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Fc fragment of IgG binding protein suppresses tumor growth by stabilizing wild type P53 in colorectal cancer cells

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Abstract

The Fc fragment of IgG binding protein (FCGBP) exhibits differential expression across various tumor types. but its role in cancer progression remains underexplored. This research discovered that FCGBP is downregulated in colorectal cancer (CRC) cells and is negatively associated with poor prognosis. Overexpression of FCGBP inhibited the growth of P53 wild-type CRC cells both in vitro and in vivo. Mechanistically, immunoprecipitation experiments revealed that FCGBP competitively binds to MDM2, thereby attenuating the formation of the P53/MDM2 complex. This, in turn, reduces P53 ubiquitination and stabilizes the protein. Our findings reveal a novel mechanism through which FCGBP significantly inhibits CRC cell growth and propose a new targeted therapeutic strategy for CRC treatment.

Keywords Fc fragment of IgG binding protein, Colorectal cancer, Wild type P53, Ubiquitination, MDM2

Introduction

Colorectal Cancer (CRC) is a leading cause of cancer-related death worldwide. Its high incidence, mortality, and poor prognosis pose significant public health challenges [1]. In China, CRC rates remain elevated, with a distinct upward trend [2, 3]. While several mechanisms underlying CRC initiation have been identified, the prognosis remains unfavorable due to its high malignancy. Therefore, a deeper understanding of the molecular mechanisms driving CRC could facilitate the development of novel and effective therapeutic strategies.

The Fc fragment of IgG binding protein (FCGBP) is a mucin initially discovered in the intestinal epithelium, where it plays a critical role in innate mucosal defense. Recent studies have highlighted its involvement in the development and progression of various cancers. FCGBP has been reported to be highly expressed in glioblastoma and associated with poor outcomes in ovarian cancer [4, 5]. Surprisingly, FCGBP expression is downregulated in gallbladder carcinoma [6]. However, many aspects of FCGBP's role in the biological context of CRC remain poorly understood.

This study demonstrates a significant reduction in FCGBP expression in CRC, which correlates with unfavorable clinical outcomes. Furthermore, cell-based assays and tumor-bearing experiments revealed that FCGBP inhibits CRC cell growth. Additionally, we identified an interaction between FCGBP and MDM2, in which FCGBP stabilizes P53 by preventing its ubiquitination by MDM2. These findings offer novel insights into

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the mechanisms by which FCGBP contributes to CRC development.

Materials and methods

General

All methods were carried out in accordance with ARRIVE guidelines and regulations.

Cells culture

CRC cell lines were all purchased from Procell Life Science & Technology Co., Ltd. Wuhan, China. HCT16 and HT29 cells were maintained in McCoy'5 A (Meilunbio, Dalian, China). LOVO, CT26 and NCM460 cells were maintained in RPMI 1640 (Meilunbio). RKO cells were maintained MEM (Meilunbio). SW620 and SW480 cells were maintained in L15 (Meilunbio). These mediums were then added with 10% FBS (Biological Industries, Israel) and cultured at 37 ℃ under the humidified atmosphere containing 5% CO_2 (SW620 was cultivated in a CO_2 free environment).

RNA interference

For siRNA-mediated silencing experiments, FCGBP SiRNA and P53 SiRNA were purchased from Origene. The SiRNAs were transfected into cells using RNAimax according to manufacturer's instructions.

Apoptosis assay

cells (3×10^5 per well) were seeded in 6-well plates. After cultivation in different times, the cells were digested with trypsin-EDTA and washed twice with 1× PBS. Analysis of apoptotic cells was performed in FL-1 and FL-2 channels of flow cytometry (BD) following by Annexin V-FITC and PI staining (BD).

Cell cycle analysis

The cell cycle experiments were conducted by using the beyotime kits (C1052). The required cells were collected, washed with 1× PBS, filtrated, stained by PI. The cells were then incubated for 30-minute at 37 °C in the dark. The flow cytometry experiments was then performed.

MTT assay

cells (1×10^3 per well) were seeded the 96-well plates and cultured in of 100 μL McCoy'5 A or RPMI 1640 containing 10% FBS for different times. The mediums were treated with 10% aqueous solution of MTT. The plates were stored at 37 °C for 4 h. DMSO was added into each well and the plates were vibrated for 30 min at ambient temperature. The absorbance at 490 nm was assessed through spectrophotometry for the evaluations.

Colony formation assay

The cells (1×10^3 per well) were seeded in 6-well plates. Following two-week period incubation, the cells were collected and rinsed twice with PBS. And the cells were then fixed by using 4% PFA for 15 min. The 0.1% aqueous solution of crystal violet (Solarbio, Beijing, China) was added into each well and the plates were vibrated for 15 min. The photographic records were used for further evaluations.

