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IL-1β mediates *Candida tropicalis*-induced immunosuppressive function of MDSCs to foster colorectal cancer

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Abstract

Background There is increasing evidence that gut fungi dysbiosis plays a crucial role in the development and progression of colorectal cancer (CRC). It has been reported that gut fungi exacerbate the severity of CRC by regulating tumor immunity. Our previous studies have shown that the opportunistic pathogenic fungal pathogen, *Candida tropicalis* (*C. tropicalis*) promotes CRC progression by enhancing the immunosuppressive function of MDSCs and activating the NLRP3 inflammasome of MDSCs. However, the relationship between IL-1β produced by NLRP3 inflammasome activation and the immunosuppressive function of MDSCs enhanced by *C. tropicalis* in CRC remains unclear.

Methods The TCGA database was used to analyze the relationship between IL-1β and genes related to immunosuppressive function of MDSCs in human CRC. The expression of IL-1β in human CRC tissues was detected by immunofluorescence staining. The proteomic analysis was performed on the culture supernatant of *C. tropicalis*-stimulated MDSCs. The experiments of supplementing and blocking IL-1β as well as inhibiting the NLRP3 inflammasome activation were conducted. A mouse colon cancer xenograft model was established by using MC38 colon cancer cell line.

Results Analysis of CRC clinical samples showed that the high expression of IL-1β was closely related to the immunosuppressive function of tumor-infiltrated MDSCs. The results of in vitro experiments revealed that IL-1β was the most secreted cytokine of MDSCs stimulated by *C. tropicalis*. In vitro supplementation of IL-1β further enhanced the immunosuppressive function of *C. tropicalis*-stimulated MDSCs and NLRP3-IL-1β axis mediated the immunosuppressive function of MDSCs enhanced by *C. tropicalis*. Finally, blockade of IL-1β secreted by MDSCs augmented antitumor immunity and mitigated *C. tropicalis*-associated colon cancer.

Conclusions *C. tropicalis* promotes excessive secretion of IL-1β from MDSCs via the NLRP3 inflammasome. IL-1β further enhances the immunosuppressive function of MDSCs to inhibit antitumor immunity, thus promoting the progression of CRC. Therefore, targeting IL-1β secreted by MDSCs may be a potential immunotherapeutic strategy for the treatment of CRC.

Keywords CRC, *Candida tropicalis*, MDSCs, NLRP3, IL-1β

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Background

Colorectal cancer (CRC) is among the most prevalent intestinal malignancies and a leading cause of cancer-related mortality worldwide [1, 2]. Both the morbidity and mortality of CRC have been gradually rising in recent years. Many risk factors (including intestinal inflammation, environmental factors, genetic factors, etc.) can contribute to development and progression of CRC [3]. It has long been widely believed that chronic inflammation or inflammatory bowel disease (IBD) is closely associated with CRC [4, 5]. The early stages of CRC are typically marked by chronic inflammation. In other words, IBD has a high likelihood of developing into CRC, and there is an "inflammation-cancer transformation" in the process of CRC development and progression. Thus, investigating the connection between chronic inflammation and the development and progression of CRC is crucial.

Previous studies have shown that patients with CRC develop a microbial dysbiosis in their gut [6–9]. The development and progression of CRC is accompanied by the dysbiosis of intestinal bacteria, fungi and viruses, and the dysbiosis of gut microbiota related to intestinal inflammation and tumorigenesis directly leads to colorectal tumorigenesis [10]. Therefore, intervening in the composition of the gut microbiome may be a promising strategy for alleviating and treating CRC. It has been extensively studied that gut bacteria contribute to progression of CRC by secreting carcinogenic bacterial toxins, interacting with host cell receptors, producing carcinogenic metabolites, inducing inflammation and recruiting immunosuppressive cells [11, 12]. Thus, CRC can be prevented through supplementing probiotics, inhibiting inflammation and reversing gut dysbiosis.

In addition to bacteria, gut fungi, as an important component of gut microbiota, also play a vital role in the occurrence and development of diseases [13]. A growing body of evidence indicates that gut fungi dysbiosis also occurs in the intestines of patients with CRC and plays an essential role in CRC development and progression [14–16]. Two studies published simultaneously in 2022 demonstrate the presence of tumor-associated fungi in human tumors by pan-cancer mycobiome analysis; moreover, fungi are involved in the development of a variety of tumors, including gastrointestinal tumors [17, 18]. A large number of *Candida* species (including *Candida tropicalis*, *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida glabrata*, etc.) are significantly enriched in human gastrointestinal tumors [18].

In general, according to our current knowledge, the vast majority (up to more than 90%) of CRC development and progression is closely related to *C. tropicalis* dysbiosis. In the advanced stages of CRC, the abundance of *C. tropicalis* in the intestines of patients with CRC increases dramatically. In addition, many studies have reported that *C. tropicalis* is significantly enriched in gastrointestinal tumors of patients with CRC [17, 18]. Our previous study has also demonstrated that the proportion of *C. tropicalis* is markedly elevated in the gut of patients with CRC and CARD9 knockout mice with CRC, and *C. tropicalis* facilitates CRC by inducing the expansion of myeloid-derived suppressor cells (MDSCs) and activating the immunosuppressive function of MDSCs [19]. Furthermore, for the first time, we elucidate the molecular mechanism by which *C. tropicalis* augments the immunosuppressive function of MDSCs by inducing the activation of glycolysis [20]. *C. tropicalis* can augment the expression of iNOS, NOX2 and COX2, as well as the production of reactive oxygen species (ROS) and nitric oxide (NO), which contribute to the immunosuppressive function of MDSCs [20]. Meanwhile, we also report that *C. tropicalis* activates NLRP3 inflammasome of MDSCs and promotes IL-1β secretion of MDSCs via glycolysis to aggravate colorectal carcinogenesis [21]. However, how IL-1β secreted by MDSCs through NLRP3 inflammasome activation is involved in the progression of *C. tropicalis*-associated CRC has not been clearly defined.

