activation, highlighting TRIM28's role in modulating protein stability. The X-linked inhibitor of apoptosis-associated factor 1 (XAF1) antagonizes TRIM28's oncogenic activity by interacting with its RING domain,

facilitating its ubiquitination and degradation.<sup>[24]</sup> TRIM28 also destabilizes XAF1 through K48-linked polyubiquitination, indicating their reciprocal regulatory relationship that affects tumor growth, migration, and epithelial-to-mesenchymal transition.<sup>[24]</sup> These findings provide direct evidence linking TRIM28 to proteasomal regulation and protein stability in cancer cells. Furthermore, oncogene research reported that TRIM28 stabilizes the proteasome activator REG1; this process accelerates the proteolytic breakdown of tumor suppressor factors and thereby facilitates malignant advancement.<sup>[25]</sup>

Despite these findings, the specific role of TRIM28 in modulating proteasomal activity in GC and its contribution to BTZ resistance remains unexplored. Given that TRIM28 can influence protein homeostasis by preventing the degradation of key regulatory proteins, we hypothesize that TRIM28 may promote BTZ resistance in GC by enhancing proteasomal function. This study aims to fill this knowledge gap by investigating whether TRIM28 upregulates proteasomal activity and thereby counteracts BTZ-induced proteotoxic stress and apoptosis. Understanding this mechanism could provide novel insights into TRIM28's role in chemotherapy resistance and identify potential therapeutic strategies to overcome BTZ resistance in GC.

## MATERIAL AND METHODS

### Ethics statement

This research received ethical clearance from the Institutional Review Board at Longgang Central Hospital of Shenzhen, China (Ethics Approval Number: 2022ECYJ022). Clinical GC samples were collected from Longgang Central Hospital of Shenzhen. Human specimen handling complied fully with international ethical standards, following the tenets established in the Helsinki Declaration,<sup>[26]</sup> and written informed consent was obtained from all the participants.

### Cell culture

MGC-803 gastric adenocarcinoma cells (Beyotime, Shanghai, China) were maintained in RPMI-1640 medium (PM150110, Procell) containing 10% fetal bovine serum (FBS, 164210-50, Procell, Wuhan, China) and 1% penicillin-streptomycin solution (PB180120, Procell). Cultures were passaged every 48-72 h at a 1:3 ratio and incubated at 37°C with 5% CO<sub>2</sub>. Cell line authenticity was verified by STR analysis, and mycoplasma testing confirmed a contamination-free status before experimentation.

### Cell transfection

Lentiviral vectors (plentiv-cmv-enhanced green fluorescent protein, produced by Beijing Zhiyuan Shenlan Technology

Co., Ltd., China) were used to construct the TRIM28-overexpressing (OE) and TRIM28-knockdown (KD) cell lines. For the TRIM28-OE cell line, the recombinant plasmid pCDH-CMV (an HIV-based lentiviral vector with a cytomegalovirus promoter)-TRIM28-EF1-CopGFP-T2A-Puro (gene length: 2508 bp; cloning vector: pCDH-CMV-MCS-EF1-copGFP-T2A (where MCS: multiple cloning site; EF1: elongation factor 1 promoter; copGFP: copepod green fluorescent protein; T2A: Thosea asigna virus 2A self-cleaving peptide; Puro: puromycin resistance gene)-Puro; cloning sites: XbaI-BamHI; resistance: ampicillin (AMP)), whose synthesis was confirmed by the corresponding gene synthesis report, was utilized. This plasmid contains the full-length coding sequence of TRIM28. For the TRIM28-KD cell line, the recombinant plasmid PLKO.1 (pLenti Lentiviral Knockdown vector, version 1)-TRIM28-short hairpin RNA (shRNA) (gene length: 55 bp; cloning vector: PLKO.1; cloning sites: AgeI-EcoRI; resistance: Amp), synthesized and verified as per its gene synthesis report, was used to provide the shRNA sequence targeting TRIM28. A short hairpin non-targeting control (sh-NC) plasmid with a scrambled sequence was also constructed. The reconstructed lentiviral vector plasmids containing the target gene (Lenti-TRIM28) and negative control (Lenti-control) were obtained from Youbio and underwent sequence verification. shRNAs targeting TRIM28 (sh-TRIM28) and sh-NC were designed on the basis of the siR-TRIM28 sequences. Lentiviral particles were produced in HEK293T cells maintained in high-glucose Dulbecco's Modified Eagle Medium (C2703, Beyotime) supplemented with 10% FBS, antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin), and 2 mM L-glutamine under standard culture conditions. Following packaging, the viral supernatants were harvested and used to transduce the MGC-803 cells. Stable transfectants were obtained through puromycin selection (2 µg/mL). The siRNA sequences are detailed in Table 1.

### Cell counting kit-8 (CCK-8) assay

Cell viability was measured by CCK-8 assay (C0038, Beyotime). Following seeding at 1000 cells/well in 96-well plates and 24 h attachment, the cells from various treatment groups were exposed to BTZ at graded concentrations (0-100 nM) in fresh complete medium, with triplicate wells per dose. At different time points (12, 24, and 48 h), 10 µL of CCK-8 solution was added to each well 2-4 h before the

end of the treatment. A microplate reader (Epoch2, BioTek, USA) was used to measure the absorbance at 450 nm after incubation at 37°C.