Western blotting and antibody

The protein lysates were resolved by SDS-PAGE gels and then transferred on a PVDF membrane. The specific primary antibodies were incubated at 4 °C overnight, including anti-FLAG (Abcam), anti-FCGBP (Abcam), anti-P21 (Proteintech), anti-CyclinD1 (Proteintech), anti-Cleaved-PARP1 (Abcam), anti-Cleaved-Caspas3 (Proteintech), anti-P53 (Abcam), anti-γH2AX (Santa Cruz

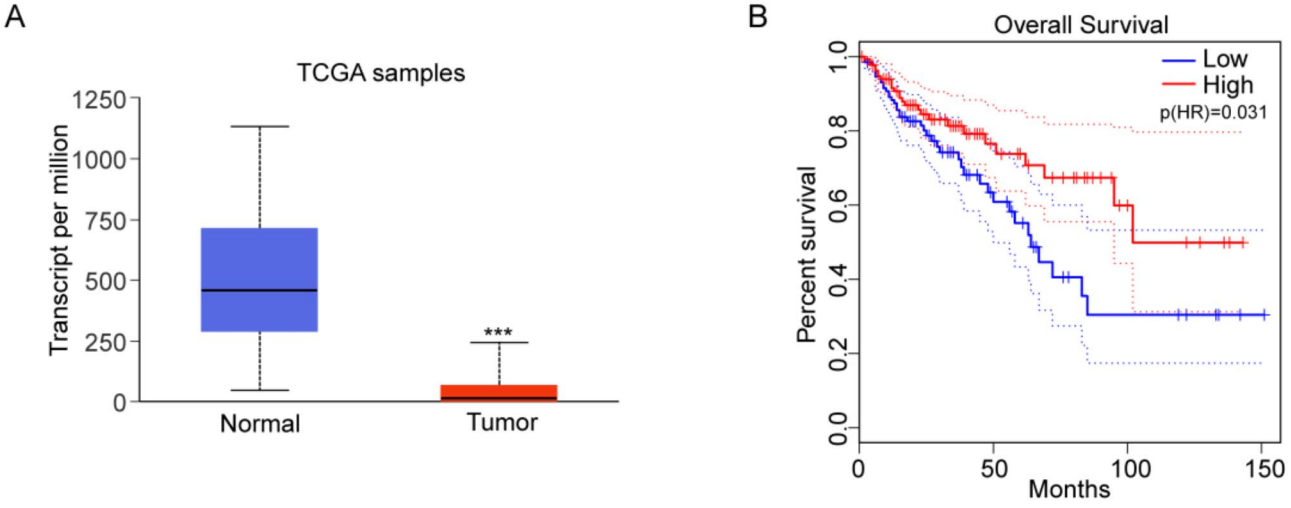

Fig. 1 FCGBP expression in CRC. (**A**) Relative mRNA levels of FCGBP in CRC compared to healthy tissues in TCGA datasets. (**B**) Kaplan-Meier analysis of OS among CRC patients with different levels of FCGBP expression (http://gepia2.cancer-pku.cn/#similar)