It has been reported that the level of IL-1β is increased in many types of tumors, including CRC, breast cancer and melanoma, and elevated IL-1β is closely related to the occurrence and progression of tumors [22–24]. As a pleiotropic cytokine, IL-1β can promote tumors development by inhibiting antitumor immunity [25]. In the tumor microenvironment, increased IL-1β accelerates tumor progression by recruiting large numbers of MDSCs. Studies have shown that overexpression of IL-1β induces spontaneous gastric cancer by mobilizing MDSCs [26]. Blocking IL-1β with Anti-IL-1β antibody prevents the progression of lung adenocarcinoma by increasing cytotoxic $CD8^+$ T cell infiltration and decreasing PMN-MDSCs infiltration [27]. In addition, a recent study uncovers that blockade of IL-1β reduces *A. sydowii*-induced immunosuppression by inhibiting the infiltration of MDSCs in lung adenocarcinoma [28]. Consistent with these findings, the latest evidence suggests that the antagonist of IL-1β exhibits synergistic antitumor benefits with the inhibitor of PD-1 and inhibits the progression of CRC by reducing the accumulation of MDSCs [29]. But it is not well understood whether IL-1β is involved in *C. tropicalis*-associated CRC by regulating the expansion and immunosuppression of MDSCs.

Here, we investigated the connection between IL-1β and the immunosuppressive function of MDSCs enhanced by *C. tropicalis* in CRC. Moreover, we suggested for the first time that *C. tropicalis* promoted the secretion of high level of IL-1β from MDSCs through the NLRP3 inflammasome. IL-1β then further augmented

the immunosuppressive function of MDSCs, resulting in suppression of antitumor immunity, thereby promoting *C. tropicalis*-associated CRC. Blocking IL-1β secreted by MDSCs through Anti-IL-1β antibodies mitigated progression of *C. tropicalis*-associated CRC, suggesting that targeting IL-1β secreted by MDSCs may be a potential immunotherapeutic strategy for CRC.

Methods

Reagents

Ultra-LEAF™ Purified Rat IgG2b, κ Isotype Ctrl (Biolegend, 400671), Ultra-LEAF™ purified anti-IL-1β mAb (Biolegend, 503516), Recombinant Murine IL-1β (PeproTech, 211-11B), CY-09 (MedChemExpress, HY-103666), Ultra-LEAF™ Purified anti-mouse Ly-6G/Ly-6C (Gr-1) antibody (Biolegend, 108453).

Bioinformatics analysis

The expression of *IL1B* in human normal colon tissues (N, $n=349$) and COAD tumor tissues (T, $n=275$) from GTEx and TCGA databases for COAD was analyzed by the GEPIA2 online analysis tool (http://gepia2.cancer-pku.cn/#index) [30]. In addition, the expression of *IL1B* in human primary COAD tumor tissues ($n=286$) and normal colon tissues ($n=41$) from the TCGA database was analyzed by UALCAN database. The expression of *IL1B* in human primary COAD tumor tissues based on individual cancer stages from the TCGA database was analyzed by UALCAN database. The correlation between *IL1b* expression and *Ccl2*, *Ccl5*, *Cxcl1*, *Ccr2*, *Ccr5*, *Cxcr2*, *Nos2*, *Ptgs2* and *Cybb* expression in TCGA database for COAD was analyzed by GEPIA2.

Human CRC samples and immunofluorescence

This study included 50 patients with CRC. Human CRC samples ($n=50$) and adjacent nontumor samples ($n=50$) were obtained from Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, China. This work was approved by the Ethics Committee of Medical School, Nanjing University. The patients included provided their written informed consent.

The methods used for immunofluorescence were comparable to those previously mentioned [20]. In brief, the sections of paraffin-embedded CRC tissues and adjacent nontumor tissues were incubated with the primary antibody against IL-1β (1:50; Abcam, ab254360) or NLRP3 (1:100; ABclonal, A5652) overnight at 4 ℃. The sections were then incubated with Goat Anti-Mouse IgG H&L (Alexa Fluor® 647) (1:500; Abcam, ab150115) secondary antibody for 1 h at room temperature in the dark. Cell nuclei were counterstained with DAPI (Working concentration: 5 μg/ml) for 10 min at room temperature. Immunofluorescence images were analyzed and obtained by a Digital Slide Scanner.

Culture and preparation of *C. tropicalis* strain

Culture and preparation of *C. tropicalis* strain were performed in accordance with our previous reports [20, 31].

Generation of bone marrow-derived MDSCs

The generation of bone marrow-derived MDSCs were induced in vitro as previously described [20]. In short, bone marrow cells were harvested from tibias and femurs of mice. Then, the bone marrow cells were cultured in complete RPMI-1640 medium with 40 ng/ml murine GM-CSF (Miltenyi Biotec, 130-095-742) and 40 ng/ml murine IL-6 (Miltenyi Biotec, 130-096-682) for 4 days. After 4 days of culture, the suspended cells were harvested for follow-up experiments.

