

RIG-I promotes immune evasion of colon cancer by modulating PD-L1 ubiquitination

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ABSTRACT

Colon cancer is one of the most prevalent cancers and exhibits high mortality worldwide. Despite the certain success in the immunotherapy of many tumor types, the limited response of colon cancer to immunotherapy remains a difficult problem. Retinoic acid-inducible gene-I (RIG-I) is a crucial component in innate antiviral immunity, but its role in antitumor immunity remains unclear. Here, in this report, we found that silencing RIG-I decreased resistance to tumor cells killed by T cells and attenuated colon tumor growth in immunocompetent mice. Meanwhile, overexpressing RIG-I promoted tumor progression, and high expression of RIG-I sensitized cells to anti-programmed cell death protein-1 (PD-1) therapy in vivo. Interestingly, we found that RIG-I influenced programmed cell death ligand 1 (PD-L1) expression to promote colon cancer immune evasion without relying on type I interferon stimulation. Mechanistically, RIG-I could compete with Speckle Type POZ protein (SPOP) to bind PD-L1, leading to attenuation of the polyubiquitination and proteasomal degradation of PD-L1. Collectively, our work reveals new insights into the contribution of RIG-I to driving immune evasion by maintaining the stability of PD-L1 through post-translational modification and provides a promising biomarker of the efficacy of immunotherapy in colon cancer.

INTRODUCTION

Colon cancer is one of the most common cancer types and the leading cause of cancer-related death worldwide.^{1 2} Cancer cells can evade immune surveillance to promote tumor progression; the underlying mechanisms include increased programmed cell death ligand 1 (PD-L1) expression. Tumor-infiltrating lymphocytes (TILs), especially CD8⁺ T cells, which express programmed cell death protein-1 (PD-1) on their surface, are currently considered the most efficient component. However, T cells can become exhausted and lose their cytotoxic function upon binding to PD-L1.³ Recently, immune checkpoint inhibitors (ICIs) therapy to block the PD-1/PD-L1 axis and restore T cells from an exhausted state has revolutionized

immunotherapy for malignancies.⁴ Unfortunately, only a minority of patients respond to ICIs therapy. Thus, more immunotherapeutic targets and biomarkers for the efficacy of immunotherapy are urgently needed. Targeting the post-translational modification (PTM) of PD-L1 has been suggested to have antitumor applications.⁵ According to clinical reports, PD-L1 expression is associated with the clinical response to ICIs.^{6 7} Therefore, an in-depth understanding of the PTMs of PD-L1 may contribute to overcoming clinical resistance to ICIs therapy.

Retinoic acid-inducible gene-I (RIG-I) is a cytosolic pattern recognition receptor that initiates innate antiviral immunity by sensing short double-stranded RNA (dsRNA) to trigger the release of type I interferons (IFNs).^{8 9} Our previous study also has shown that RIG-I can serve as a binding protein to regulate the PTMs of other proteins, confers resistance to IFN- α -induced apoptosis in melanoma.¹⁰

Polyinosinic-polycytidylic acid (polyI:C), a synthetic dsRNA analog of double-stranded 5'-triphosphate RNA (3pRNA) that serves as one of the most widely studied clinical antitumor vaccine adjuvants,^{11 12} can activate RIG-I and subsequently increase immunogenic cell death in a manner dependent on the classic type I IFN pathway.^{13 14} Meanwhile, RIG-I has been reported to regulate STAT1 phosphorylation through a non-classical pathway to exert antitumor activity in hepatocellular carcinoma.¹⁵ However, high RIG-I expression was related to tumor size, TNM (tumor-node-metastasis) stage, metastasis, and shorter survival time in patients with colorectal cancer,¹⁶ suggesting that RIG-I can promote colon cancer progression, but need to be further investigated.

In this study, we show RIG-I serves as an unexpected driving factor of cancer immune evasion by attenuating the ubiquitination and

proteasomal degradation of PD-L1 in colon cancer. We also show that anti-PD1 therapy achieves greater efficacy in tumors with high RIG-I expression in vivo. Thus, these results provide a novel biomarker for immunotherapy efficacy and a promising therapeutic strategy for colon cancer.

MATERIALS AND METHODS

Animal and cell lines

BALB/c, C57BL/6, and nude mice (6–8 weeks, female) were purchased from Vital River Laboratory Animal Technology Company (Beijing). OT-I TCR-transgenic mice (C57BL/6-Tg (Tcr α Tcr β)1100Mjb/J) were kind gifts from Dr Liang Xiaoyu (Sun Yat-sen University Cancer Center, Guangzhou). Studies were approved by the Animal Ethics Committee of Wuhan University (approval No. ZN2021184). The human cell lines HT29 and HER293T and the murine cell line CT26 and MC38 were obtained from the China Center for Type Culture Collection (Wuhan); the MC38-OVA cell line was a kind gift from Dr Liang Xiaoyu. All cells were screened for *Mycoplasma* using PCR. Cells were cultured in RPMI-1640 medium or DMEM medium (HyClone) supplemented with 10% fetal bovine serum (Gibco) at 37°C with 5% CO₂ in a humidified incubator.