### Colony formation assay

Cells from each experimental group were plated in six-well plates and allowed to adhere for 24 h. Following attachment, the cultures were treated with 20 nM BTZ in fresh complete medium and maintained for 10-14 days, with medium renewal every 3-4 days. After incubation, the cells were washed with phosphate-buffered saline (PBS) (C0221A, Beyotime), fixed with 4% paraformaldehyde (P0099, Beyotime) for 15-20 min, and stained with crystal violet (C0121, Beyotime) for an equivalent duration. After water rinsing and air-drying, the colonies were quantified microscopically.

### Flow cytometry

Following 24 h exposure to 20 nM BTZ, the cells were harvested, washed with PBS twice, and adjusted to  $1 \times 10^6$  cells/mL in  $1 \times$  binding buffer. For apoptosis detection, 100 µL of aliquots were stained with 5 µL each of annexin fluorescein isothiocyanate-conjugated annexin V (V-FITC) and propidium iodide (C1062S, Beyotime) for 15-20 min at room temperature (RT) in the dark. After 400 µL of buffer was added, the samples were immediately analyzed by flow cytometry (BD LSRFortessa SORP).

### Western blot analysis

Posttreatment cells were harvested and lysed in ice-cold radioimmunoprecipitation assay buffer (P0013B, Beyotime) containing protease/phosphatase inhibitors (ST506, Beyotime) for 30 min on ice. Following centrifugation (12,000 rpm, 15 min), the supernatants were collected for protein quantification using bicinchoninic acid assay (P0010S, Beyotime). Proteins of equal amounts were prepared with loading buffer, denatured (5-10 min boiling), and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred to polyvinylidene fluoride membranes, blocked with 5% skim milk (P0216, Beyotime; 1-2 h, RT), and probed overnight at 4°C with the primary antibodies against 20S proteasome subunits ( $\alpha 1/4$ : sc-166073;  $\beta 1/2/5$ : sc-271297/

**Table 1:** Sequences of siRNAs.

Name	Sense	Antisense
sh-NC	UUCUCCGAACGAGUCACGUTT	ACGUGACUCGUUCGGAGAATT
sh - TRIM28	AGACATCGTGGAGAATTATTT	AAATAATTCTCCACGATGTCT

Sh-NC: short hairpin non-targeting control, sh-TRIM28: short hairpin-tripartite motif-containing protein 28, A: Adenine, T: Thymine, G: Guanine, C: Cytosine

sc-374405/sc-515066/sc-393931; all 1:500; Santa Cruz) and  $\beta$ -actin (66009-1-Ig, 1:500; Proteintech, Wuhan, China). After tris-buffered saline with tween washes ( $3-5 \times 10$  min), the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (ab205719, 1:3000; Abcam; 1-2 h, RT), washed again, and visualized by chemiluminescence. Band intensities were quantified using ImageJ (NIH, USA).

### Real-time quantitative polymerase chain reaction (PCR)

Total RNA extraction was performed using a Trizol reagent (15596018, Ambion, Shanghai, China) through a standard chloroform-isopropanol protocol (Acros Organics 327270010, USA). Purified RNA pellets were washed with 75% ethanol, air-dried, and resuspended in RNase-free water before quantification. Reverse transcription was carried out with PrimeScript™ RT reagent Kit (TAKARA RR037Q, Japan), and the synthesized cDNA was preserved at  $-80$  °C. TB Green™ Premix Ex Taq™ II (TAKARA RR820S, Japan) was employed in quantitative PCR, with  $\beta$ -actin serving as the endogenous control. Gene expression was quantified through the  $2^{-\Delta\Delta CT}$  method using the primers detailed in Table 2.

### Enzyme-linked immunosorbent assay (ELISA)

Following treatment, cellular proteasome activity was quantified using a commercial ELISA assay kit (ab107921, Abcam, UK). In brief, cell lysates were combined with the provided substrate and incubated under specified conditions. Reaction product absorbance was determined with a BioTek microplate reader, with activity levels calculated against the kit's standard curve.

### Immunohistochemistry (IHC) analysis

The clinical GC specimens were preserved in 4% paraformaldehyde, progressively dehydrated in ethanol gradients, and embedded in paraffin blocks. Tissue sections ( $4 \mu\text{m}$ ) were prepared on slides, followed by deparaffinization and rehydration. Epitope retrieval was conducted before the application of 5% goat serum for 30 min to minimize background staining. The tissue sections were processed and analyzed by a professional third-party service provider (Wuhan Servicebio Technology Co., Ltd., Wuhan, China), which specializes in histopathological assays, to ensure optimal quality and consistency in IHC staining and

imaging. The procedures were as follows: Incubation with primary antibodies against TRIM28 (1:500, PA5-27648, ThermoFisher, USA) and proteasome subunits ( $\beta 1/2/5$ ) (1:100, sc-374405; 1:100, sc-515066; 1:100, sc-393931; primary antibodies from Santa Cruz Biotechnology (USA) were applied overnight at  $4^\circ\text{C}$ , after which the samples were treated with HRP-linked secondary antibodies (ab205719, Abcam, UK; 1:3000 dilution) for 30 min at RT. Chromogenic detection was performed using 3,3'-diaminobenzidine (P0203, Beyotime, China). The stained sections were imaged under a microscope (Olympus Corp, Japan, CX31), and protein expression levels were quantified using ImageJ software (NIH, USA, Version 1.8.0).