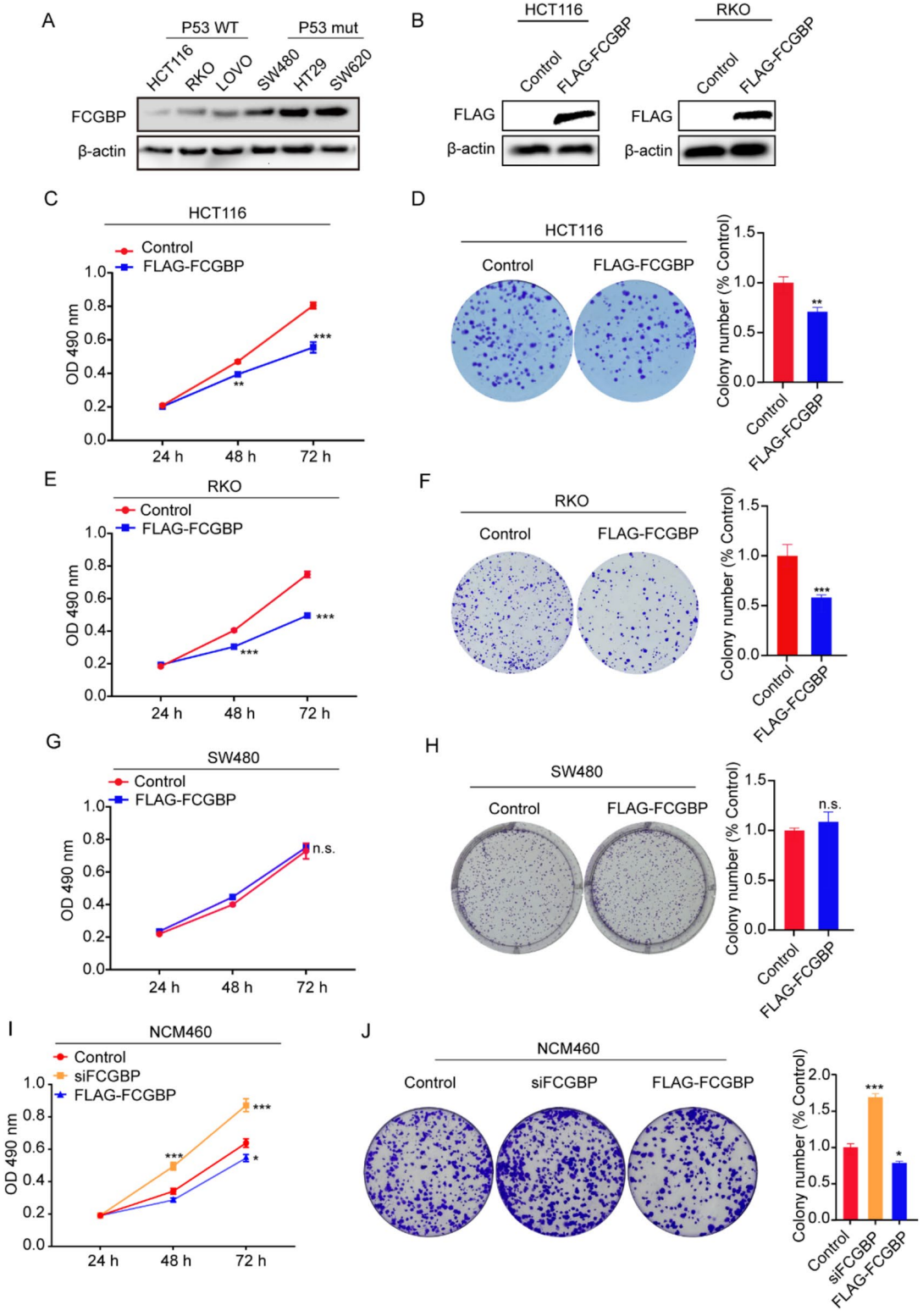

Fig. 2 FCGBP suppresses the proliferation of P53^{wt} CRC cells in vitro. (**A**) Western blot analysis of FCGBP expression in P53^{wt} and mutant CRC cells. (**B**) Cell lysates from HCT116 and RKO cells stably expressing either empty vector or FLAG-FCGBP were subjected to Western blotting. (**C**, **E**, **G**, **I**) MTT assays were performed in HCT116, RKO, SW480, and NCM460 cells with differential FCGBP expression. (**D**, **F**, **H**, **J**) Colony formation assays of the indicated cells. Data are presented as mean ± standard deviation (SD), $n = 3$. ***$p < 0.001$, **$p < 0.01$

Biotechnology), anti-MDM2 (Cell Signaling Technology) and anti-β-actin (Proteintech, to be used for control). The