Human samples

Resected human colon cancer tissues were obtained from patients at Zhongnan Hospital (Wuhan). All the patients' clinical information is provided in online supplemental table S1.

Western blotting and co-immunoprecipitation assays

For western blotting, cell lysate preparation, SDS-PAGE, immunoblotting and chemiluminescent detection were performed as described previously.¹⁰ For co-immunoprecipitation (co-IP), cell lysates were precleared with protein A/G-Sepharose beads (Santa Cruz), and immunoprecipitates were incubated with antibodies overnight and pulled down using protein A/G-Sepharose beads at 4°C for 3 hours. The beads were washed and collected. Complexes were eluted by boiling the beads in SDS loading buffer and then centrifuged, and the supernatants were subjected to immunoblotting. The gray value of each blot was quantified by the ImageJ software (V.1.0, USA), with GAPDH being the control.

Ubiquitination assay

HT29 and HEK293T cells were transfected with His-ubiquitin, FLAG-PD-L1 and scramble or HA-RIG-I for 48 hours and treated with MG132 (20 μ M, 6 hours). The cell lysates were incubated with anti-Flag magnetic beads (BioLinkedin) for 4 hours at room temperature and washed with an IP wash buffer twice. Proteins eluted from the beads in the loading buffer were resolved by SDS-PAGE for immunoblotting.

Statistical analysis

All quantitative data are expressed as the mean \pm SEM. Intergroup comparisons were performed using unpaired Student's t-test or one-way or two-way analysis of variance. Correlation analyses were performed by the Pearson correlation test. Differences for which the p value<0.05 were considered statistically significant. All experiments were performed at least three times. Other detailed experimental procedures are described in the online supplemental materials and methods.

RESULTS AND DISCUSSION

RIG-I promotes immune evasion of colon cancer by suppressing T-cell antitumor immunity

First, we investigated the biological function of RIG-I in colon cancer, and found there was no significant difference in cell proliferation between RIG-I-KO and control CT26 cells, shRIG-I and control HT29 cells in vivo (online supplemental figure S1A,B). Then, we established a subcutaneous tumor model and found that the growth of RIG-I-KO CT26 cell-derived tumors was consistently and significantly repressed in immunocompetent mice but not in immunodeficient mice (figure 1A and B and online supplemental figure S1C,D). The same results were obtained in the subcutaneous tumor models of another mouse colon cancer cell MC38 (online supplemental figure S1E-H). Meanwhile, RIG-I knockout significantly decreased the number of lung metastatic nodes in the lung metastasis model in BALB/c mice (figure 1C and online supplemental figure S1I). To further delineate changes in the tumor microenvironment (TME) on reducing RIG-I expression in the tumors, we analyzed TILs in mouse-bearing RIG-I-KO tumors. The infiltration of a significantly larger number of CD3⁺ CD8⁺ T cells was detected in RIG-I-KO tumors compared with wild-type (WT) tumors (figure 1D and online supplemental figure S1J). In addition, the levels of CD3⁺ CD8⁺ T cells among peripheral blood mononuclear cells were elevated in the RIG-I-KO lung metastatic model group compared with the WT group (online supplemental figure S2K,L). To better simulate the TME in vivo, we generated stable RIG-I-overexpressing CT26 cells and established an orthotopic colon cancer model in BALB/c mice. Fluorescence images in vivo showed that overexpression of RIG-I dramatically promoted tumor progression in the mice (figure 1E). These results suggested that RIG-I could promote the progression of colon tumors in an immune-dependent manner in vivo.

BALB/c nude mice are normal mice that carry the FoxN1 mutation, which causes dysplasia of the thymus leading to T-cell dysfunction. Given the vast differences between nude and normal mice, we proposed that the biological function of RIG-I is associated with T-cell anti-tumor immunity. Thus, we treated a mouse colon cancer cell line (MC38-OVA) stably expressing ovalbumin (OVA) with RIG-I plasmid or siRNA and cocultured the cells with OVA-specific CD8⁺ T cells that had been separated

