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The TCF3-FBXL18 axis regulates KRAS stability to determine cetuximab response in colorectal cancer

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Article

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Abstract

Background

Resistance to anti-EGFR therapy represents a major clinical challenge in metastatic colorectal cancer. Despite the use of RAS mutation status to guide cetuximab administration, a substantial proportion of RAS wild-type tumors develop primary or acquired resistance, underscoring the need to elucidate alternative mechanisms governing EGFR signaling. While protein ubiquitination critically regulates oncogenic protein stability, the specific E3 ubiquitin ligases mediating KRAS turnover in cetuximab resistance remain poorly defined.

Objective

This study aimed to identify novel molecular determinants of cetuximab resistance and elucidate the underlying transcriptional and post-translational regulatory mechanisms controlling KRAS protein stability in colorectal cancer.

Methods

We examined FBXL18 expression in cetuximab-sensitive and -resistant clinical specimens and established resistant cell models (HCT-8/CET-R and Caco-2/CET-R). Functional assays including CCK-8, colony formation, EDU incorporation, and flow cytometry were performed to evaluate the biological role of FBXL18. Ubiquitination assays, cycloheximide chase experiments, and co-immunoprecipitation were conducted to investigate KRAS degradation mechanisms. Bioinformatics analysis and dual-luciferase reporter assays were employed to identify upstream transcriptional regulators. In vivo xenograft experiments validated the therapeutic effects of FBXL18 restoration.

Results

FBXL18 was significantly downregulated in cetuximab-resistant CRC tissues and cell lines at both transcriptional and translational levels. Functionally, FBXL18 suppressed cell proliferation, promoted apoptosis, and enhanced cetuximab sensitivity. Mechanistically, FBXL18 directly interacted with KRAS and catalyzed its ubiquitin-mediated proteasomal degradation, thereby reducing KRAS protein stability and attenuating oncogenic signaling. Furthermore, integrated bioinformatics analysis identified transcription factor 3 (TCF3) as a critical upstream regulator that directly binds to the FBXL18 promoter and transcriptionally activates its expression. Disruption of the TCF3–FBXL18 axis led to KRAS accumulation and cetuximab resistance.

Conclusion

Our findings establish the TCF3–FBXL18–KRAS axis as a novel transcriptional–posttranslational regulatory network governing cetuximab response in colorectal cancer. These results provide a potential predictive biomarker and therapeutic target for overcoming anti-EGFR resistance.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide and remains a leading cause of cancer-related mortality. Targeted therapy with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies, such as cetuximab (CET), has significantly improved clinical outcomes in patients with RAS wild-type metastatic CRC. However, primary or acquired resistance to CET occurs in a substantial proportion of patients, which severely limits its therapeutic efficacy and remains a major clinical challenge[1,2].

Aberrant activation of the RAS signaling pathway is a key determinant of resistance to anti-EGFR therapy. KRAS functions as a central molecular switch that regulates downstream MAPK and PI3K/AKT signaling cascades, thereby promoting tumor cell proliferation and survival[3]. Even in tumors without KRAS mutations, sustained KRAS protein stability or activation has been reported to confer resistance to EGFR-targeted therapies[4]. These findings suggest that post-translational regulation of KRAS may play an important role in cetuximab resistance.

Protein ubiquitination is a critical mechanism controlling protein stability and signaling homeostasis. Several E3 ubiquitin ligases have been shown to regulate oncogenic signaling pathways through targeted protein degradation[5]. Recent studies indicate that ubiquitin-mediated degradation of RAS proteins represents a potential strategy to suppress RAS-driven tumor progression and overcome therapeutic resistance[6]. However, the specific E3 ligases responsible for regulating KRAS stability in colorectal cancer remain incompletely understood.

F-box proteins are substrate-recognition components of the SCF (SKP1-Cullin-F-box) E3 ubiquitin ligase complex and play important roles in tumor biology by mediating the ubiquitination and degradation of oncogenic proteins[7]. FBXL18, a member of the F-box protein family, has recently been implicated in cancer progression, but its biological function and regulatory mechanisms in colorectal cancer, particularly in the context of targeted therapy resistance, remain largely unknown.

In addition to post-translational regulation, transcriptional control represents another critical layer governing gene expression. Transcription factor 3 (TCF3), a member of the basic helix–loop–helix (bHLH) family, has been reported to participate in tumor development, stemness maintenance, and therapy resistance through transcriptional regulation of downstream target genes[8,9]. Nevertheless, whether TCF3 regulates FBXL18 expression and contributes to cetuximab resistance has not been investigated.

In the present study, we demonstrate that FBXL18 is significantly downregulated in cetuximab-resistant colorectal cancer and functions as a tumor suppressor by promoting ubiquitin-mediated proteasomal degradation of KRAS. Mechanistically, TCF3 directly binds to the FBXL18 promoter and transcriptionally activates its expression. Loss of the TCF3-FBXL18 axis leads to KRAS stabilization and contributes to cetuximab resistance. Our findings reveal a novel transcriptional-posttranslational regulatory pathway controlling KRAS signaling and provide a potential therapeutic target for overcoming anti-EGFR resistance in colorectal cancer.