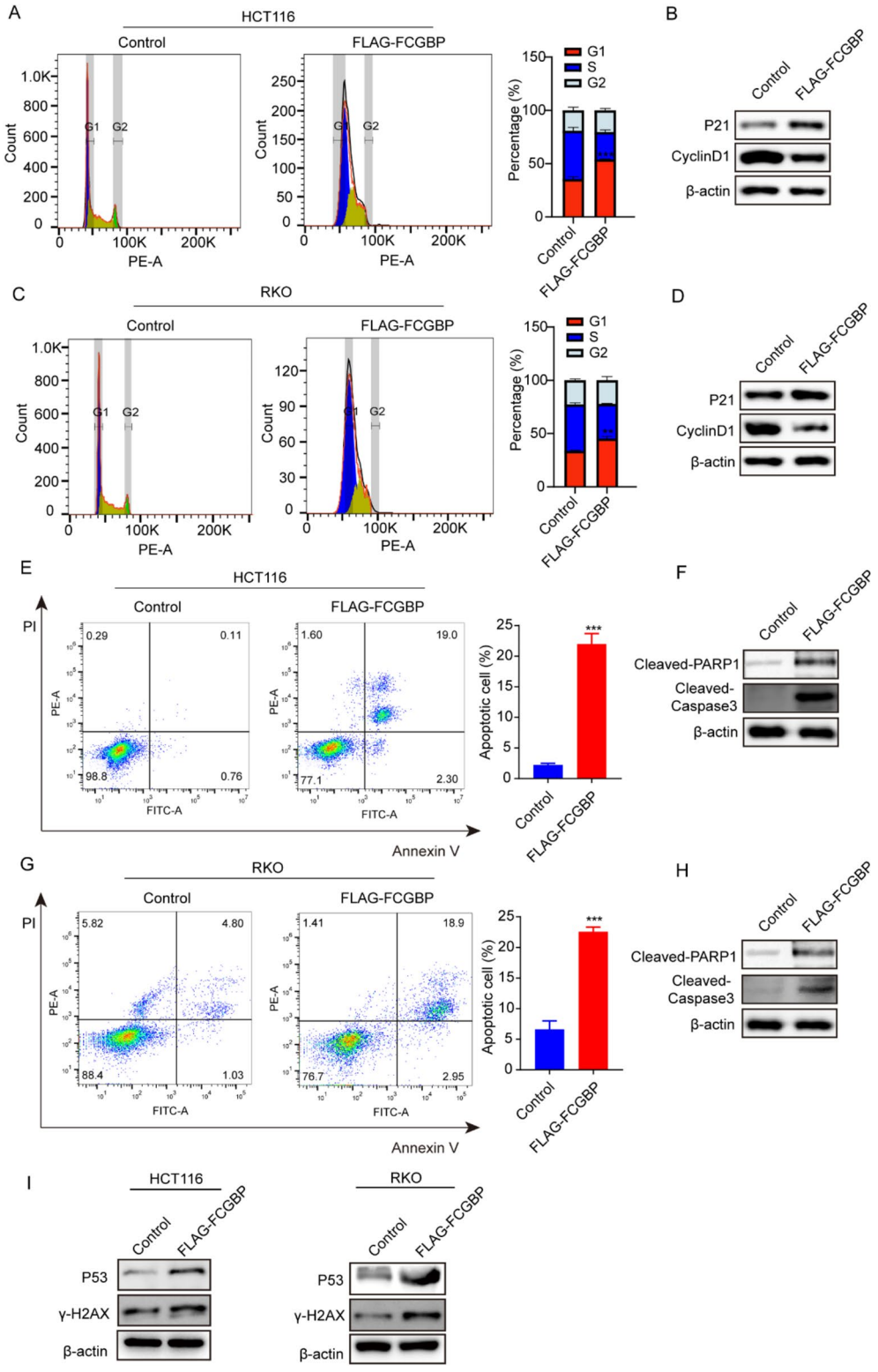

Fig. 3 FCGBP enhances apoptosis of CRC cells in vitro. (**A** and **C**) Flow cytometry analysis of DNA content in HCT116 and RKO cells following staining with propidium iodide. Data are presented as mean ± SD for $n = 3$; $^{**}p < 0.01$, $^{***}p < 0.001$ (Student's t test). (**B** and **D**) Expression of P21 and Cyclin D1 detected by by Western blotting in selected cells. (**E** and **G**) Control cells or cells with FCGBP overexpression were subjected to flow cytometry assays. Data are presented as mean ± SD for $n = 3$; $^{***}p < 0.001$ (Student's t test). (**F** and **H**) Cleaved caspase-3 and cleaved PARP1 were assessed by Western blotting. β-actin was used as a reference control. (**I**) Western blot analysis of P53 expression in HCT116 and RKO cells infected with control or FLAG-FCGBP

membranes were hybridized with a secondary antibody (KPL) at room temperature for 2 h. The protein bands were visualized by Omni-ECL™ chemiluminescence detection kit (epizyme).

Mouse xenograft models

The mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Injected CT26 (1×10^6), HCT116 cells (5×10^6) expressing either FLAG-FCGBP or control were subcutaneously into female nude mice (BALB/c; 6–8 weeks; 5 mice/set). The tumor volumes were monitored, calculated and recorded every three days ($V = 1/2 \times \text{length} \times \text{width}^2$). After 16 or 28 days, these mice were euthanized with CO_2. The tumors tissues were collected and photographed. All animal experimental protocols and ethics were approved by Tianjin Medical University Cancer Institute & Hospital.

Immunoprecipitation

The required cells were collected and treated with IP buffer at 4℃ for half an hour. The cell lysis were collected by vortex at 4℃ at 12,000 rpm for 15 min. The suspensions were incubated with antibodies at 4℃ overnight. The protein A/G agarose beads were added and incubated (Bimake, USA) at 4℃ for 2 h. The pellets were collected by rinsing with IP buffer at 0℃ for three times. WB analysis were then proceeded.

Results

FCGBP is downregulated in CRC tissues

We first analyzed RNA-seq data from the TCGA database to assess FCGBP expression in CRC tissues. The results showed a significant decrease in the mRNA level of FCGBP in CRC tissues compared to healthy colorectal tissues (Fig. 1A). Subsequently, utilizing the GEPIA2 database, we performed Kaplan-Meier survival analysis, demonstrating that CRC patients with low FCGBP expression had poorer overall survival (OS) (Fig. 1B). These findings suggest a reduced expression of FCGBP in CRC, which correlates with a worse prognosis for patients. This implies that FCGBP plays a critical role in CRC progression.

FCGBP inhibits the growth of P53 wild-type CRC cells in vitro

Previous studies have highlighted a significant association between the expression of wild-type P53 and FCGBP [7]. To further investigate this, we performed western blotting to assess FCGBP expression in P53 wild-type ($P53^{wt}$) and P53 mutant ($P53^{mut}$) CRC cells. The results revealed low FCGBP expression in P53 wild-type cells, but significantly increased expression in P53 mutant cells (Fig. 2A). To evaluate the effect of FCGBP on CRC cell growth, we engineered lentiviral vectors expressing either control or FLAG-FCGBP and transfected them into HCT116 and RKO cells. Stable cell lines were established, and expression levels were confirmed (Fig. 2B). MTT assays showed a significant inhibition of cell survival in HCT116 and RKO cells upon FCGBP overexpression compared to the control (Fig. 2C and E). Additionally, colony formation assays confirmed the suppressive effect of FCGBP overexpression on cell proliferation (Fig. 2D and F). To assess the impact of FCGBP in $P53^{mut}$ CRC cells, MTT and colony formation assays were also performed on SW480 cells, and the results showed that FCGBP overexpression did not inhibit cell proliferation in these cells (Fig. 2G-H). To investigate the role of FCGBP in normal colonic epithelial cells, MTT and clonogenic assays were conducted following knocking down and overexpression in NCM460 cells. The results showed that FCGBP knockdown enhanced cell proliferation and clonogenic capacity, whereas FCGBP overexpression inhibited cell proliferation to a certain extent (Fig. 2I-J). Collectively, these findings indicate that under in vitro conditions, FCGBP inhibits the proliferation of $P53^{wt}$ CRC cells.