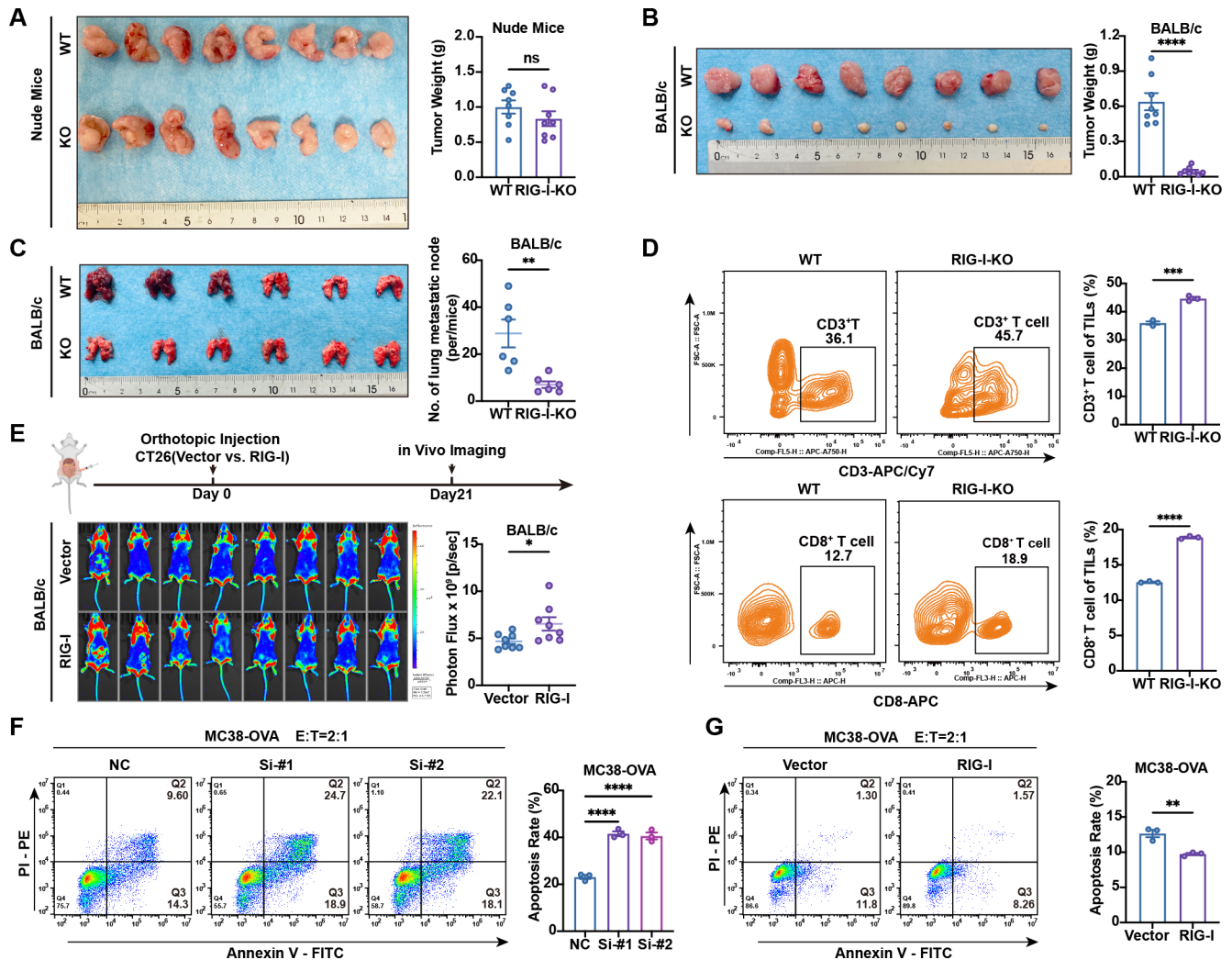


Figure 1 RIG-I promotes immune evasion by suppressing T-cell antitumor immunity. (A) Representative images and weights of tumors from WT and RIG-I-KO CT26 cells in nude mice (n=8). (B) Representative images and weights of tumors from WT and RIG-I-KO CT26 cells in BALB/c mice (n=8). (C) Representative lung images and the metastatic node numbers from tumors from WT and RIG-I-KO CT26 cells in BALB/c mice (n=6). (D) Flow cytometry analysis of the frequency of CD3⁺ T-cell and CD8⁺ T-cell subsets in subcutaneous tumors in BALB/c mice (n=3). (E) Schematic protocols, in vivo images and quantitative analysis following orthotopic injection of CT26 cells transfected with vector and RIG-I overexpression plasmid into BALB/c mice (n=8). (F) The indicated MC38-OVA cells were cocultured with T cells from OT-1 mice for 48 hours (effector: target=2:1), and tumor cell apoptosis was measured by flow cytometry. Data are shown as the mean ± SEM. All data are representative of three independent experiments (n=3). ns, no significant difference; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. FITC, fluorescein isothiocyanate; KO, knockout; NC, normal control; OVA, ovalbumin; PI, propidium iodide; PE, phycoerythrin; RIG-I, retinoic acid-inducible gene-I; TIL, tumor-infiltrating lymphocyte; WT, wild-type.

from the spleens of OT-1 mice. Strikingly, RIG-I overexpression significantly decreased the killing rate of CD8⁺ T cells, whereas RIG-I silencing significantly increased the killing rate (figure 1F and G and online supplemental figure S1M,N). The T-cell-mediated killing assay further indicated that RIG-I affected T-cell cytotoxicity and had an effect on T-cell-mediated antitumor immunity in vitro. Overall, these results suggest that RIG-I promotes immune evasion in colon cancer mainly by affecting T-cell-mediated antitumor immunity.