Materials and Methods

Clinical specimens and immunohistochemistry

Colorectal cancer tissues were obtained from patients who received cetuximab-based therapy at the First Affiliated Hospital of Gannan Medical University. Patients were categorized as cetuximab-sensitive or cetuximab-resistant based on RECIST (version 1.1) criteria. All samples were collected with written informed consent, and the study was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Gannan Medical University (Approval No. LLSC-2025333) and performed in accordance with the Declaration of Helsinki.

Paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval in citrate buffer (pH 6.0). Following blocking with 5% bovine serum albumin, sections were incubated with primary antibodies against FBXL18 or TCF3 overnight at 4°C, followed by horseradish peroxidase-conjugated secondary antibodies. Immunoreactivity was visualized using 3,3'-diaminobenzidine and counterstained with hematoxylin. Protein expression levels were quantified using the H-score method.

All human participants' names and other HIPAA identifiers were removed from all sections of the manuscript. No identifying images or details that could lead to the identification of participants are included in this study.

Cell culture and establishment of cetuximab-resistant cell lines

Human colorectal cancer cell lines HCT-8 and Caco-2 were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C with 5% CO_2. Cetuximab-resistant sublines (HCT-8/CET-R and Caco-2/CET-R) were established by continuous exposure to gradually increasing concentrations of cetuximab (0.1–10 μg/mL) over 3–6 months. Resistance was confirmed by CCK-8 assays.

Plasmids, shRNA, and transfection

Gene-specific short hairpin RNAs (shRNAs) targeting FBXL18 or TCF3 and corresponding negative controls were obtained from GeneChem (Shanghai, China). Full-length FBXL18 and TCF3 cDNAs were

subcloned into pcDNA3.1 expression vectors for gain-of-function studies. Transient transfections were performed using Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's protocol. Knockdown and overexpression efficiencies were validated by Western blot analysis at 48–72 h post-transfection.

Western blot analysis

Cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors. Equal amounts of protein (30–50 μg) were separated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). Membranes were incubated with primary antibodies against FBXL18, TCF3, KRAS, Bax, and β-actin overnight at 4°C, followed by HRP-conjugated secondary antibodies. Protein bands were visualized using enhanced chemiluminescence (ECL) and quantified by ImageJ software.

Cell viability and colony formation assays

Viability in response to cetuximab was assessed using the Cell Counting Kit-8 (CCK-8) assay. For colony formation assays, cells were seeded in six-well plates at a density of 500 cells per well and cultured for 10–14 days. Colonies were fixed with 4% paraformaldehyde (Biosharp), stained with 0.1% crystal violet (Servicebio), and ImageJ was used for counting statistics..

5-ethynyl-2'-deoxyuridine (EdU) proliferation assay

Proliferation was evaluated using the EdU incorporation assay (Beyotime). Cells were incubated with 10 μM EdU for 2 h, fixed, permeabilized, and stained according to the manufacturer's instructions. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI), and the percentage of EdU-positive cells was calculated from at least five random fields.

Apoptosis analysis

Apoptosis was analyzed using Annexin V-FITC/propidium iodide (PI) double staining (Elabscience) followed by flow cytometry. The percentage of apoptotic cells (Annexin V-positive, PI-positive) was quantified using FlowJo software.

Ubiquitination assay

Cells were co-transfected with hemagglutinin (HA)-tagged ubiquitin and relevant plasmids. Twenty-four hours post-transfection, cells were treated with the proteasome inhibitor MG132 (10 μM) for 6 h. Cell lysates were subjected to immunoprecipitation with anti-KRAS antibodies, and ubiquitinated KRAS was detected by Western blot using anti-HA antibodies.

Cycloheximide (CHX) chase assay

Cells were treated with cycloheximide (100 μg/mL) to inhibit de novo protein synthesis. Cells were harvested at indicated time points (0, 0.5, 1, and 2 h), and KRAS protein levels were analyzed by Western blot to evaluate protein half-life.

Co-immunoprecipitation (Co-IP)

Cell lysates were incubated with antibodies against FBXL18 or KRAS overnight at 4°C, followed by incubation with protein A/G agarose beads. Immunoprecipitation were washed and analyzed by Western blot.

Dual-luciferase reporter assay

The FBXL18 promoter fragments and mutant constructs were cloned into the pGL3-basic vector. Cells were co-transfected with firefly luciferase reporter plasmids, TCF3 expression vectors, and Renilla luciferase plasmid (internal control). Luciferase activities were measured 48 h post-transfection using the Dual-Luciferase Reporter Assay System (Promega) and normalized to Renilla activity.

Xenograft model

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the First Affiliated Hospital of Gannan Medical University (Approval No. LLSC-2026007) and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines

BALB/c nude mice (female, 4–6 weeks old) were purchased from ZhuHai Bestest Biotechnology Co., Ltd. (Zhuhai, China) and maintained under specific pathogen-free conditions with a 12-hour light/dark cycle at 22 ± 2°C and 55 ± 5% relative humidity, with free access to food and water *ad libitum*.

For tumor xenograft studies, mice were subcutaneously injected with 2×10^6 cetuximab-resistant cells stably expressing empty vector or FBXL18. Tumor dimensions were measured every 3 days using calipers, and volume was calculated as (length × width²)/2.

Mice were monitored every 3 days for signs of distress, including > 20% body weight loss, lethargy, or tumor ulceration. Humane endpoints were applied, and mice were euthanized by isoflurane inhalation anesthesia followed by cervical dislocation when tumor volume reached 2,000 mm³ or at 21 days post-inoculation, whichever occurred first. Euthanasia methods were consistent with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020).