### Analysis of the correlation between TRIM28 expression and clinicopathological features

TRIM28 expression profiles in gastric adenocarcinoma were systematically evaluated by utilizing the UALCAN bioinformatic platform (<https://ualcan.path.uab.edu>). Clinical datasets comprising 34 normal gastric samples and 415 primary tumor specimens were obtained from the Cancer Genome Atlas (TCGA) (<https://www.cancer.gov/tcga>) for comprehensive analysis.<sup>[27]</sup> The correlation between TRIM28 expression and clinicopathological features, including sample types, cancer stages, tumor grade, and node metastasis status, was examined to explore TRIM28's potential role in GC progression.

### Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, USA). Continuous variables were expressed as mean  $\pm$  standard deviation. Intergroup comparisons were conducted using two-tailed unpaired *t*-tests, and multigroup analyses employed one-way analysis of variance with Tukey's *post hoc* test. Statistical significance was set at  $P < 0.05$ .

## RESULTS

### TRIM28 expression in GC based on clinicopathological features

To elucidate the role of TRIM28 in GC progression, we analyzed 449 samples from TCGA, including 34 normal tissues and 415 primary tumor tissues. TRIM28 was significantly upregulated in the tumor tissues compared

**Table 2:** Sequences of RNA primers.

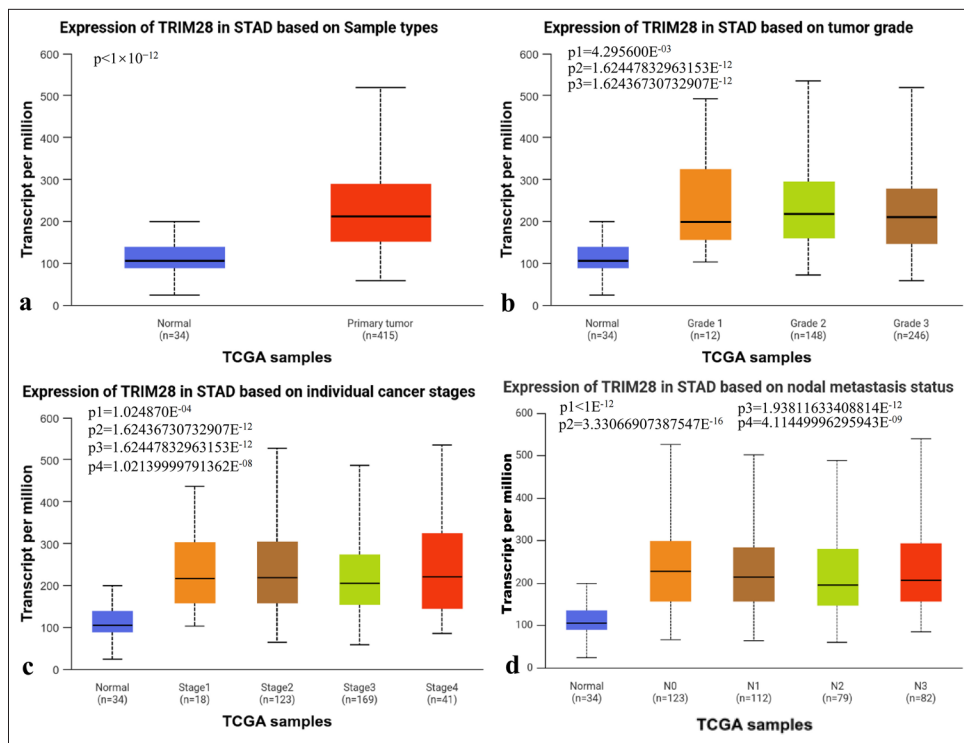
Names	Forward primer	Reverse primer
$\beta$ -actin	CAGGAAGTCCCTTGCCATCC	ACCAAAAGCCTTCATACATCTCA
TRIM28	ATGGTGGCCTCCGCGGCGGC	GCCGCCGCGGAGGCCACCAT

TRIM28: Tripartite motif-containing protein 28, A: Adenine, T: Thymine, G: Guanine, C: Cytosine