RIG-I positively regulates the PD-L1 protein and sensitizes colon cancer to immunotherapy

PD-L1 is well known to be a negative regulator of anti-tumor immune responses via its ability to bind PD-1 to reduce T-cell activation signals and facilitate the evasion of immune surveillance by cancer cells.^{4,17} Thus, we further investigated whether RIG-I promotes immune evasion by regulating PD-L1. First, we verified that RIG-I activation by polyI:C could stimulate the expression of genes in type I IFN pathways, such as IRF and STAT genes (online supplemental file 4), to further promote the transcriptional regulation of immune checkpoint genes, especially PD-L1 in HT29, CT26 and MC38 cells (figure 2A and B and online supplemental figure

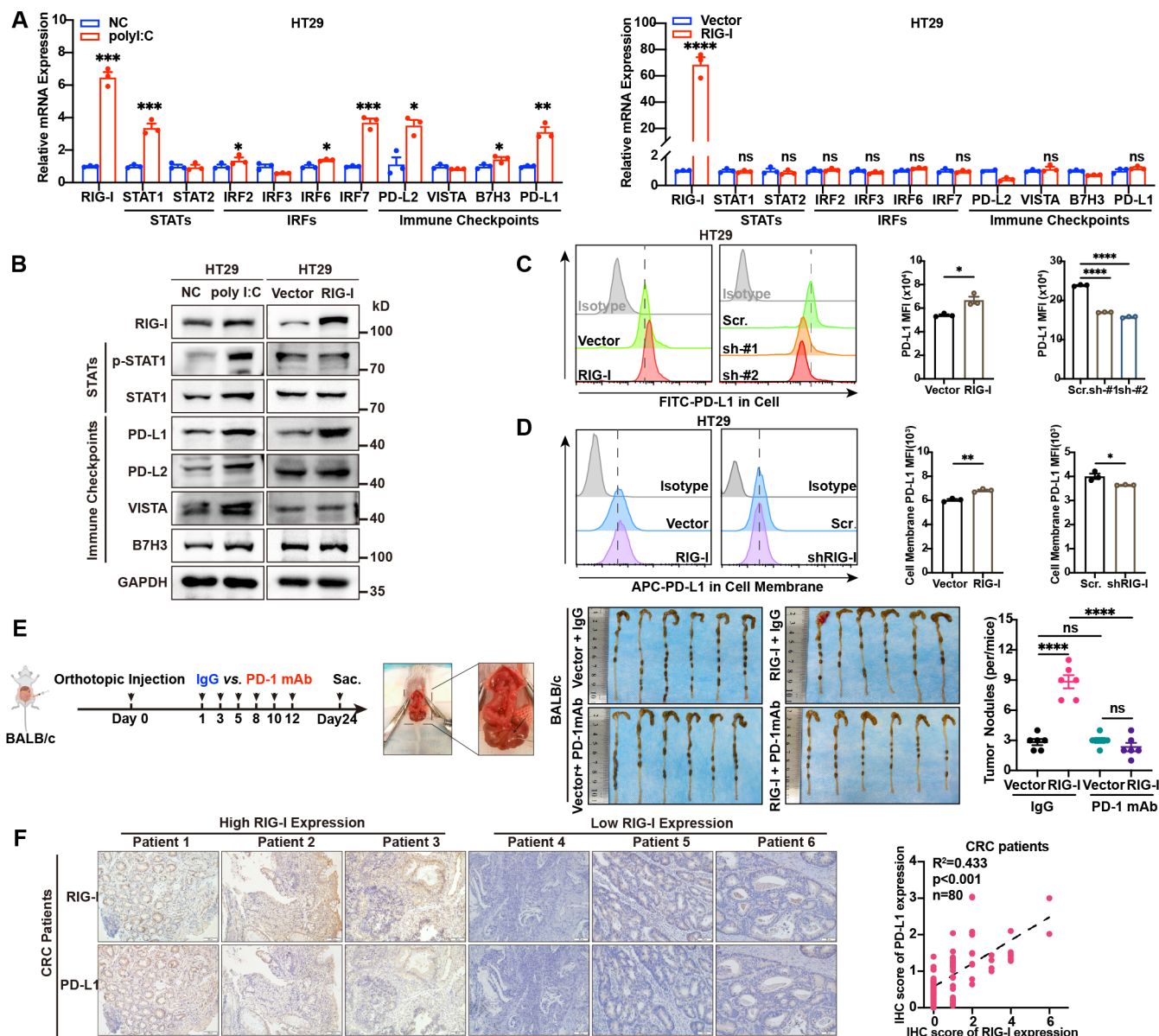


Figure 2 RIG-I positively regulates PD-L1 protein expression and sensitizes colon cancer to immunotherapy. (A) mRNA and protein expression of type I IFN-associated genes and immune checkpoint genes in HT29 cells on stimulation with polyI:C (2 μ g/mL, 24 hours, $n=3$). (B) mRNA and protein expression of type I IFN-associated genes and immune checkpoint genes in HT29 cells on RIG-I overexpression. (C) Representative protein staining and mean fluorescence intensity (MFI) of total PD-L1 in HT29 cells by flow cytometry ($n=3$). (D) Representative protein staining and MFI of surface PD-L1 on HT29 cells by flow cytometry ($n=3$). (E) Schematic of the orthotopic colon cancer model and treatment protocol (left panel), representative colon images (middle panel), and quantification of the tumor nodules (right panel) from BALB/c mice injected with vector and RIG-I-overexpressing CT26 cells with or without IgG/anti-PD1 mAb treatment ($n=6$). (F) Representative images and correlation analysis between RIG-I and PD-L1 protein expression detected by IHC in colon cancer tissue from Zhongnan Hospital ($n=80$). Scale bar, 50 μ m. All data are representative of three independent experiments ($n=3$). ns, no significant difference; * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. APC, allophycocyanin; CRC, colorectal cancer; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IHC, immunohistochemistry; IRF, interferon regulatory factor; mAb, monoclonal antibody; MFI, mean fluorescence intensity; mRNA, messenger RNA; NC, normal control; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand 1; polyI:C, polyinosinic-polycytidylic acid; RIG-I, retinoic acid-inducible gene-I; STAT, signal transducer and activator of transcription; VISTA, V-type immunoglobulin domain-containing suppressor of T cell activation.

S2A,C,D). Then, we investigated whether RIG-I could affect PD-L1 expression without polyI:C. Interestingly, RIG-I overexpression did not increase the transcript level of PD-L1 or IFN-associated genes, but the protein levels remained significantly increased (figure 2A and

B and online supplemental figure S2B-D). Meanwhile, the level of IFN- α increased significantly after RIG-I activation by polyI:C but did not change after RIG-I overexpression (online supplemental figure S2E). In addition, the PD-L1 protein level was similarly downregulated, but