Bioinformatics analysis

Potential transcription factors regulating FBXL18 were predicted using FIMO-JASPAR, ENCODE, GTRD, ChIP-Atlas, and hTFtarget databases. Intersection analysis was performed to identify candidate regulators.

Statistical analysis

All experiments were performed independently at least three times. Data are presented as mean ± standard deviation (SD). Statistical significance was determined using Student's t-test for two-group comparisons or one-way analysis of variance (ANOVA) for multiple-group comparisons. A two-tailed P-value < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism software.

Results

FBXL18 is significantly downregulated in cetuximab-resistant colorectal cancer

To investigate the potential involvement of FBXL18 in cetuximab (CET) resistance in colorectal cancer, its expression was examined in clinical specimens and cell models. Immunohistochemical analysis revealed that FBXL18 expression was markedly reduced in tumors from CET-resistant patients compared with CET-sensitive cases, which was further confirmed by H-score quantification ($P < 0.001$) (Fig. 1A). Consistently, Western blot analysis demonstrated significantly lower FBXL18 protein levels in CET-resistant clinical samples (Fig. 1B). In addition, qRT-PCR analysis showed that FBXL18 mRNA expression was also markedly decreased in resistant tissues, suggesting that its downregulation may occur at the transcriptional level (Fig. 1C). To further validate these findings, CET-resistant sublines were established from KRAS wild-type colorectal cancer cell lines HCT-8 and Caco-2. CCK-8 assays showed that resistant cells exhibited significantly higher viability than parental cells upon CET treatment across a range of concentrations, confirming successful establishment of resistance models (Fig. 1D). Western blot analysis further demonstrated that FBXL18 protein expression was significantly reduced in CET-resistant cells compared with their parental counterparts (Fig. 1E). Collectively, these results demonstrate that FBXL18 is consistently downregulated in both clinical samples and in vitro models of CET resistance, suggesting that loss of FBXL18 may contribute to cetuximab resistance in colorectal cancer and may be regulated at the transcriptional level.

FBXL18 suppresses proliferation and promotes apoptosis, thereby enhancing cetuximab sensitivity in colorectal cancer

To further investigate the functional role of FBXL18 in colorectal cancer, FBXL18 knockdown models were established in HCT-8 and Caco-2 parental cells, while FBXL18 overexpression models were generated in cetuximab-resistant cells (HCT-8/CET-R and Caco-2/CET-R). Western blot analysis confirmed that sh-FBXL18 markedly reduced FBXL18 protein levels, whereas ectopic expression efficiently increased its expression (Fig. 2A). Colony formation assays showed that FBXL18 knockdown significantly increased clonogenic capacity in parental cells, whereas FBXL18 overexpression markedly

reduced colony formation in resistant cells (Figs. 2B–C). EDU incorporation assays further demonstrated that silencing FBXL18 enhanced cell proliferation, while FBXL18 overexpression significantly decreased the percentage of EDU-positive cells, indicating an inhibitory effect on cell growth (Figs. 2D–E). Flow cytometry analysis revealed that FBXL18 knockdown significantly reduced apoptotic rates, whereas FBXL18 overexpression markedly increased apoptosis (Figs. 2F–G). Consistently, Western blot analysis showed that FBXL18 overexpression upregulated the pro-apoptotic protein Bax, while FBXL18 silencing decreased Bax expression (Fig. 2H). Furthermore, in vivo experiments demonstrated that overexpression of FBXL18 significantly suppressed the growth of subcutaneous tumors derived from cetuximab-resistant cells compared with the control group (Fig. 2I). Collectively, these results indicate that FBXL18 functions as a tumor suppressor in colorectal cancer. Loss of FBXL18 promotes proliferation and inhibits apoptosis, whereas restoration of FBXL18 expression reverses resistant phenotypes and enhances sensitivity to cetuximab.

FBXL18 promotes proteasomal degradation of KRAS through ubiquitination and directly interacts with KRAS

TCF3 directly binds to the FBXL18 promoter and transcriptionally activates its expression

To identify potential upstream transcription factors of FBXL18, integrated bioinformatics analyses were performed using FIMO_JASPAR, ENCODE, GTRD, ChIP-Atlas, and hTFtarget databases. Intersection analysis identified several candidate transcription factors, among which TCF3 was selected as a key regulator (Fig. 4A). Western blot analysis showed that silencing TCF3 significantly reduced FBXL18 protein levels in both HCT-8 and Caco-2 cells (Fig. 4B), suggesting that TCF3 positively regulates FBXL18 expression. JASPAR prediction revealed a conserved TCF3-binding motif within the FBXL18 promoter region (Figs. 4C-D). Dual-luciferase reporter assays demonstrated that TCF3 overexpression significantly increased the transcriptional activity of the FBXL18 promoter, with the major regulatory region located within − 1000 bp upstream of the transcription start site (Fig. 4E). Mutation of the predicted binding site markedly attenuated or abolished the TCF3-induced transcriptional activation (Figs. 4F–G), indicating that this site is critical for TCF3-mediated regulation. Immunohistochemical analysis of clinical samples further showed that TCF3 expression was significantly higher in tumors with high FBXL18 expression (Fig. 4H). Collectively, these results demonstrate that TCF3 directly binds to the FBXL18 promoter and transcriptionally activates its expression.