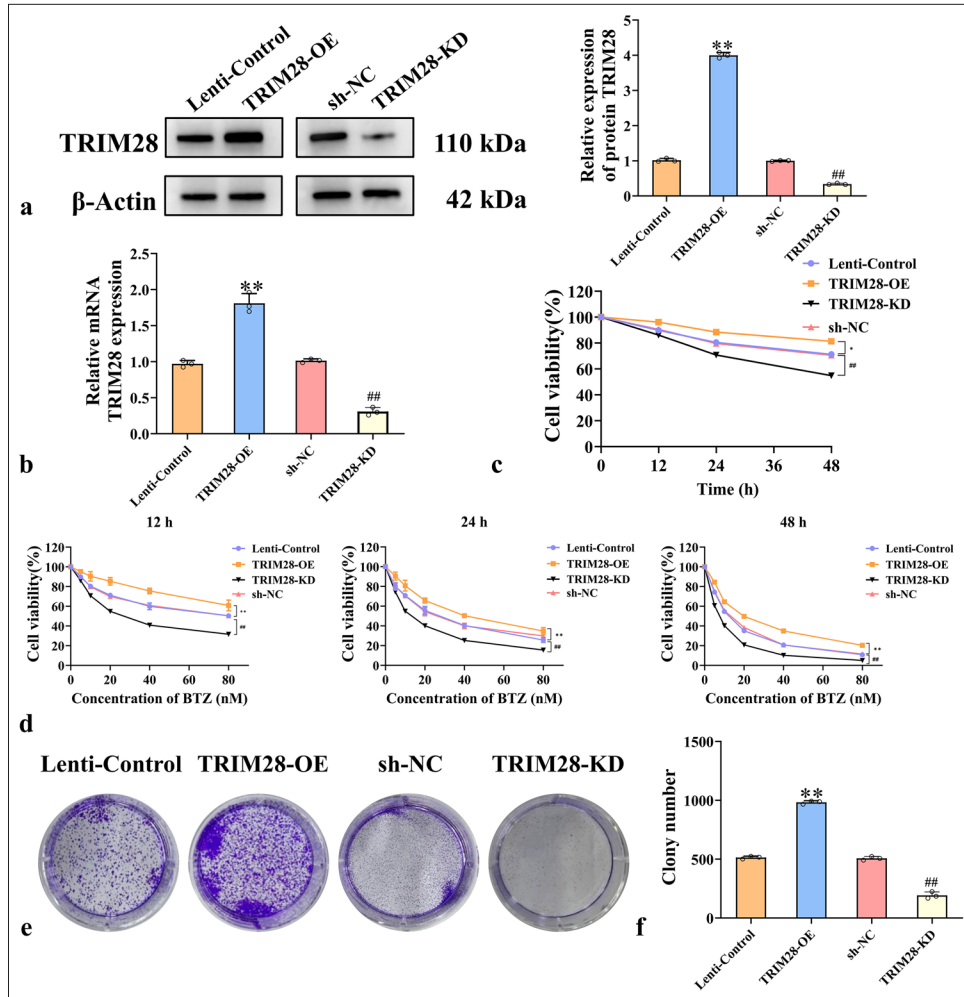
with that in the normal tissues, suggesting its potential involvement in GC development [Figure 1a]. Analysis of expression levels across tumor grades showed that TRIM28 was elevated in high-grade tumors (grades 2 and 3) relative to normal tissues, with the highest median expression observed in grade 2 [Figure 1b]. Although no strict linear increase with grade was observed, the overall high expression of TRIM28 in advanced grades implies its potential link to tumor aggressiveness. TRIM28 expression also varied across clinical stages, with stage 4 tumors exhibiting a relatively higher median expression compared with stages 1-3, which may indicate TRIM28's association with late-stage progression [Figure 1c]. Regarding lymph node status, although the TRIM28 expression in N1 and N2 was not significantly different from that in N0, the highest levels were observed in N3 tumors [Figure 1d]. This trend suggests that TRIM28 may be associated with nodal metastasis at advanced stages. These data collectively demonstrate TRIM28's critical role in driving GC progression and metastasis, positioning it as an attractive candidate for molecular targeted therapy.

### TRIM28 promotes cell proliferation and BTZ resistance in GC cells

To investigate TRIM28's cellular functions in GC, we genetically manipulated its expression in MGC-803 cells through OE and knockdown (KD), approaches. Successful modulation was confirmed by Western blot and quantitative PCR analyses [Figure 2a and b]. Subsequent functional analyses, including colony formation and CCK-8, revealed TRIM28's effect on tumor cell proliferation [Figure 2c-f]. CCK-8 assay, which assesses cell viability at multiple time points, revealed that the TRIM28-OE cells demonstrated significantly higher viability compared with the control cells, particularly under BTZ treatment. This finding indicates that TRIM28 OE confers resistance to BTZ-induced cytotoxicity [Figure 2c and d]. In the colony formation assay, the TRIM28-OE GC cells exhibited a markedly enhanced ability to form colonies, highlighting a substantial increase in their proliferative capacity [Figure 2e]. By contrast, the TRIM28-KD cells showed a notable reduction in colony



**Figure 1:** TRIM28 expression in gastric cancer based on clinicopathological features. (a) TRIM28 expression in primary gastric tumor samples and normal tissues; p: Primary tumor versus normal. (b) TRIM28 expression across different tumor grades; p1: Grade 1 versus normal; p2: Grade 2 versus normal; p3: Grade 3 versus normal. (c) TRIM28 expression in different cancer stages; p1: Stage 1 versus normal; p2: Stage 2 versus normal; p3: Stage 3 versus normal; p4: Stage 4 versus normal. (d) TRIM28 expression in tumors with or without nodal metastasis; p1: N0 versus normal; p2: N1 versus normal; p3: N2 versus normal; p4: N3 versus normal. TCGA: The cancer genome atlas; STAD: Stomach adenocarcinoma, TRIM28: Tripartite motif-containing protein 28.