FCGBP promotes apoptosis of CRC cells in vitro

To evaluate the effect of FCGBP on the cancer cell cycle, we first performed flow cytometry. The results showed that overexpression of FCGBP induced G1 phase arrest in HCT116 and RKO cells compared to the control (Fig. 3A and C). Cell cycle markers were detected by western blotting (Fig. 3B, D). Flow cytometry analysis was conducted on HCT116 and RKO cells stably expressing control or FLAG-FCGBP to assess the impact of overexpression on apoptosis. The findings indicated a significant increase in cell apoptosis upon overexpression of FCGBP (Fig. 3E-H). Additionally, immunoblotting experiments were conducted to examine the association between FCGBP and P53. The results showed that in FCGBP-overexpressing cell lines, the expression of P53 was significantly elevated. Concurrently, the expression of γ-H2AX, a marker involved in DNA damage repair along with P53, was also increased (Fig. 3I).

FCGBP stabilizes wild-type P53 through inhibiting MDM2

Numerous reports have shown that MDM2 downregulates P53 levels by increasing P53 ubiquitination [8, 9]. To determine whether FCGBP interferes with this function and stabilizes P53, we conducted gene correlation analysis, which revealed a positive correlation between FCGBP and MDM2 in $P53^{wt}$ CRC cells (Fig. 4A). Additionally, total proteins were extracted from HCT116 cells for immunoprecipitation (IP) assays, which revealed an interaction between FCGBP and MDM2 in these cells (Fig. 4B and C). To further support the role of FCGBP in regulating P53, anti-MDM2 co-immunoprecipitation (Co-IP) assays were performed in cells expressing