Proteomics analysis

WT MDSCs were stimulated with *C. tropicalis* (MOI = 1) for 24 h. Then the cell culture supernatant was collected for proteomic analysis. The proteomic analysis was performed by Shanghai Lu-Ming Biotech Co., Ltd. (Shanghai, China). Firstly, the protein components were analyzed and identified by liquid chromatography-mass spectrometry. Then the qualitative, quantitative and functional analysis of the proteins were carried out. The thresholds of P-value < 0.05 and fold change ≥ 1.5 or fold change ≤ 1/1.5 were employed to screen differentially expressed proteins. Differentially expressed proteins were further utilized for KEGG signaling pathway enrichment analysis.

Flow cytometry

For the determination of MDSCs, the single-cell suspensions were incubated with APC anti-mouse Ly-6G/Ly-6C (Gr-1) antibody (2.5 μg/ml; BioLegend, 108412) and FITC anti-mouse/human CD11b antibody (2.5 μg/ml; BioLegend, 101206) for 30 min. For the detection of $CD8^+$ T cells in spleen, the single-cell suspensions were incubated with FITC anti-mouse CD3 antibody (10 μg/ml; BioLegend, 100204) and APC anti-mouse CD8α antibody (2.5 μg/ml; BioLegend, 100712) for 30 min. The cells were then washed with PBS and examined with a FACSCalibur flow cytometer (BD Biosciences). FlowJo software version V10 was used to analyze the collected data.

Quantitative real-time PCR (qPCR)

Following the guidance of manufacturer, TRIzol Reagent was utilized to extract total RNA from MDSCs or tumor tissues. Then cDNA was synthesized using HiScript III RT SuperMix for qPCR Kit (Vazyme, R323-01). qPCR was conducted using SYBR green PCR master mix

on a Step One Plus sequence detection system (Applied Biosystems, Thermo Fisher Scientific, US). The relative genes expression was determined by the $2^{-\Delta\Delta CT}$ method and normalized to *β-actin*. The primer sequences for qPCR are listed in Table 1.

Western blotting analysis

MDSCs and tumor tissues were lysed with RIPA Lysis Buffer. The protein concentrations were tested by BCA protein concentration assay kit (Beyotime Biotechnology, P0010) following the instructions of manufacturer. An equal amount of protein of each sample was separated through SDS-PAGE and transferred onto a PVDF membrane. Subsequently, the PVDF membrane was blocked with 5% skim milk or 5% BSA. The PVDF membrane was incubated overnight with the specific primary antibody, followed by the HRP-linked secondary antibody for 1.5 h. The bands were visualized using enhanced chemiluminescence solution on the Multicolor Fluorescence and Chemiluminescence Imaging Systems (Champ-Chemi Top 420). The primary antibodies were as follows: β-actin (1:1000; Cell Signaling Technology, 8457S), iNOS (1:2000; Proteintech, 18985-1-AP), COX2 (1:1000; Cell Signaling Technology, 12282 T), NOX2 (1:4000; Proteintech, 19013-1-AP), CD8α (1:1000; Cell Signaling Technology, 98941 T), PD-1 (1:1000; Cell Signaling Technology, 84651S). Full uncropped membrane blots images are exhibited in Additional file 2.

NO and ROS measurement

NO assay kit (Beyotime Biotechnology, S0021) was used to determine the concentration of NO in the cell culture supernatants of MDSCs according to the protocols of manufacturer. For detection of ROS production in MDSCs, MDSCs were incubated with 10 μM DCFH-DA (Beyotime Biotechnology, S0033S) at 37 ℃ for 30 min. Then flow cytometry was used to assess the fluorescence intensity of DCF.

$CD8^+$ T cells suppression experiment

The $CD8^+$ T cells isolated from splenocytes were pre-incubated with 5 μM CFSE (eBioscience, C34570) at 37 ℃ for 10 min away from light. MDSCs were then cocultured with $CD8^+$ T cells at a 1:1 ratio in 96-well plates supplemented with 2 μg/mL anti-CD28 mAbs (eBioscience, 16-0281-81) and 4 μg/mL anti-CD3 (eBioscience, 16-0031-81) for 3 days. Finally, the proliferation of $CD8^+$ T cells was measured by flow cytometry.

Enzyme-linked immunosorbent assay (ELISA)

Murine IFNγ and Granzyme B were determined with Mouse IFNγ ELISA Kit (MULTI SCIENCES, EK280/3–96) and Mouse Granzyme B ELISA Kit (MULTI SCIENCES, EK2173-96) according to the instructions of manufacturer.

Pretreatment of bone marrow-derived MDSCs

The induced MDSCs were harvested and cultured in cell culture plates. Then these MDSCs were treated with Anti-IgG antibody (1 μg/ml), *C. tropicalis* (MOI = 1) combined with Anti-IgG antibody (1 μg/ml), Anti-IL-1β antibody (1 μg/ml), *C. tropicalis* (MOI = 1) combined with Anti-IL-1β antibody (1 μg/ml) for 24 h, respectively. It was used for in vitro experiment and murine subcutaneous colon cancer xenograft model experiment.

Murine subcutaneous colon *cancer* xenograft model

Male C57BL/6 J mice aged 6-8 weeks were purchased from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co., Ltd. (Taizhou, China). These mice were raised in a specific pathogen-free facility (SPF) at Medical School of Nanjing University. All mouse experiments were approved by the Institutional Animal Care and Use Committee (IACUC-D2202134) of Nanjing University.