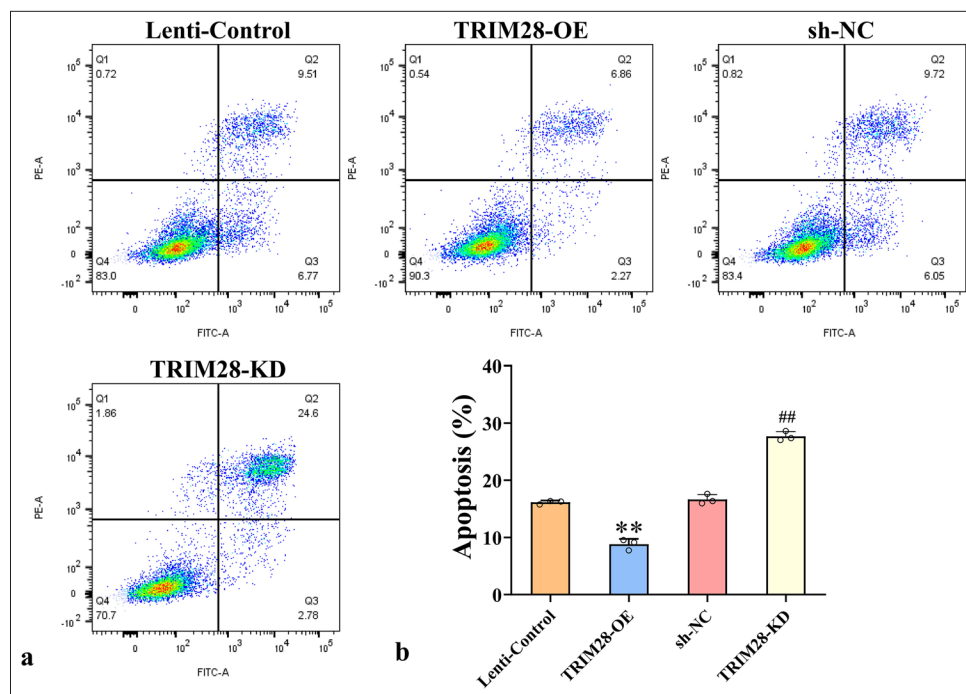
**Figure 2:** TRIM28 promotes cell proliferation and bortezomib resistance in gastric cancer cells. (a) Western blot assays were used to verify the overexpression and knockdown efficiency of TRIM28 in MGC-803 cells.  $\beta$ -Actin served as the loading control. (b) Real-time quantitative polymerase chain reaction assays were used to detect the messenger RNA expression of TRIM28 in MGC-803 cells. (c) CCK-8 assays were used to assess cell viability in TRIM28-KD/OE MGC-803 cells not treated with bortezomib at 12, 24, 36 and 48h. (d) CCK-8 assays were used to assess the viability of TRIM28-KD and TRIM28-OE MGC-803 cells treated with varying concentrations of bortezomib at 12, 24, and 48 h. (e) Representative images of colony formation assay in control, TRIM28-OE, and TRIM28-KD groups. (f) Quantification of colony number. All data were obtained from at least three repeated experiments and presented as mean  $\pm$  standard deviation.  $n = 3$ , \* $P < 0.05$ . \*\* $P < 0.01$  versus Lenti-control; ## $P < 0.01$  versus sh-NC (Student's  $t$ -test). OE: over expression; KD: knock down, TRIM28: Tripartite motif-containing protein 28, CCK-8: Cell counting kit-8, sh-NC: Short hairpin non-targeting control.

formation, further supporting the role of TRIM28 in promoting cellular proliferation [Figure 2f]. Collectively, our data demonstrate that TRIM28 is pivotal in regulating growth and chemoresistance to BTZ in GC cells.

#### TRIM28 confers resistance to BTZ-induced apoptosis in GC cells

Flow cytometry analysis was conducted to assess the impact of TRIM28 OE and knockout on apoptosis rates

in GC cells following BTZ treatment [Figure 3a and b]. In the TRIM28-OE cells, a significant decrease in apoptotic cells (early and late stages) was observed compared with the controls, demonstrating that TRIM28 OE confers resistance to BTZ-induced apoptosis. By contrast, the TRIM28-KD cells exhibited a pronounced increase in apoptotic cells, with elevated proportions in early and late apoptotic stages. These findings conclusively demonstrate that TRIM28 depletion sensitizes GC cells to BTZ, resulting in enhanced apoptosis and cell death. They



**Figure 3:** TRIM28 confers resistance to bortezomib-induced apoptosis in gastric cancer cells. (a) Flow cytometry analysis showing the distribution of apoptotic cells in TRIM28-OE/KD MGC-803 cells following bortezomib treatment. (b) Quantification of apoptotic cells in different groups detected by flow cytometry. All data were obtained from at least three repeated experiments and presented as mean  $\pm$  standard deviation.  $n = 3$ ,  $**P < 0.01$  versus Lenti-control;  $**P < 0.01$  versus sh-NC. (Student's *t*-test). OE: over expression, KD: knock down, TRIM28: Tripartite motif-containing protein 28, sh-NC: short hairpin non-targeting control.

underscore TRIM28's role as a protective factor against BTZ-induced apoptosis and its contribution to drug resistance.