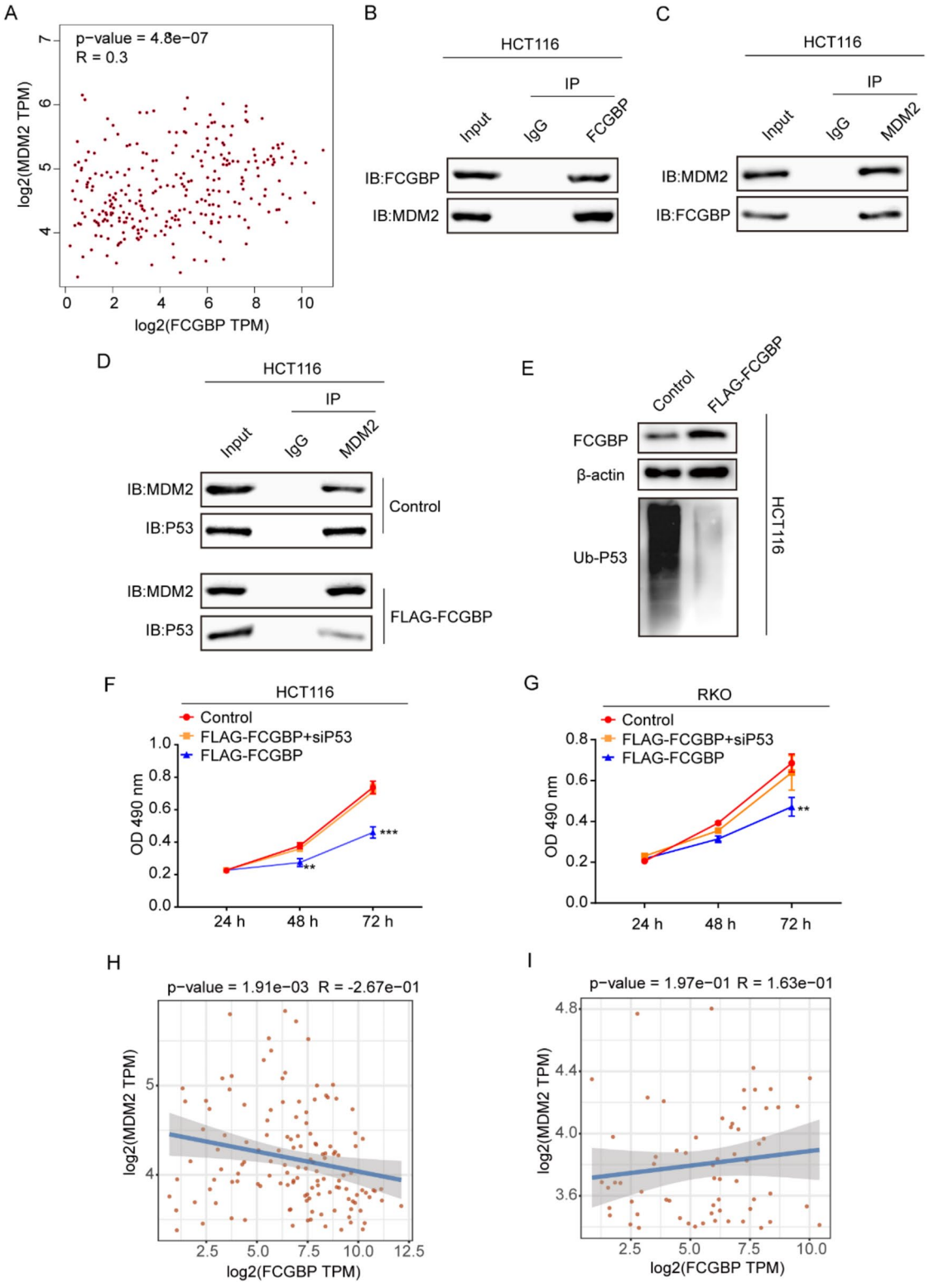

Fig. 4 FCGBP enhances the stability of wild-type P53. (**A**) Spearman correlation between FCGBP and MDM2 mRNA expression in CRC. R: Pearson's correlation coefficient. (**B**-**C**) Immunoprecipitation (IP) experiments were conducted using anti-FCGBP or anti-MDM2, followed by western blot analysis on HCT116 cells. (**D**) IP experiments were performed using anti-FLAG, followed by western blot analysis on HCT116 cells. (**E**) Cell lysates from HCT116 cells under control conditions and those expressing FLAG-FCGBP were subjected to western blotting. (**F**-**G**) MTT assays of the indicated cells. The TCGA database analyzed the association between MDM2 and FCGBP in samples of colorectal cancer patients with $MDM2^{high}/P53^{wt}$ (**H**) or $MDM2^{high}/P53^{mut}$ (**I**). Data are presented as mean ± SD, $n = 3$. ***$p < 0.001$, **$p < 0.01$