Figure 4. TCF3 transcriptionally activates FBXL18 by binding to its promoter. (A) Intersection analysis of potential transcription factors regulating FBXL18 based on FIMO_JASPAR, ENCODE, GTRD, ChIP-Atlas, and hTFtarget databases, identifying TCF3 as a candidate regulator; (B) Western blot analysis of FBXL18 expression following TCF3 knockdown in HCT-8 and Caco-2 cells, with quantitative analysis; (C) Predicted TCF3-binding motif obtained from the JASPAR database; (D) Predicted TCF3-binding sites within the FBXL18 promoter region; (E) Dual-luciferase reporter assays using truncated FBXL18

promoter fragments to evaluate transcriptional activation by TCF3; (F) Schematic illustration of wild-type (WT) and mutant (MUT) TCF3-binding site constructs within the FBXL18 promoter; (G) Luciferase assays assessing the regulatory effect of TCF3 on WT and MUT promoter activity; (H) Immunohistochemical staining of TCF3 in clinical samples with low or high FBXL18 expression, followed by H-score analysis. Data are presented as mean ± SD (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant. All original gels are presented in Supplementary Fig. 4.

Discussion

Resistance to anti-EGFR therapy remains a major obstacle in the treatment of metastatic colorectal cancer. Although RAS mutation status is currently used to guide cetuximab administration, a substantial proportion of patients with RAS wild-type tumors still develop primary or acquired resistance, indicating the presence of alternative regulatory mechanisms affecting EGFR signaling[10,11]. In the present study, we identified a novel regulatory axis involving TCF3, FBXL18, and KRAS that contributes to cetuximab resistance in colorectal cancer.

Our results demonstrated that FBXL18 was significantly downregulated in cetuximab-resistant colorectal cancer tissues and cell models. Functional experiments further showed that FBXL18 suppresses cell proliferation, promotes apoptosis, and enhances sensitivity to cetuximab, suggesting that FBXL18 functions as a tumor suppressor in this context. F-box proteins serve as substrate-recognition components of the SCF E3 ubiquitin ligase complex and play critical roles in cancer progression by regulating the stability of key oncogenic proteins[12]. However, the role of FBXL18 in colorectal cancer and targeted therapy resistance has not been previously reported.

One of the major findings of this study is that FBXL18 regulates KRAS protein stability through the ubiquitin–proteasome pathway. Aberrant activation of KRAS signaling is a central driver of resistance to EGFR-targeted therapy[13]. While KRAS mutations are well-characterized, increasing evidence suggests that post-translational mechanisms regulating KRAS stability also contribute to therapeutic resistance[14]. Here, we demonstrated that FBXL18 directly interacts with KRAS, promotes its ubiquitination, and accelerates its proteasomal degradation. These findings reveal a previously unrecognized mechanism controlling KRAS protein turnover and suggest that loss of FBXL18 may lead to KRAS accumulation and sustained downstream signaling, thereby conferring resistance to cetuximab.

In addition to post-translational regulation, transcriptional control of E3 ligases represents another important layer of regulation. Through integrated bioinformatics analysis and experimental validation, we identified TCF3 as a transcriptional activator of FBXL18. TCF3 knockdown reduced FBXL18 expression, whereas luciferase assays confirmed direct binding of TCF3 to the FBXL18 promoter. TCF3 is a member of the basic helix–loop–helix transcription factor family and has been implicated in tumor progression, stemness, and therapy resistance in multiple cancers[8,15]. Our findings expand the functional network of TCF3 and identify the TCF3–FBXL18 axis as a critical upstream regulatory pathway controlling KRAS stability.

From a clinical perspective, this study provides several potential implications. First, FBXL18 expression may serve as a predictive biomarker for cetuximab response in colorectal cancer. Second, therapeutic strategies aimed at restoring FBXL18 expression or enhancing KRAS degradation may improve the efficacy of anti-EGFR therapy. More broadly, targeting protein stability through the ubiquitin–proteasome system represents a promising approach for overcoming resistance to targeted therapies[16].

Several limitations should be noted. The clinical sample size was relatively limited, and larger cohort studies are needed to validate the prognostic and predictive value of FBXL18. In addition, although our data support a role for the TCF3–FBXL18–KRAS axis in cetuximab resistance, the downstream signaling alterations and potential interactions with other resistance pathways warrant further investigation

Conclusion

In summary, our study identifies FBXL18 as a novel tumor suppressor that is downregulated in cetuximab-resistant colorectal cancer. FBXL18 promotes ubiquitin-mediated proteasomal degradation of KRAS, thereby suppressing oncogenic signaling and enhancing cetuximab sensitivity. Mechanistically, TCF3 directly binds to the FBXL18 promoter and transcriptionally activates its expression. Loss of the TCF3–FBXL18 axis leads to KRAS stabilization and contributes to therapeutic resistance. These findings reveal a previously unrecognized transcriptional–posttranslational regulatory network controlling KRAS signaling and provide a potential biomarker and therapeutic target for overcoming anti-EGFR resistance in colorectal cancer.

Declarations

Funding

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Data and material availability

All data will be available on request.

Declaration of competing interest

All authors declare that they have no conflict of interest.