the messenger RNA (mRNA) levels were not significantly altered on RIG-I knockdown or knockout (online supplemental figure S2F,G). Subsequent flow cytometry experiments showed the same results (figure 2C and online supplemental figure S2H). We also detected the expression level of PD-L1 in the cell membrane and found that the expression of PD-L1 on the cell surface was downregulated on RIG-I knockdown but upregulated after RIG-I overexpression (figure 2D and online supplemental figure S2I).

Given that the level of PD-L1 expression directly affects the therapeutic effects of ICIs, high PD-L1 expression can enhance the efficacy of anti-PD-1 treatment.^{3 7 18 19} We then speculated that high RIG-I expression may affect the efficacy of ICIs. An orthotopic colon cancer model was used to determine whether RIG-I expression could influence the efficacy of an anti-PD1 monoclonal antibody (mAb). We found that RIG-I overexpression promoted tumor progression, but RIG-I overexpression plus anti-PD1 mAb treatment dramatically decreased the metastatic burden and number of tumor nodules in the lungs and intestines of mice, remarkably, RIG-I-overexpressing CT26 tumors exhibited a better response to the anti-PD-1 mAb (figure 2E and online supplemental figure S2J). Next, we assessed levels of RIG-I and PD-L1 in the tissues of 80 patients with colon cancer, which demonstrated that RIG-I expression positively correlated with PD-L1 expression in clinical samples (figure 2F and online supplemental figure S2K,L).

Collectively, these results illustrate that RIG-I promotes immune evasion by modulating the PTM of PD-L1 and that high RIG-I expression can enhance the efficacy of anti-PD1 therapy.

RIG-I attenuates the polyubiquitination and proteasomal degradation of PD-L1 in colon cancer

We demonstrated that interference with RIG-I expression did not affect the mRNA levels of PD-L1 but dramatically reduced the protein levels of PD-L1. PTMs of the PD-L1 protein have emerged as important regulatory mechanisms in previous studies, and PTMs are often therapeutic targets for pharmacological inhibition in cancer by agents such as CDK4/6 and NEK inhibitors.^{18–21} To test whether RIG-I enhances PD-L1 protein levels through the regulation of its protein stability, we used CHX, a common protein synthesis inhibitor (200 µM), to block further synthesis of the protein and found that the PD-L1 protein levels decreased significantly faster in RIG-I-knockdown or knockout HT29 and CT26 cells (figure 3A and online supplemental figure S3A). Sequentially, on the treatment of RIG-I-knockdown cells with the proteasome inhibitor MG132, PD-L1 protein levels were remarkably restored, and similar results were observed by flow cytometry analysis of PD-L1 (figure 3B and online supplemental figure S3B,C). Additional ubiquitination assays demonstrated that RIG-I overexpression inhibited PD-L1 ubiquitination, while RIG-I knockdown increased PD-L1 ubiquitination in HT29, CT26 and HEK293T cells (figure 3C and

online supplemental figure S3D,E). These results indicated that RIG-I regulates the ubiquitination and proteasomal degradation of PD-L1 in colon cancer.