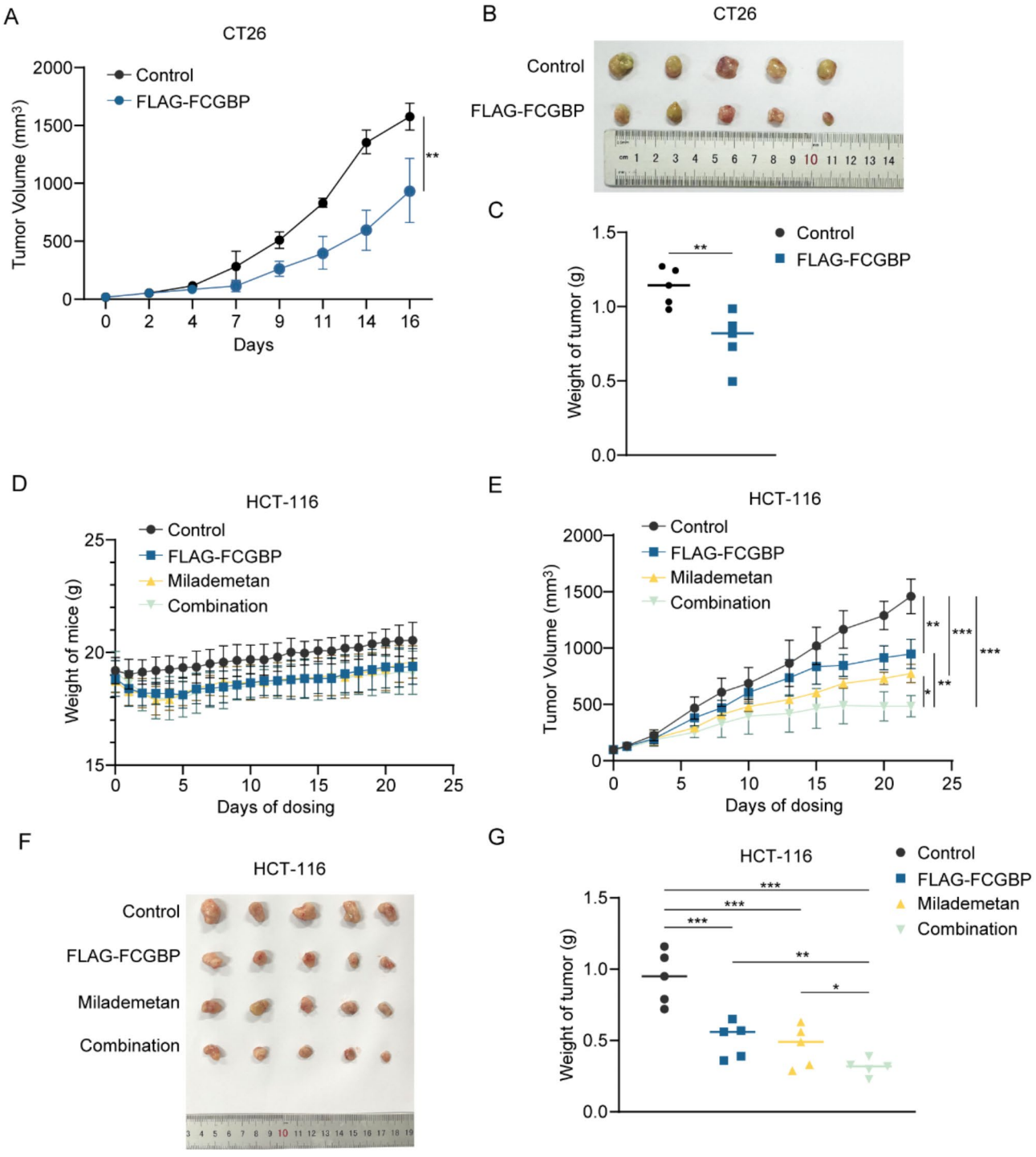

Fig. 5 Overexpression of FCGBP combined with MDM2 inhibitors suppressed CRC growth in vivo. CT26 cells expressing control or Flag-FCGBP were subcutaneously transplanted into nude mice. Tumors were excised 16 days later and photographed (**B**). Tumor volume (**A**) and mass (**C**) were measured. HCT116 tumor alterations in body weight (**A**) and tumor size (**B**). (**C**-**D**) Tumor tissue weights from the four groups on the terminated days. Data are presented as mean ± SD, $n = 5$. ***$p < 0.001$, **$p < 0.01$

control or FLAG-FCGBP. We observed that increased FCGBP expression suppressed the interaction between MDM2 and P53 (Fig. 4D). Western blotting analysis demonstrated that FCGBP overexpression reduced the ubiquitination level of P53 (Fig. 4E). Furthermore, rescue experiments using MTT assays revealed that knocking down P53 counteracted the proliferation-inhibitory effect of FCGBP in both HCT116 and RKO cells (Fig. 4F and

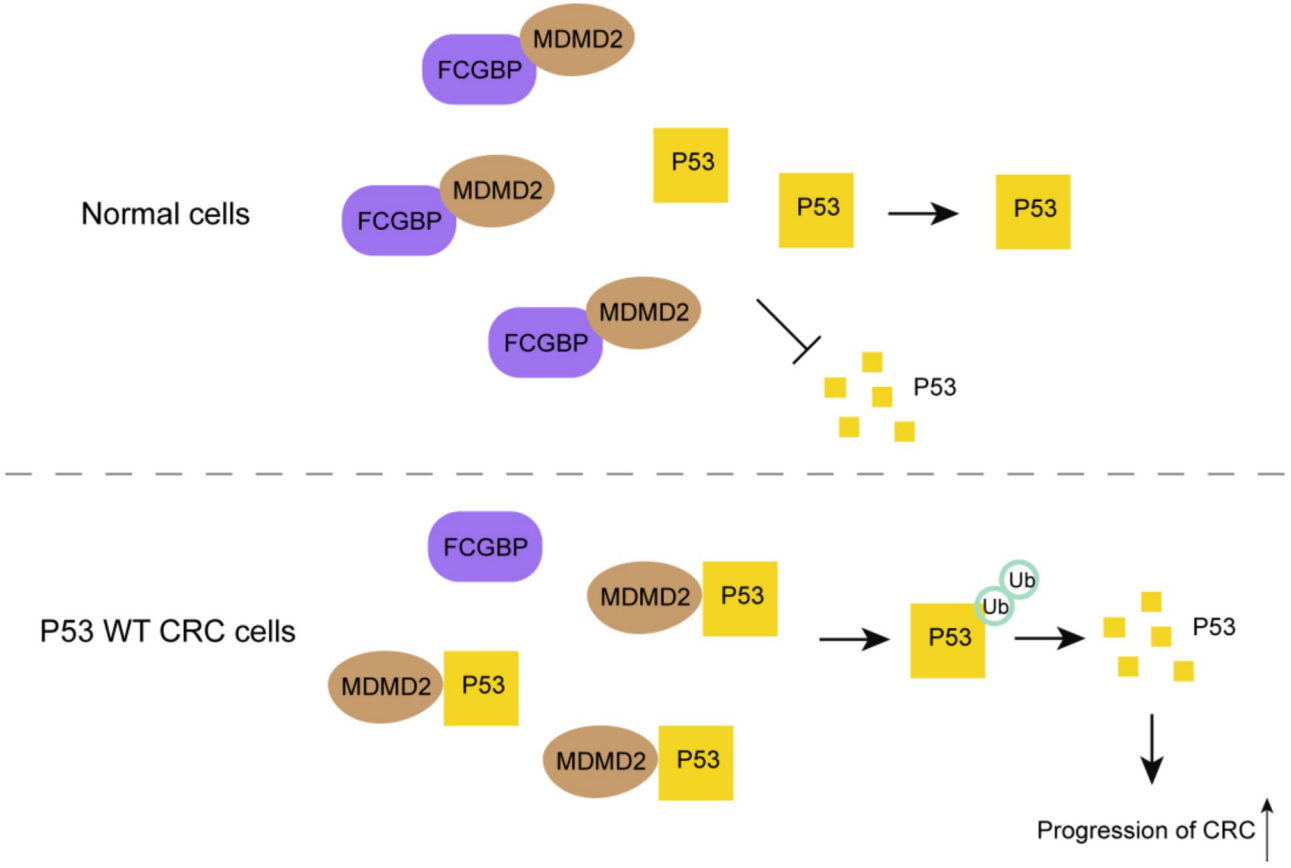

Fig. 6 Schematic representation of the mechanism by which decreased FCGBP expression promotes CRC progression