For the construction of mouse colon cancer xenograft model, 6- to 8-week-old male C57BL/6J mice were randomly divided into four groups: Anti-IgG MDSCs group, *C. tropicalis* + Anti-IgG MDSCs group, Anti-IL-1β MDSCs group and *C. tropicalis* + Anti-IL-1β MDSCs

Table 1 Primer sequences for qPCR

Gene (Mouse)	Forward Primer (5′→3′)	Reverse Primer (5′→3′)
β-actin	ATTGTTACCAACTGGGACGACATG	CTTCATGAGGTAGTCTGTCAGGTC
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Ptgs2	TACAGGAGAGAAGGAAATGGC	TTGAGGAGAACAGATGGGATT
Cybb	TGCCCCAAGGTATCCAAGTT	CCTCCGTCCAGTCTCCCACA
Gzmb	CCACTCTCGACCCTACATGG	GGCCCCCAAAGTGACATTTATT
Ifng	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
Pdcd1	ACCCTGGTCATTCACTTGGG	CATTTGCTCCCTCTGACACTG

group. On day 0, 5×10^5 MC38 cells in 100 μL sterile PBS were administered subcutaneously into the flank of each mouse. On day 5, each mouse was treated with intraperitoneal injection of 200 μg Anti-Gr1 antibody to deplete MDSCs. On day 7, 4 groups of mice were intravenously injected with 5×10^6 different pretreated MDSCs, respectively: MDSCs pretreated with Anti-IgG antibody, MDSCs pretreated with *C. tropicalis* and Anti-IgG antibody, MDSCs pretreated with Anti-IL-1β antibody, and MDSCs pretreated with *C. tropicalis* and Anti-IL-1β antibody. When the tumors were visible, their width and length were measured every 3 days. The volume of the tumors were then calculated with the formula: $0.5 \times \text{length} \times (\text{width})^2$. On day 22, the mice were euthanized. Serum, spleen and tumors of all mice were collected, and the tumors were photographed and weighed.

Hematoxylin and eosin (H&E) and Immunohistochemistry (IHC) analysis

Paraffin sections of tumor tissues were stained with hematoxylin and eosin (H&E). The histological score was evaluated as previously mentioned [20]. For IHC staining, paraffin sections of tumor tissues were stained individually with primary antibodies against PCNA (1:8000; Cell Signaling Technology, 13110S), Ki-67 (1:400; Cell Signaling Technology, 12202S) and CD8α (1:400; Cell Signaling Technology, 98941 T) at 4 ℃ overnight. ImageJ was used to determine the percentages of positive cells.

Statistical analysis

Data are presented as mean ± SEM. To compare the differences between 2 groups, two-tailed unpaired Student's *t*-test was conducted. For comparison of multiple groups, one-way ANOVA with Bonferroni's multiple comparisons test for post-hoc test was performed. Correlation analysis was performed by Spearman's rank tests. $P < 0.05$ was regarded as statistically significant. ns (not significant), $P > 0.05$; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. GraphPad Prism 8.0 was used to conduct statistical analysis.

Results

The elevated expression of IL-1β is associated with the infiltration and immunosuppressive function of MDSCs in human CRC

To determine the expression of IL-1β in human CRC tissues, we first analyzed Colon adenocarcinoma (COAD) data from the TCGA and GTEx databases using the GEPIA2 online tool. The results showed that the mRNA level of *IL1B* was significantly elevated in COAD tumor tissues compared to normal colon tissues (Fig. 1A). The analysis of UALCAN database further confirmed the above results (Fig. 1B). We also analyzed the expression of *IL1B* in human primary COAD tumor tissues based on individual cancer stages. The results showed that the expression of *IL1B* in stage 1, stage 2, stage 3 and stage 4 COAD was significantly higher than that in normal colon tissues (Fig. 1C). This suggested that the level of IL-1β expression was correlated with the pathological stages of CRC. Moreover, our immunofluorescence investigation revealed that human CRC tissues had substantially greater levels of IL-1β expression than adjacent nontumor tissues (Fig. 1D). Our previous study has indicated that the abundance of *C. tropicalis* was significantly elevated in patients with CRC [19]. Therefore, we analyzed the correlation between the abundance of *C. tropicalis* and IL-1β expression in human CRC tissues. We found that the abundance of *C. tropicalis* was positively correlated with IL-1β expression (Fig. 1E). In addition, our immunofluorescence staining also showed that the expression of NLRP3 was markedly increased in human CRC tissues (Additional file 1: Fig. S1A). And correlation analysis demonstrated that there was a positive correlation between the abundance *C. tropicalis* and the expression of NLRP3 in human CRC tissues (Additional file 1: Fig. S1B).

Next, we further investigated the relationship between IL-1β and MDSCs recruitment in human CRC. It has been reported that tumor-associated CCL2, CCL5, and CXCL1 promote the recruitment of MDSCs by interacting with the receptors CCR2, CCR5, and CXCR2 on the surface of MDSCs, respectively [27, 32–35]. Therefore, our analysis of the TCGA database by GEPIA2 indicated a positive correlation between the expression of *IL1B* mRNA and that of *Ccl2*, *Ccl5*, *Cxcl1*, *Ccr2*, *Ccr5*, *Cxcr2* mRNA in COAD (Fig. 1F). Furthermore, there was a significant correlation between the expression of *IL1B* mRNA and the genes *Nos2*, *Ptgs2*, and *Cybb*, which are associated with the immunosuppressive activity of MDSCs (Fig. 1G). Collectively, these findings suggest that the high expression of IL-1β is related to the infiltration and immunosuppressive function of MDSCs in human CRC.