RIG-I competes with SPOP to bind PD-L1, maintaining PD-L1 stability

Our previous study verified that RIG-I can competitively bind with phosphorylase to regulate the phosphorylation of STAT1.¹⁰ Thus, we speculated that RIG-I can bind PD-L1 and affect its expression. First, we visualized the conformation of protein–protein docking using PyMol (V.2.2.0) and LigPlot+ (V.2.2.4), which revealed a possible interaction between the RIG-I and PD-L1 proteins (online supplemental figure S3F). Co-IP confirmed the binding between the endogenous RIG-I and PD-L1 proteins in HT29 and CT26 cells (figure 3D and online supplemental figure S3G). Then, we introduced expression plasmids for FLAG-PD-L1 and HA-RIG-I into HEK293T cells and confirmed the interaction between these two proteins when expressed ectopically (online supplemental figure S3H). Consistent with this finding, Immunofluorescence (IF) staining validated that endogenous RIG-I colocalized with PD-L1 in HT29 and CT26 cells (figure 3E and online supplemental figure S3I). We also investigated the change in the membrane localization of PD-L1 through confocal immunofluorescence. We found that the PD-L1 expression was reduced in the membrane and cytoplasm in HT29 and CT26 cells, consistent with the flow cytometry results (online supplemental figure S4A,B).

Based on the above observations, we sought to identify which PD-L1 domains are critically required for its interaction with RIG-I. We generated three PD-L1 truncations according to its molecular structure (1–238, 1–259, 260–290), and through co-IP assays, we found that the transmembrane domain (TM, 239–259) of PD-L1 mediated its interaction with RIG-I (figure 3F). Collectively, our data indicates that RIG-I could directly interact with the TM domain of PD-L1 (239–259), and this interaction may contribute to RIG-I-mediated regulation of PD-L1 in colon cancer.

The ubiquitin-proteasome system plays an important role in the regulation of protein stability, and accumulating evidence has demonstrated that PD-L1 is extensively regulated by the ubiquitin–proteasome pathway through many E3 ligases.^{19 22} Considering the dynamic change in the ubiquitination level of PD-L1 and its binding with RIG-I, we hypothesized that RIG-I regulates the binding of both E3 ligase and PD-L1. Strikingly, immunoprecipitation mass spectrometry (IP-MS) data identified 258 significantly differentially expressed PD-L1-interacting proteins in RIG-I-knockdown compared with control HT29 cells. To identify E3 ubiquitin ligases that interact with PD-L1, we evaluated the intersection of PD-L1 interactors identified by IP-MS analysis with a list of human E3 ubiquitin ligases, which revealed that the E3 ubiquitin ligases speckle-type pox virus and zinc finger (SPOP) and CUL1 might be competitive regulators that affect the RIG-I-mediated regulation of PD-L1 expression