Author contributions

Li Jian and Zhijing Zhang conceived the study, designed and conducted the experiments, and wrote the initial draft of the manuscript. Yudong Li and Yao Tian performed data analysis, interpreted the results, and contributed to manuscript writing. Xin Deng assisted with data interpretation and manuscript revision and obtained funding. Yixiang Wen assisted with experimental work and data collection. Xiaowen Tan assisted with specimen and data collection. Qi Qi, Zhen Bao, Jin Gong, Peng Ouyang and Guoan Xiang share corresponding authorship. They supervised the project, provided critical feedback, obtained funding and were responsible for the final approval of the manuscript. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Ethics declaration

Informed consent was obtained from all participants, and the study was approved by the Ethics Committee of First Affiliated Hospital of Gannan Medical University (approval number: LLSC-2025333). This study adhered to the principles of the Declaration of Helsinki.

Animals were housed in a pathogen-free environment, and all experiments conformed to ethical principles and guidelines approved by Institutional Animal Care and Use Committee (IACUC) of the First Affiliated Hospital of Gannan Medical University (Approval No. LLSC-2026007).

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Competing interests

The authors declare that they have no competing interests.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

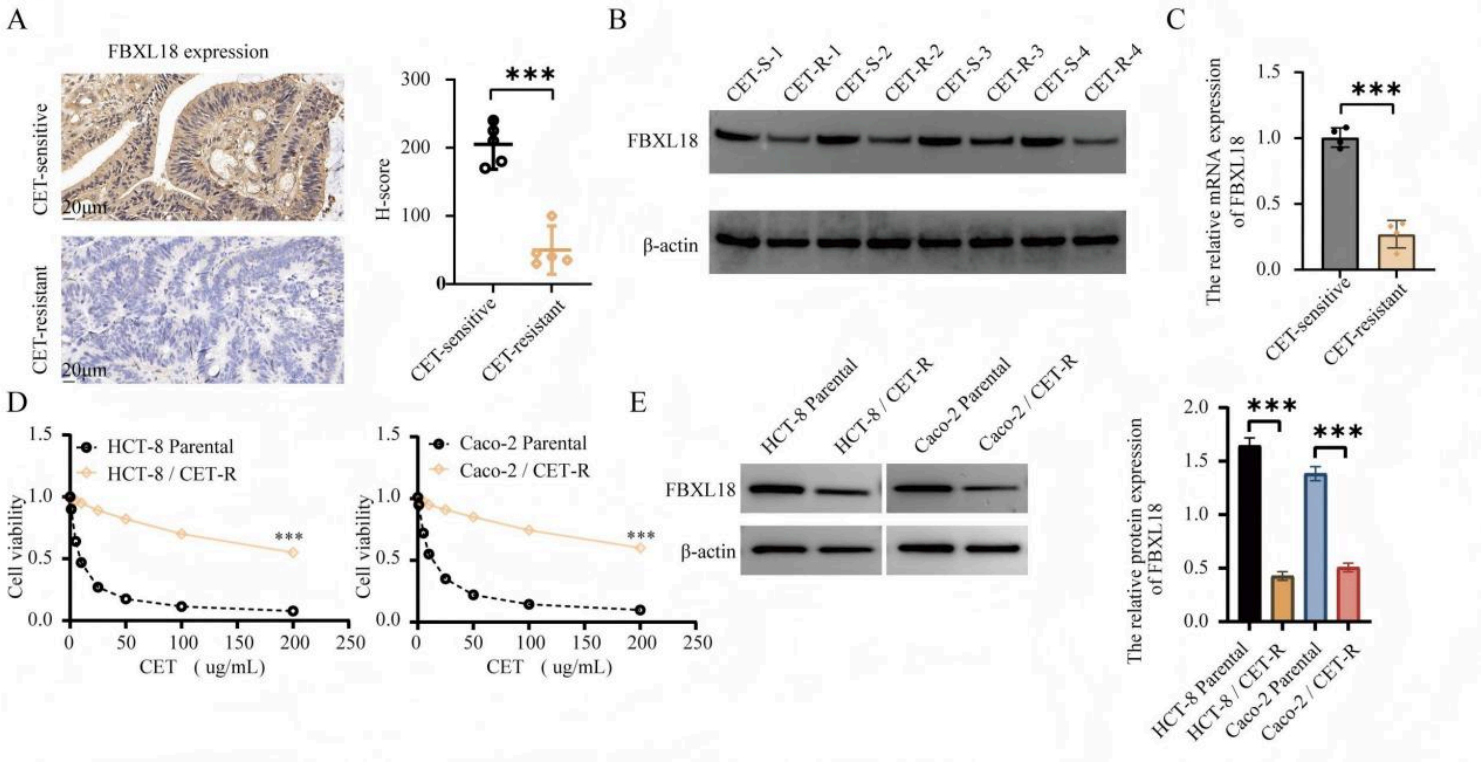


Figure 1

Downregulation of FBXL18 in cetuximab-resistant colorectal cancer. (A) Representative immunohistochemical staining and H-score quantification of FBXL18 expression in CET-sensitive and CET-resistant colorectal cancer tissues; (B) Western blot analysis of FBXL18 protein levels in CET-sensitive and CET-resistant patient samples; (C) qRT-PCR analysis of FBXL18 mRNA expression in CET-sensitive and CET-resistant tissues; (D) Cell viability of parental and CET-resistant HCT-8 and Caco-2 cells treated with increasing concentrations of cetuximab, assessed by CCK-8 assay; (E) Western blot analysis and quantification of FBXL18 protein expression in parental and CET-resistant cell lines. Data are presented as mean ± SD. ***$P < 0.001$. All original gels are presented in Supplementary Figure 1.