IL-1β is the most secreted cytokine among those secreted by *C. tropicalis*-stimulated MDSCs

To reveal the effect of *C. tropicalis* on cytokines secreted by MDSCs, we treated MDSCs directly with *C. tropicalis* in vitro and then harvested the cell culture supernatant for proteomic analysis. Principal component analysis (PCA) demonstrated that there were significant differences between the *C. tropicalis* treatment group and the control group (Fig. 2A). Among the protein molecules secreted by *C. tropicalis*-stimulated MDSCs, 1897 protein molecules were significantly increased and 1488 protein molecules were significantly decreased (Fig. 2B and C). Among the top ranked protein molecules secreted

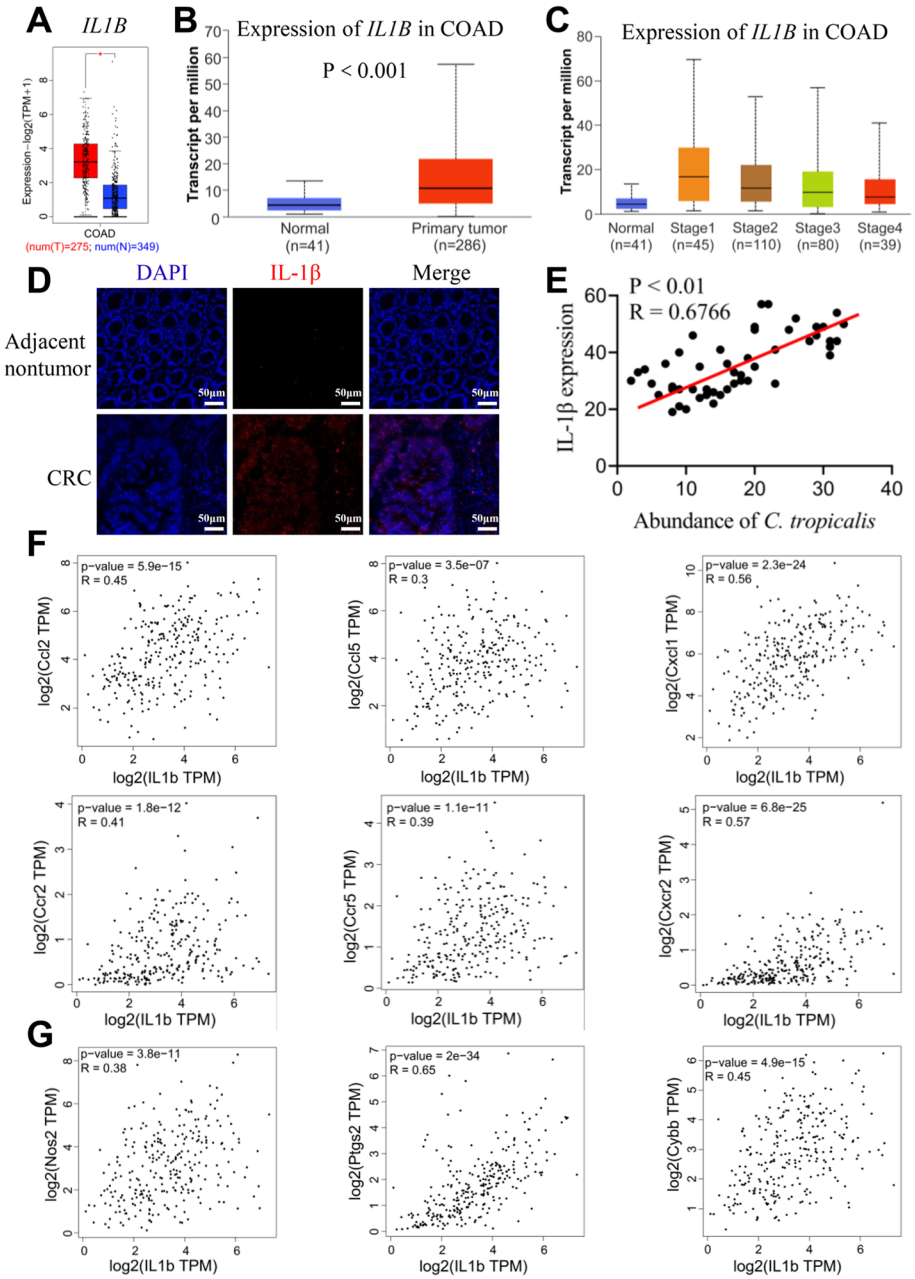

Fig. 1 The high expression of IL-1β is related to the infiltration and immunosuppressive function of MDSCs in patients with CRC. (**A**) The expression of *IL1B* in human normal colon tissues (N, $n = 349$) and COAD tumor tissues (T, $n = 275$) from GTEx and TCGA databases for COAD was analyzed by the GEPIA2 online analysis tool (http://gepia2.cancer-pku.cn/#index). (**B**) The expression of *IL1B* in human primary COAD tumor tissues ($n = 286$) and normal colon tissues ($n = 41$) from the TCGA database was analyzed by UALCAN database. (**C**) The expression of *IL1B* in human primary COAD tumor tissues based on individual cancer stages from the TCGA database was analyzed by UALCAN database. (**D**) The expression level of IL-1β in human CRC tissues and adjacent nontumor tissues was detected by immunofluorescence staining, $n = 50$. Scale bars, 50 μm. (**E**) The correlation between the abundance of *C. tropicalis* and IL-1β expression in human CRC tissues ($n = 50$). (**F** and **G**) The correlation between *IL1b* expression and *Ccl2*, *Ccl5*, *Cxcl1*, *Ccr2*, *Ccr5*, *Cxcr2*, *Nos2*, *Ptgs2* and *Cybb* expression in COAD from TCGA database was analyzed by GEPIA2. Data are presented as mean ± SEM. Statistical analysis was performed using the unpaired Student's *t*-test (**A** and **B**) and one-way ANOVA with Bonferroni's multiple comparisons test for post-hoc test (**C**). Correlation analysis was performed by Spearman's rank tests (**E**-**G**). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