### TRIM28 regulation of proteasome activity in GC cells

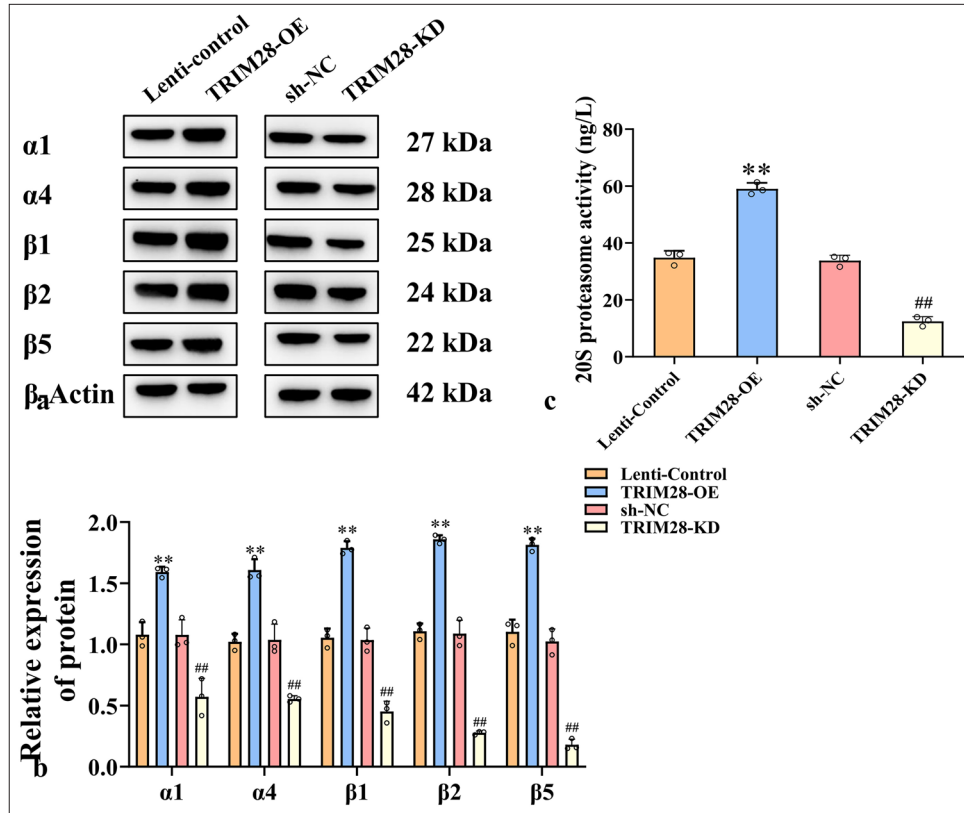
Considering that BTZ exerts its antitumor effects primarily through the inhibition of proteasome activity, we next investigated whether TRIM28 influences proteasomal function, which may underlie its role in BTZ resistance. Western blot analysis and proteasome activity assays were conducted [Figure 4]. Western blot analysis revealed that TRIM28 OE resulted in the upregulated expression of proteasome subunits, including 20S  $\alpha$ 1/4 and  $\beta$ 1/2/5, relative to those in the control cells [Figure 4a and b]. This upregulation was accompanied by a marked increase in 20S proteasome activity, as quantified by proteasome activity assays [Figure 4c]. Conversely, TRIM28 knockout caused a substantial reduction in proteasome subunit levels and a corresponding decline in proteasome activity [Figure 4]. These findings suggest that TRIM28 enhances proteasomal function, potentially explaining its role in mitigating BTZ-induced cytotoxicity and promoting drug resistance.