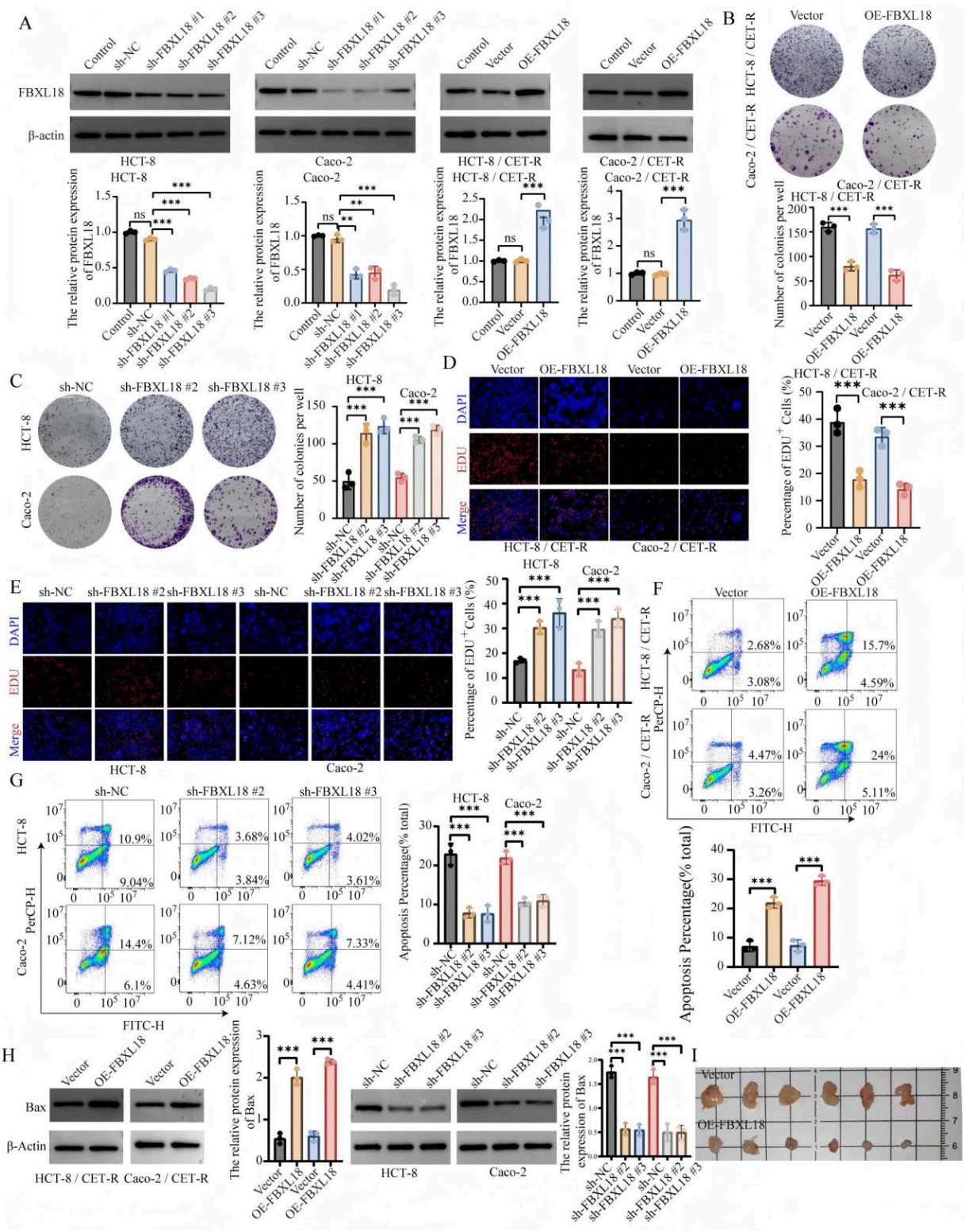


Figure 2

FBXL18 suppresses proliferation and promotes apoptosis, thereby enhancing cetuximab sensitivity in colorectal cancer. (A) FBXL18 knockdown was established in parental HCT-8 and Caco-2 cells, and FBXL18 overexpression was generated in cetuximab-resistant HCT-8/CET-R and Caco-2/CET-R cells. FBXL18 protein levels were detected by Western blot and quantified; (B–C) Colony formation assays were performed to evaluate the effects of FBXL18 knockdown or overexpression on clonogenic capacity,

followed by quantification of colony numbers; (D–E) EDU incorporation assays were conducted to assess the role of FBXL18 in regulating cell proliferation, and the percentage of EDU-positive cells was quantified; (F–G) Cell apoptosis was analyzed by Annexin V/PI staining followed by flow cytometry, and apoptotic rates were quantified; (H) Western blot analysis of the pro-apoptotic protein Bax and its quantitative analysis; (I) Subcutaneous xenograft assays were performed to evaluate the effect of FBXL18 overexpression on tumor growth derived from cetuximab-resistant cells in vivo. Representative tumor images are shown. Data are presented as mean ± SD (n = 3). ***P* < 0.01, ****P*< 0.001; ns, not significant. All original gels are presented in Supplementary Figure 2.