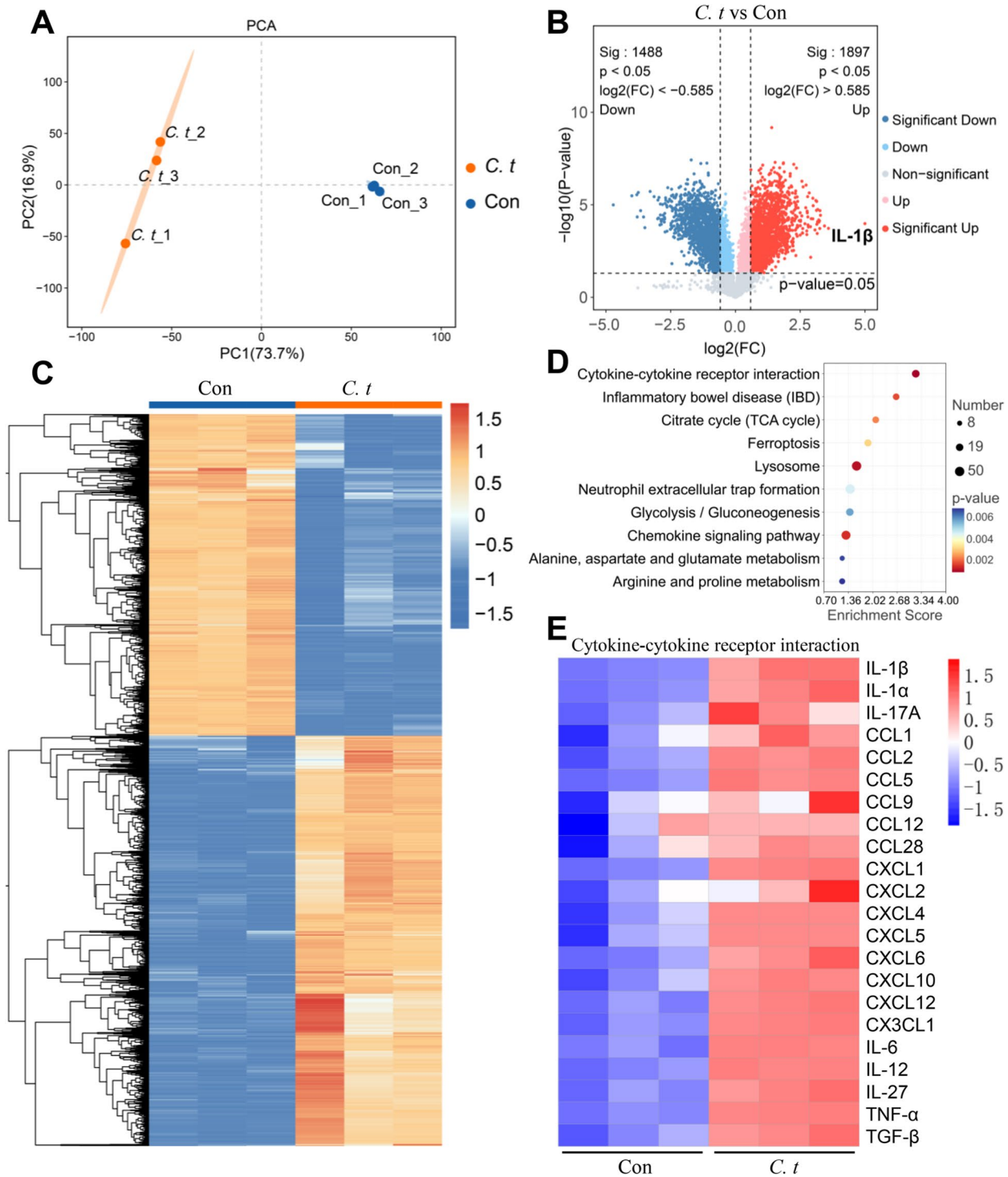

Fig. 2 Among the cytokines secreted by MDSCs stimulated by *C. tropicalis*, the secretion of IL-1β is the most. After stimulating WT MDSCs with *C. tropicalis* (MOI = 1) for 24 h, the cell culture supernatant was collected for proteomic analysis ($n = 3$). (**A**) Principal component analysis (PCA) was used to analyze the differences between the *C. tropicalis* treatment group and the control group. (**B** and **C**) Volcano map and heat map showed protein molecules with significant differences in the *C. tropicalis* treatment group compared with the control group. (**D**) KEGG signal pathway enrichment analysis revealed significant changes in signaling pathways. (**E**) Heat map indicated the significant differential factors mainly enriched in Cytokine-cytokine receptor interaction pathway. Con (Control), *C. t* (*C. tropicalis*)

by *C. tropicalis*-stimulated MDSCs, IL-1β was the most secreted cytokine (Fig. 2B). Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis also showed that *C. tropicalis* markedly upregulated Cytokine-cytokine receptor interaction pathway and Inflammatory bowel disease (IBD) pathway (Fig. 2D). We further screened for the significant differential factors mainly enriched in Cytokine-cytokine receptor interaction pathway. The results indicated that *C. tropicalis* significantly promoted the secretion of 22 cytokines, including IL-1β, CCL2, CCL5, CXCL1 and other cytokines (Fig. 2E). This suggested that *C. tropicalis* promoted excessive secretion of IL-1β from MDSCs and then IL-1β might be involved in the progression of CRC through Cytokine-cytokine receptor interaction. Thus, our results indicate that *C. tropicalis* induces MDSCs to secrete the highest levels of IL-1β.