G). In addition, correlation analysis was performed in samples with MDM2^{high}/TP53^{wt} and MDM2^{high}/TP53^{mut} of colorectal cancer patients from the TCGA database (https://www.aclbi.com/static/index.html#/). The results indicated that there was a significant negative correlation between FCGBP and MDM2 in MDM2^{high}/TP53^{wt} specimens (Fig. 4H). However, none significant correlations were indicated in MDM2^{high}/TP53^{mut} patients (Fig. 4I). Collectively, the above findings demonstrated that FCGBP might exert anti-cancer effects by stabilizing wild-type P53 by disrupting the interaction between MDM2 and P53.

Overexpression of FCGBP combined with MDM2 inhibitors inhibits CRC growth in vivo

To eliminate potential interactions related to MSS/MSI status, we performed in vivo animal experiments using CRC cells with P53^{wt}. Firstly, FCGBP-overexpressing CT26 cells and control cells were subcutaneously injected into 6-week-old nude mice, and tumor size was measured every three days. After 16 days, the mice were euthanized, and the tumors were excised for weighing and imaging. The results showed that the volume and weight of tumors in the FCGBP overexpression group were significantly reduced compared to the control group (Fig. 5A-C). In addition, we used a subcutaneous tumor xenograft model to assess the impact of FCGBP overexpression combined with MDM2 inhibitors on CRC growth. Both overexpression and control cells were injected into nude mice. Once the tumors reached a volume of 80–100 mm^3, they were randomly assigned to receive treatment with MDM2 inhibitors. After 23 d, the mice were euthanized, and the tumors were excised for measurement and imaging. The results revealed that the body weight of the mice remained within a normal range throughout the experiment (Fig. 5D). Compared to the control group, both the volume and weight of tumors in the FCGBP overexpression and MDM2 inhibitor groups were significantly reduced. Notably, the combination of FCGBP overexpression and MDM2 inhibitors resulted in superior tumor suppression (Fig. 5E and G). These findings suggest that, consistent with the in vitro data, FCGBP overexpression suppresses CRC growth in vivo. Taken together, our study indicates that FCGBP expression is decreased in P53^{wt} CRC cells, reducing its competitive binding with MDM2, which promotes P53 degradation and cancer progression (Fig. 6).

Discussion

This research analyzed TCGA data and identified a downregulation of FCGBP in CRC. This reduced expression was associated with unfavorable outcomes in CRC, suggesting an anti-cancer role for FCGBP. Although previous reports have highlighted the significance of FCGBP in liver, ovarian cancer, and osteosarcoma [5, 10, 11], its specific functions in CRC and the underlying mechanisms require further investigation. Experimental results from this study indicated that overexpression of FCGBP inhibits the proliferation of CRC cells. To explore the mechanism by which FCGBP suppresses CRC progression, we conducted correlation analysis, revealing a direct association between FCGBP and MDM2 in CRC. IP assays demonstrated an interaction between FCGBP and MDM2, with FCGBP preventing the ubiquitin-mediated degradation of P53 by MDM2. These findings provide insight into the mechanism by which FCGBP suppresses CRC. In healthy tissues, FCGBP competitively binds to MDM2, preventing MDM2-mediated ubiquitination of $P53^{wt}$ and stabilizing it. In $P53^{wt}$ CRC cells, FCGBP expression is reduced, allowing MDM2 to degrade P53 via ubiquitination, thereby facilitating tumor progression. We also demonstrated overexpression of FCGBP significantly enhances the anti-tumor efficacy of MDM2 inhibitors in a mouse model. Our study reveals a novel mechanism by which FCGBP inhibits the malignant progression of CRC.

Abbreviations

ARRIVE	Animal Research Reporting of In Vivo Experiments
CRC	Colorectal Cancer
FBS	Fetal bovine serum
FCGBP	Fc fragment of IgG binding protein
MSI	Microsatellite instable
MSS	Microsatellite stable
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Wt	Wild type

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13873-y.

Supplementary Material 1

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Author contributions

J. W. did the most of experiments and wrote the manuscript. Z. G. and J. L. did the biological evaluation in vitro. W. W., K. L., Y. Y. and X. L. help to do the animal experiments. J. W. designed the whole project and revised the manuscript. All authors reviewed the manuscript.

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Data availability

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

Declarations

Ethics approval and consent to participate

The ethics was approved by ethics committee of Tianjin Medical University Cancer (NSFC-AE-2024149).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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