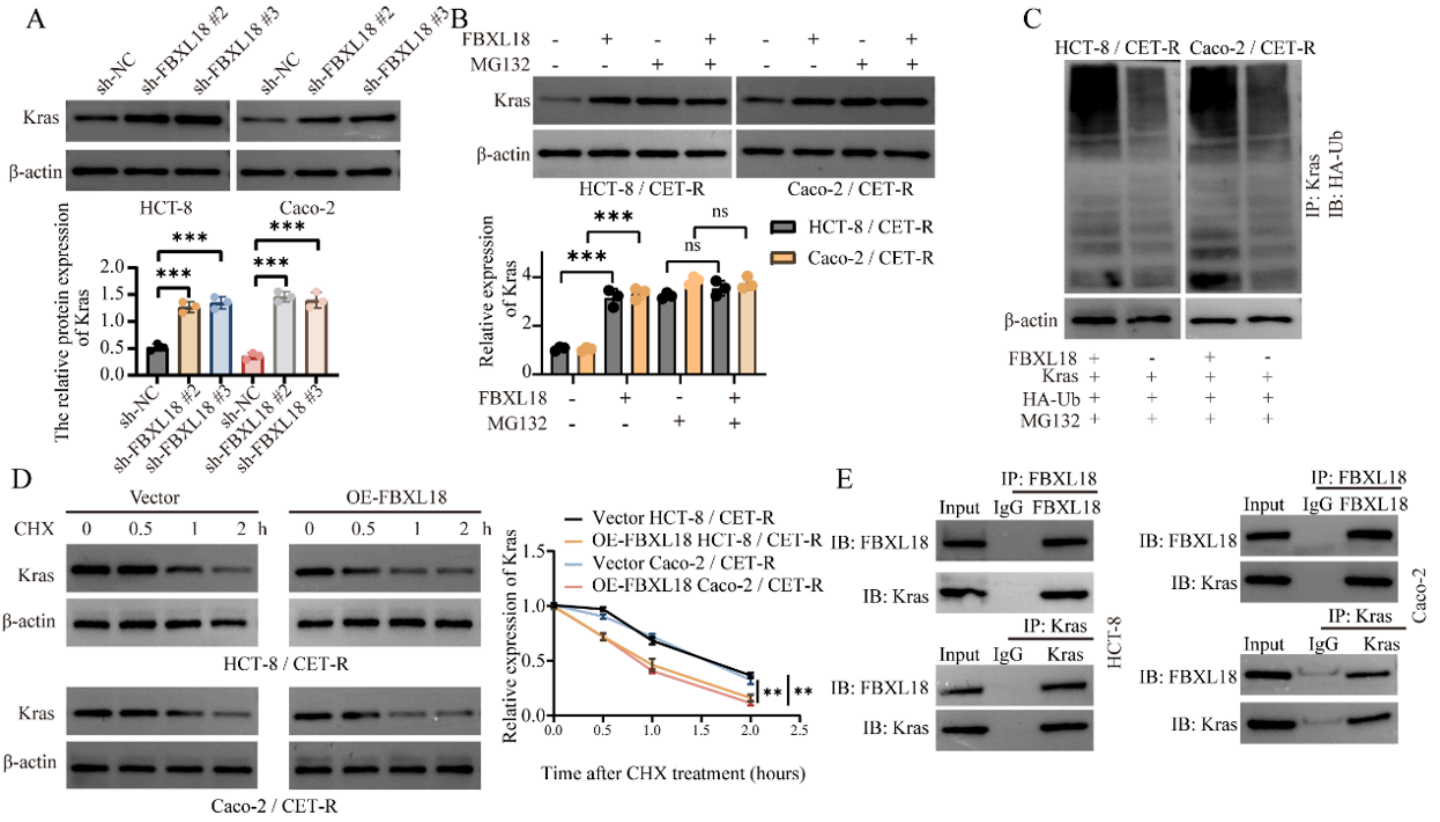


Figure 3

FBXL18 promotes ubiquitin-mediated proteasomal degradation of KRAS. (A) KRAS protein levels were examined by Western blot after FBXL18 knockdown in HCT-8 and Caco-2 cells, followed by quantitative analysis; (B) FBXL18 was overexpressed in HCT-8/CET-R and Caco-2/CET-R cells with or without treatment with the proteasome inhibitor MG132. KRAS expression was analyzed by Western blot and quantified; (C) In vivo ubiquitination assays showing the effect of FBXL18 overexpression on KRAS ubiquitination (IP: KRAS; IB: HA-Ub); (D) Cycloheximide (CHX) chase assays were performed to evaluate the effect of FBXL18 overexpression on KRAS protein stability, followed by quantification of degradation kinetics; (E) Co-immunoprecipitation (Co-IP) assays demonstrating the interaction between FBXL18 and KRAS (IP: FBXL18 or KRAS; IB: KRAS or FBXL18). Data are presented as mean ± SD (n = 3). ***P* < 0.01, ****P*< 0.001; ns, not significant. All original gels are presented in Supplementary Figure 3.