IL-1β and *C. tropicalis* synergistically promote the generation and immunosuppressive function of MDSCs

We first examined whether IL-1β and *C. tropicalis* could induce the generation of MDSCs from bone marrow (BM) cells. We added GM-CSF and IL-6 to the BM cells culture system as a positive control for inducing the generation of MDSCs. In addition, we treated BM cells with either GM-CSF plus *C. tropicalis* or GM-CSF plus IL-1β. We found that either GM-CSF plus *C. tropicalis* or GM-CSF plus IL-1β observably increased the percentage of MDSCs (Fig. 3A and B). To further investigate the effect of IL-1β on *C. tropicalis*-stimulated MDSCs, we treated MDSCs with *C. tropicalis* and IL-1β alone or *C. tropicalis* plus IL-1β combination. Our qPCR and Western blot analysis showed that both *C. tropicalis* and IL-1β could markedly upregulated the expression of iNOS, COX2 and NOX2 (Fig. 3C and D). What's more, IL-1β further enhanced the expression of iNOS, COX2 and NOX2 upregulated by *C. tropicalis* (Fig. 3C and D). We then measured the levels of NO and ROS, the products of iNOS and NOX2, and found that IL-1β and *C. tropicalis* synergistically augmented the production of NO and ROS (Fig. 3E-G). Finally, we investigated the inhibitory effect of MDSCs on the proliferation of $CD8^+$ T cells, and cocultured MDSCs with $CD8^+$ T cells. The results demonstrated that IL-1β and *C. tropicalis* synergistically enhanced the suppressive effect of MDSCs on the proliferation of $CD8^+$ T cells (Fig. 3H and I). And IL-1β and *C. tropicalis* also synergistically promoted MDSCs to inhibit the secretion of IFNγ by $CD8^+$ T cells (Fig. 3J). Together, these data reveal that IL-1β and *C. tropicalis* synergistically enhance the expansion of MDSCs and the inhibition of MDSCs on $CD8^+$ T cells.

Blockade of IL-1β reverses the immunosuppressive function of MDSCs enhanced by *C. tropicalis*

To further study whether IL-1β mediates the immunosuppressive function of MDSCs enhanced by *C. tropicalis*, we first added GM-CSF and *C. tropicalis* to the BM cells culture system, along with Anti-IL-1β antibody or isotype Anti-IgG antibody. We observed that Anti-IL-1β antibody significantly inhibited the generation of MDSCs induced by *C. tropicalis* (Fig. 4A and B). We then added Anti-IL-1β antibody to the medium cocultured with MDSCs and *C. tropicalis* to neutralize IL-1β secreted by MDSCs. The results indicated that IL-1β neutralization markedly decreased the expression of iNOS, COX2 and NOX2 up-regulated by *C. tropicalis* (Fig. 4C and D). In addition, Anti-IL-1β antibody treatment notably suppressed NO and ROS production of *C. tropicalis*-stimulated MDSCs (Fig. 4E-G). Consistent with the above results, blocking IL-1β with Anti-IL-1β antibody reversed the immunosuppressive effect of *C. tropicalis*-stimulated MDSCs on $CD8^+$ T cells (Fig. 4H-J). Therefore, our findings conclusively suggest that IL-1β mediates the expansion and immunosuppressive function of MDSCs promoted by *C. tropicalis*.

Activation of NLRP3 inflammasome is required for the immunosuppressive function of MDSCs augmented by *C. tropicalis*

Since the activation of NLRP3 inflammasome promotes the maturation and secretion of IL-1β, we further explored whether the activation of NLRP3 inflammasome is involved in the expansion and immunosuppression of MDSCs induced by *C. tropicalis*. A selective and direct NLRP3 inhibitor, CY-09, has been identified to inhibit the activation of NLRP3 inflammasome [36]. In consequence, the BM cells were pretreated with CY-09. These cells were then treated with GM-CSF and *C. tropicalis*. The results demonstrated that inhibition of NLRP3 inflammasome activation significantly reduced the percentage of MDSCs increased by *C. tropicalis* (Fig. 5A and B). Subsequently, we pretreated MDSCs with CY-09 and then stimulated them with *C. tropicalis*. We found that CY-09 significantly reduced the expression of iNOS, COX2 and NOX2 promoted by *C. tropicalis* (Fig. 5C and D). Moreover, blockade of NLRP3 inflammasome activation markedly inhibited the production of NO and ROS induced by *C. tropicalis* (Fig. 5E-G). In addition, CY-09 treatment attenuated the immunosuppressive function of MDSCs stimulated by *C. tropicalis*, evidenced by increased proliferation and IFNγ secretion of $CD8^+$ T cells. (Fig. 5H-J). To sum up, these results further prove that *C. tropicalis* promotes the production and