### Immunohistochemistry analysis of TRIM28 and proteasome subunits in GC tissues

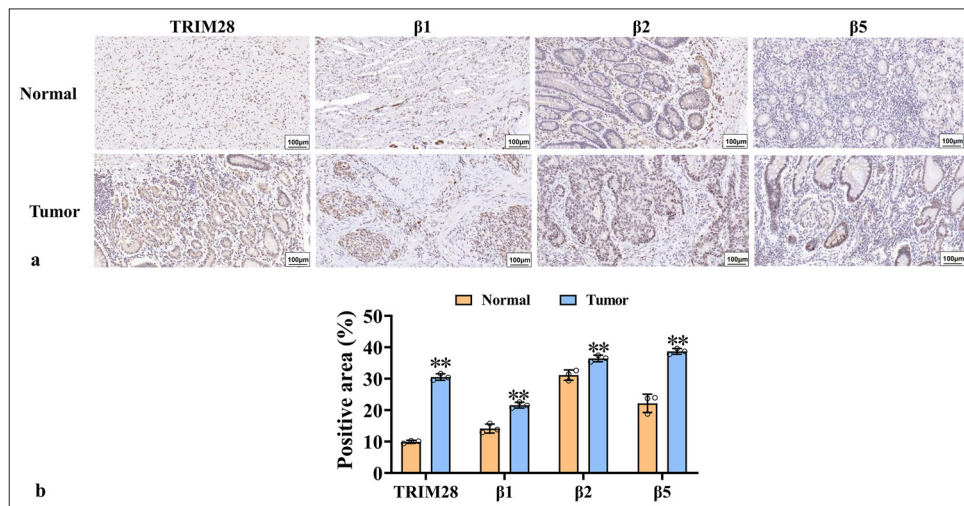
IHC staining was conducted on GC tissues to evaluate TRIM28 and proteasome subunit expression in tumor versus normal tissues [Figure 5]. IHC analysis revealed significantly elevated TRIM28 expression in the tumor tissues compared with that in the normal tissues, with pronounced staining intensity in the tumor samples [Figure 5a]. Quantification analysis of proteasome subunit ( $\beta$ 1/2/5) expression demonstrated a similar trend [Figure 5b]. Proteasome subunit expression was also significantly upregulated in the tumor tissues and correlated strongly with the TRIM28 levels. These results indicate that the TRIM28-mediated regulation of proteasomal components is closely associated with GC progression and may underlie resistance to proteasome inhibitors such as BTZ.

### DISCUSSION

Our findings highlight TRIM28 as a key mediator of BTZ resistance in GC. TCGA-based evaluation revealed that TRIM28 expression correlates with advanced disease



**Figure 4:** Tripartite motif-containing protein 28's impact on proteasome activity in gastric cancer cells. (a) Western blot analysis of proteasome subunits in MGC-803 cells. (b) Quantitative analysis of the Western blot results presented in panel A. (c) Proteasome activity assay results measured by enzyme-linked immunosorbent assay. All data were obtained from at least three repeated experiments and presented as mean  $\pm$  standard deviation.  $n = 3$ , \*\* $P < 0.01$  versus Lenti-control; # $P < 0.01$  versus short hairpin non-targeting control (Student's  $t$ -test).



**Figure 5:** IHC analysis of TRIM28 and proteasome subunits in gastric cancer tissues. (a) IHC staining of TRIM28 and proteasome subunits ( $\beta 1/2/5$ ) in gastric cancer tissues and normal tissues (scale bar: 100  $\mu\text{m}$ ). (b) Quantitative analysis of the IHC results shown in panel a. All data were obtained from at least three repeated experiments and presented as mean  $\pm$  standard deviation.  $n = 3$ , \*\* $P < 0.01$  versus normal group (Student's  $t$ -test). TRIM28: Tripartite motif-containing protein 28, IHC: Immunohistochemistry.

features, showing progressive upregulation from normal tissues to primary tumors and further elevation in metastatic lesions. This graded OE pattern, consistent with tumor aggressiveness, supports TRIM28's established oncogenic properties in cancer pathogenesis.<sup>[20,28,29]</sup>

Cellular functional assays revealed that TRIM28 OE significantly increases proliferative capacity and attenuates BTZ-mediated cytotoxic effects on GC cells, as evidenced by CCK-8 viability tests and clonogenic survival assays. These results suggest that TRIM28 enables tumor cell survival and expansion despite its proteasome inhibition.<sup>[30]</sup> Conversely, TRIM28 knockout reduces cell proliferation and increases sensitivity to BTZ, further supporting its role in promoting drug resistance.

Flow cytometry analysis showed that TRIM28 OE inhibits BTZ-induced apoptosis, whereas its knockout sensitizes cells to apoptosis. This finding suggests that TRIM28 acts as a protective factor against the apoptotic effects of BTZ, which is a key mechanism for the antitumor activity of this drug. The ability of TRIM28 to regulate apoptosis may be related to its influence on proteasome function, as proteasome inhibition by BTZ typically leads to the accumulation of pro-apoptotic proteins.<sup>[31,32]</sup>

Western blot and proteasome activity assays revealed that TRIM28 upregulates the expression of proteasome subunits and increases proteasome activity. This upregulation of proteasome function may explain how TRIM28 mitigates the cytotoxic effects of BTZ.<sup>[33]</sup> By enhancing proteasome activity, TRIM28 may facilitate the degradation of the proteins targeted by BTZ, thereby reducing the drug's effectiveness.

IHC analysis of GC tissues further supported the *in vitro* findings. The elevated expression of TRIM28 and proteasome subunits in tumor tissues, along with their strong correlation, suggests that TRIM28-mediated proteasomal regulation is involved in GC progression and may contribute to resistance to proteasome inhibitors such as BTZ. Furthermore, TRIM28 regulates the tumor immune microenvironment by repressing type I interferon signaling and modulating immune cell infiltration, which may further facilitate immune evasion and resistance *in vivo*.<sup>[30]</sup> These roles underscore TRIM28's potential as a dual therapeutic target acting on tumor cells and the immune milieu.