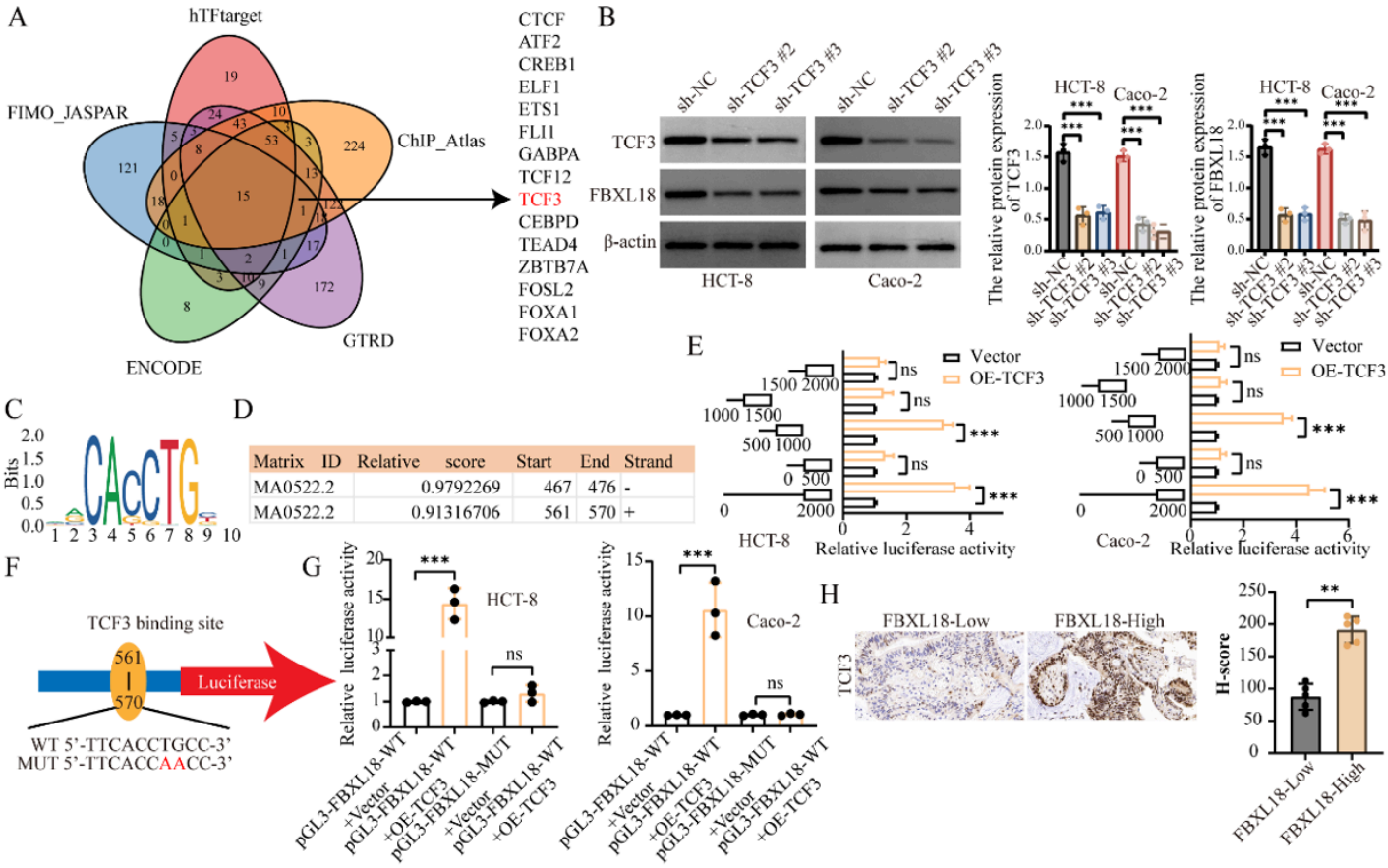


Figure 4

TCF3 transcriptionally activates FBXL18 by binding to its promoter. (A) Intersection analysis of potential transcription factors regulating FBXL18 based on FIMO_JASPAR, ENCODE, GTRD, ChIP-Atlas, and hTFtarget databases, identifying TCF3 as a candidate regulator; (B) Western blot analysis of FBXL18 expression following TCF3 knockdown in HCT-8 and Caco-2 cells, with quantitative analysis; (C) Predicted TCF3-binding motif obtained from the JASPAR database; (D) Predicted TCF3-binding sites within the FBXL18 promoter region; (E) Dual-luciferase reporter assays using truncated FBXL18 promoter fragments to evaluate transcriptional activation by TCF3; (F) Schematic illustration of wild-type (WT) and mutant (MUT) TCF3-binding site constructs within the FBXL18 promoter; (G) Luciferase assays assessing the regulatory effect of TCF3 on WT and MUT promoter activity; (H) Immunohistochemical staining of TCF3 in clinical samples with low or high FBXL18 expression, followed by H-score analysis. Data are presented as mean ± SD (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant. All original gels are presented in Supplementary Figure 4.

Supplementary Files

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- SupplementaryFigure1.OriginaluncroppedWesternblotsforFigure1.pdf

- SupplementaryFigure2.OriginaluncroppedWesternblotsforFigure2.pdf
- SupplementaryFigure3.OriginaluncroppedWesternblotsforFigure3.pdf
- SupplementaryFigure4.OriginaluncroppedWesternblotsforFigure4.pdf