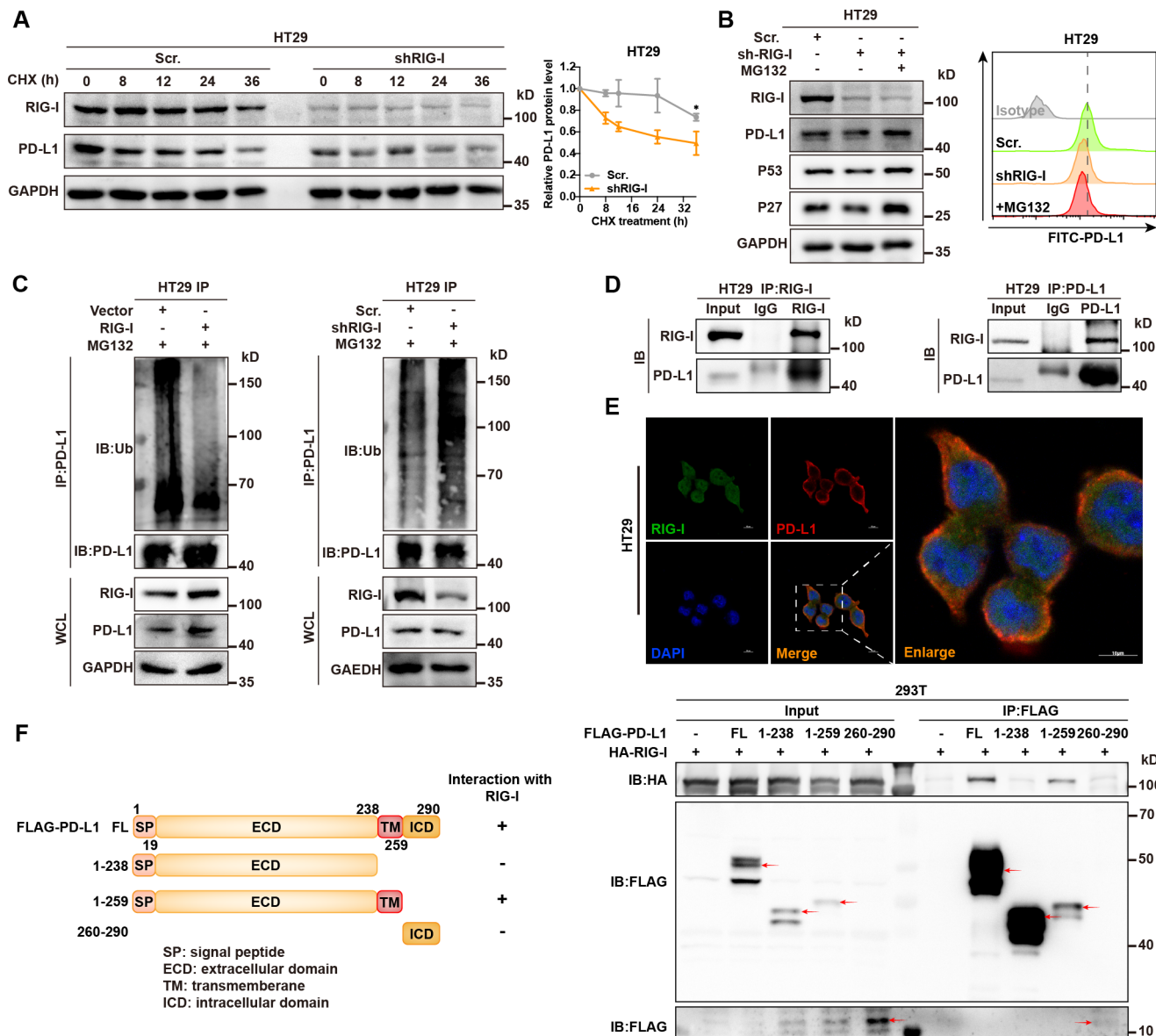


Figure 3 RIG-I attenuates the polyubiquitination and proteasomal degradation of PD-L1 in colon cancer. (A) PD-L1 protein levels in HT29 cells with RIG-I knockdown that were treated with CHX (200 ng/mL) for the indicated time (left panel). Graph showing the amount of PD-L1 protein that remained after CHX treatment (right panel, n=3). (B) PD-L1 protein levels in HT29 cells with RIG-I knockdown that were treated with MG132 (20 μ M) for 6 hours (left panel). Representative PD-L1 protein staining is shown in the right panel by flow cytometry (n=3). (C) The polyubiquitination of PD-L1 in HT29 cells with RIG-I overexpression or knockdown was examined by immunoprecipitation. Cells were treated with MG132 (20 μ M) for 6 hours before being harvested. (D) Co-IP analysis of the interaction between endogenous PD-L1 and RIG-I within HT29 cells. (E) Representative confocal microscopy images from IF analysis of RIG-I and PD-L1 expression and the colocalization between RIG-I (green) and PD-L1 (red) in HT29 cells. Scale bar, 10 μ m. (F) Schematic representation of FLAG-PD-L1 full-length (FL) and three truncations (FLAG-1-238, 1-259, 260-290) (left panel, + indicates that HA-RIG-I has a positive binding with FLAG-PD-L1), and the interaction between HA-RIG-I and FLAG-PD-L1 FL or three truncations detected by co-IP assays in HEK293T cells (right panel, red arrow represents the specific molecular position of the truncated FLAG-PD-L1). All data are representative of three independent experiments (n=3). ns, no significant difference; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. co-IP, co-immunoprecipitation; CHX, cycloheximide; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IB, immunoblotting; IP, immunoprecipitation; PD-L1, programmed cell death ligand 1; RIG-I, retinoic acid-inducible gene-I; shRIG-I, knockdown of RIG-I; WCL, whole cell lysates.

(figure 4A). A co-IP assay confirmed the binding between endogenous SPOP and CUL1 with PD-L1 (figure 4B), and only overexpression of SPOP decreased the PD-L1 protein level in HT29 and HEK293T cells (figure 4C and online supplemental figure S4C). SPOP is a typical substrate

adaptor of the Cullin 3-RING ligase (CRL3) family,²³ and a series of studies have reported that SPOP substrates mediate the degradation of various proteins in cells.²⁴ Therefore, we focused on SPOP for further investigation. We found by co-IP experiments that FLAG-PD-L1 and