Although our findings demonstrate that TRIM28 enhances proteasome activity and confers resistance to BTZ in GC cells, the underlying molecular mechanisms remain to be fully elucidated. Emerging evidence suggests that TRIM28 may modulate proteasome function through multiple signaling axes and posttranslational regulatory mechanisms.

One plausible mechanism involves the phosphatidylinositol 3-kinase/protein kinase B (PI3K-Akt) pathway, which is pivotal in regulating proteasome biogenesis and maintaining proteostasis under cellular stress. TRIM28 interacts with the upstream components of this pathway, potentially enhancing Akt phosphorylation and downstream signaling. This activation may indirectly upregulate proteasome subunit expression or stabilize proteasome assembly, contributing to a sustained proteolytic environment that favors tumor survival and drug resistance.<sup>[34]</sup> In addition, TRIM28 may intersect with the nuclear factor kappa B (NF- $\kappa$ B) signaling cascade, a key regulator of inflammation and proteasome-dependent transcriptional activity. By promoting nuclear translocation of NF- $\kappa$ B subunits or preventing their proteasomal degradation, TRIM28 could facilitate the transcription of proteasome-related genes, thereby enhancing the proteasomal capacity of cancer cells and attenuating the cytotoxic effects of BTZ.<sup>[34]</sup> As a scaffold protein and transcriptional coregulator, TRIM28 may exert direct control over the ubiquitin, proteasome system. Through its association with E3 ubiquitin ligases, TRIM28 could regulate the ubiquitination and degradation of proteasome suppressors or proteasome subunit modulators. Recent studies highlighted the role of TRIM family proteins in maintaining proteasome integrity, suggesting that TRIM28 may contribute to the stabilization of 20S core subunits or influence their posttranslational modifications.<sup>[35,36]</sup>

Further research is necessary to delineate these pathways in great detail. The pharmacological inhibition of PI3K-Akt or NF- $\kappa$ B in TRIM28-OE cells may help define their contribution to TRIM28-mediated proteasome regulation. In parallel, co-immunoprecipitation and ubiquitination assays could determine whether TRIM28 interacts directly with proteasome components or their upstream regulators. Finally, *in vivo* validation using xenograft or genetically modified mouse models will be critical to confirm the mechanistic relevance of TRIM28 in BTZ resistance and to evaluate the therapeutic potential of targeting this axis in GC.

## SUMMARY

Our findings establish TRIM28 as a key mediator of BTZ resistance in GC. Elevated TRIM28 expression correlates with accelerated tumor progression, heightened proteasome function, and diminished apoptotic response to BTZ. These results position TRIM28 as a promising therapeutic target to circumvent proteasome inhibitor resistance. Additional investigations are warranted to fully delineate the molecular mechanisms

of TRIM28-associated chemoresistance and its cross-talk with other signaling networks. Future studies should also focus on validating these findings in multiple GC cell lines and *in vivo* models to further understand the clinical implications of targeting TRIM28 in combination with existing therapies.

## AVAILABILITY OF DATA AND MATERIALS

All data and materials generated or analyzed during this study are included in this article. Data are available from the corresponding author upon reasonable request. Correspondence should be addressed to Jinjun Ye at yejj610@163.com.

## ABBREVIATIONS

ANOVA: Analysis of variance  
 BCA: Bicinchoninic acid  
 BTZ: Bortezomib  
 CCK-8: Cell counting kit-8  
 DMEM: Dulbecco's Modified Eagle Medium  
 ELISA: Enzyme-linked immunosorbent assay  
 FBS: Fetal bovine serum  
 GC: Gastric cancer  
 IHC: Immunohistochemistry  
 PBS: Phosphate-buffered saline  
 PI: Propidium iodide  
 PVDF: Polyvinylidene fluoride  
 RIPA: Radioimmunoprecipitation assay  
 SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis  
 STAD: Stomach adenocarcinoma  
 TBST: Tris-buffered saline with Tween  
 TCGA: The Cancer Genome Atlas  
 TRIM28: Tripartite motif containing 28

## AUTHOR CONTRIBUTION

LX: Conceived and designed the study, supervised the project, critically revised the manuscript, approved the final version, and is accountable for all aspects of the work; SYH, ZXW, XYY, and YWY: Acquired and analyzed data, interpreted results, critically revised the manuscript, approved the final version, and are accountable for all aspects of the work; JDL, XB, and JYJ: Participated in data analysis and interpretation, drafted the manuscript, revised it critically, approved the final version, and are accountable for all aspects of the work. All authors contributed to critical revision of the manuscript (meeting ICMJE criteria for revising), approved the final version, and are accountable for the work's accuracy and integrity.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Medical Ethics Committee of Longgang Central Hospital of Shenzhen (Approval No. 2022ECYJ022). Clinical gastric cancer samples were all collected from Longgang Central Hospital of Shenzhen. All procedures involving human samples were conducted in accordance with ethical guidelines and regulations, strictly adhered to the principles of the Declaration of Helsinki, and written informed consent was obtained from all participants.

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Not applicable.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## EDITORIAL/PEER REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded for reviewers and vice versa) through an automatic online system.

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