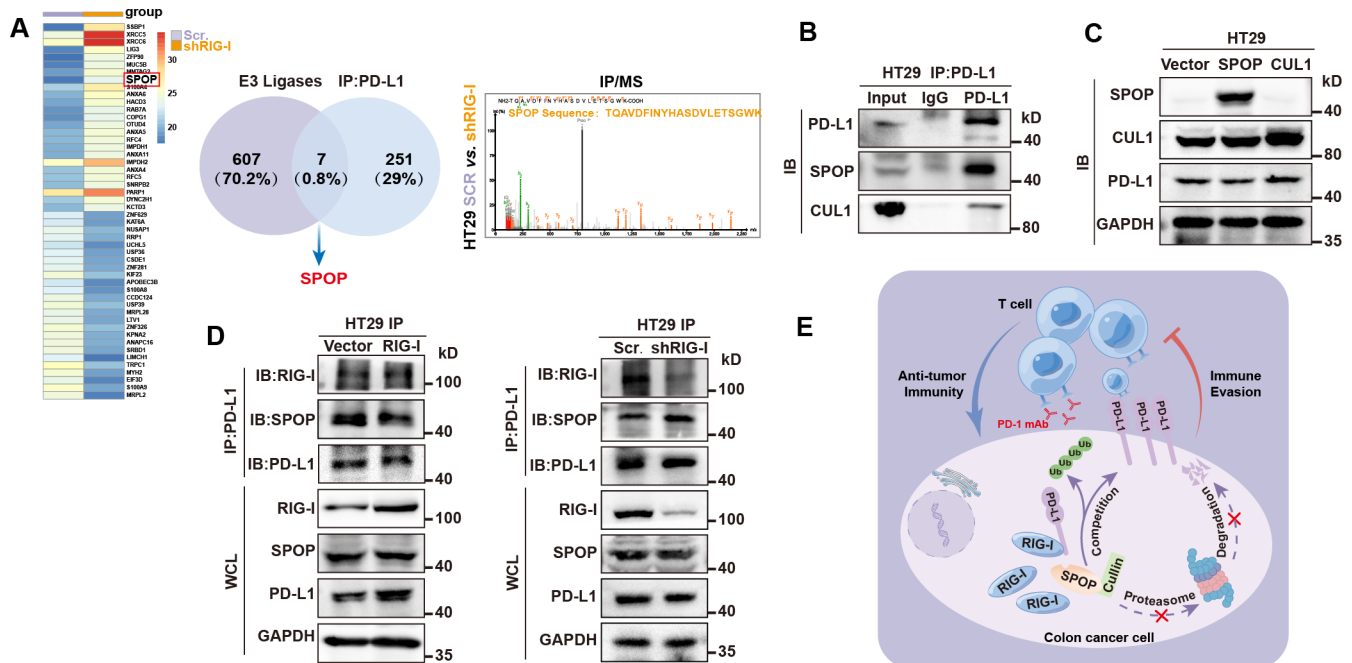


Figure 4 RIG-I competes with SPOP to bind PD-L1, maintaining PD-L1 stability. (A) Heatmap of differentially expressed PD-L1-interacting proteins in RIG-I-knockdown and control HT29 cells by IP/MS analysis (left panel). Venn diagram showing IP-MS-detected potential PD-L1-interacting protein and E3 ligases (middle panel) and the secondary mass spectrum of SPOP (right panel). (B) Co-IP analysis of the interaction of endogenous PD-L1 with SPOP and CUL1 in HT29 cells. IgG was used as the negative control. (C) PD-L1 protein expression on overexpression of SPOP and CUL1 in HT29 cells. (D) Co-IP analysis of the interaction between endogenous PD-L1, SPOP, and RIG-I within HT29 cells on overexpression (left panel) or knockdown of RIG-I (right panel). All data are representative of three independent experiments (n=3). (E) Schematic model showing that RIG-I promotes immune evasion by modulating PD-L1 ubiquitination in colon cancer. All data are representative of three independent experiments (n=3). ns, no significant difference; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. CUL1, cullin 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IP-MS, immunoprecipitation mass spectrometry; IB, immunoblotting; mAb, monoclonal antibody; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand 1; RIG-I, retinoic acid-inducible gene-I; shRIG-I, knockdown of RIG-I; SPOP, speckle-type POZ protein; WCL, whole cell lysates.

Myc-SPOP formed a complex in a heterologous system, and the polyubiquitination of PD-L1 was increased in the presence of SPOP in HEK293T cells (online supplemental figure S4D). These results verified that SPOP is the E3 ligase of PD-L1 and can promote the ubiquitination and degradation of the PD-L1 protein and to impact PD-L1 function. However, whether RIG-I alters PD-L1 expression by altering SPOP expression, we found that neither knockdown nor overexpression of RIG-I changed the expression of SPOP (online supplemental figure S4E,F). Importantly, the interaction between SPOP and PD-L1 was decreased in the RIG-I-knockdown group but stronger in the RIG-I-overexpression group in HT29 and HEK293T cells, consistent with a heatmap of the IP-MS results (figure 4D and online supplemental figure S4G). Consistent with our results, SPOP was recently reported to recruit PD-L1 as a substrate and increase its polyubiquitination, thus promoting PD-L1 degradation.^{18 25} These findings indicated that RIG-I attenuates the ubiquitination of PD-L1 and maintains its stability by competing with SPOP for PD-L1 interaction to promote colon cancer immune evasion.

In summary, in the present study, we have verified that RIG-I contributes to immune evasion by regulating the polyubiquitination and proteasomal degradation

of PD-L1 in colon cancer. Furthermore, high RIG-I expression sensitizes colon cancer to anti-PD1 therapy (figure 4D).

CONCLUSION

Overall, our findings reveal that tumor RIG-I expression may serve as a novel biomarker of immunotherapy efficacy and provide a potential therapeutic strategy for use in combination with ICIs in colon cancer treatment. Although RIG-I drives immune evasion via an immune checkpoint, the regulation of cancer immunity by RIG-I may proceed via other mechanisms, and the effect of RIG-I expression on immunotherapy sensitivity needs to be further verified in human clinical trials.

Contributors YZ contributed to the experimental design, methodology, analysis, and writing the draft. YZ and LZ performed the experiments and acquired data. MW, ZY, and HZ contributed to revise the figures. LG and RZ evaluated the pathological reports. JL and WS performed updating the reference lists. YC, LL, and QZ supervised statistical analyses. YL and JL provided supervision and revised the manuscript.

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Competing interests No, there are no competing interests.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Clinical Trial Ethics Committee of Zhongnan Hospital (approval No. 2021032). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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