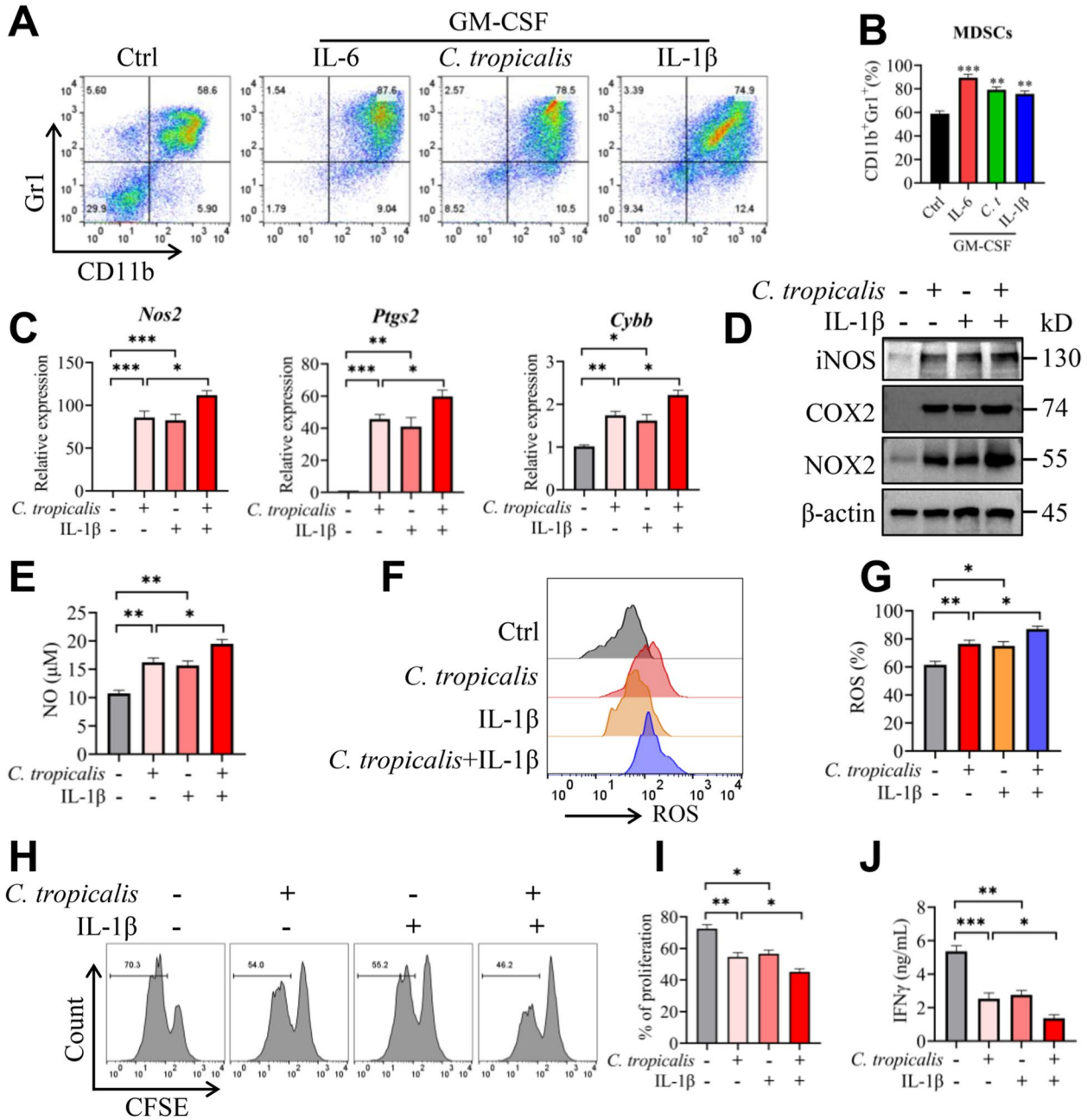

Fig. 3 IL-1β and *C. tropicalis* synergistically augment the expansion and immunosuppressive function of MDSCs. (**A** and **B**) BM cells were treated with medium (Ctrl), GM-CSF (40 ng/ml) + IL-6 (40 ng/ml), GM-CSF (40 ng/ml) + C.t (*C. tropicalis*, MOI = 1), GM-CSF (40 ng/ml) + IL-1β (10 ng/ml) for 4 days, respectively. The percentage of MDSCs ($CD11b^+Gr1^+$) was detected by flow cytometry. (**C**) MDSCs were treated with *C. tropicalis* (MOI = 1), IL-1β (10 ng/ml) or *C. tropicalis* (MOI = 1) + IL-1β (10 ng/ml) for 6 h. Then the expression of *Nos2*, *Ptgs2* and *Cybb* were measured by qPCR. (**D**) MDSCs were treated with *C. tropicalis* (MOI = 1), IL-1β (10 ng/ml) or *C. tropicalis* (MOI = 1) + IL-1β (10 ng/ml) for 48 h. Then the expression of iNOS, COX2 and NOX2 were detected by Western blot. (**E-G**) MDSCs were treated with *C. tropicalis* (MOI = 5), IL-1β (10 ng/ml) or *C. tropicalis* (MOI = 5) + IL-1β (10 ng/ml) for 48 h. The concentration of NO in the cell culture supernatant was detected (**E**), and the production of ROS in MDSCs was analyzed by flow cytometry (**F** and **G**). (**H** and **I**) The immunosuppressive effect of MDSCs on $CD8^+$ T cell proliferation was analyzed by flow cytometry. (**J**) MDSCs were treated with *C. tropicalis* (MOI = 1), IL-1β (10 ng/ml) or *C. tropicalis* (MOI = 1) + IL-1β (10 ng/ml) for 24 h, and then these MDSCs and $CD8.^+$ T cells were cocultured in 96-well plates in a ratio of 1:1 for 48 h. The concentration of IFNγ in the cell culture supernatant was detected by ELISA. Data are presented as mean ± SEM, $n = 3$. Each panel displays at least three biological replicates from a representative experiment. Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple comparisons test for post-hoc test. ns (not significant), $P > 0.05$; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

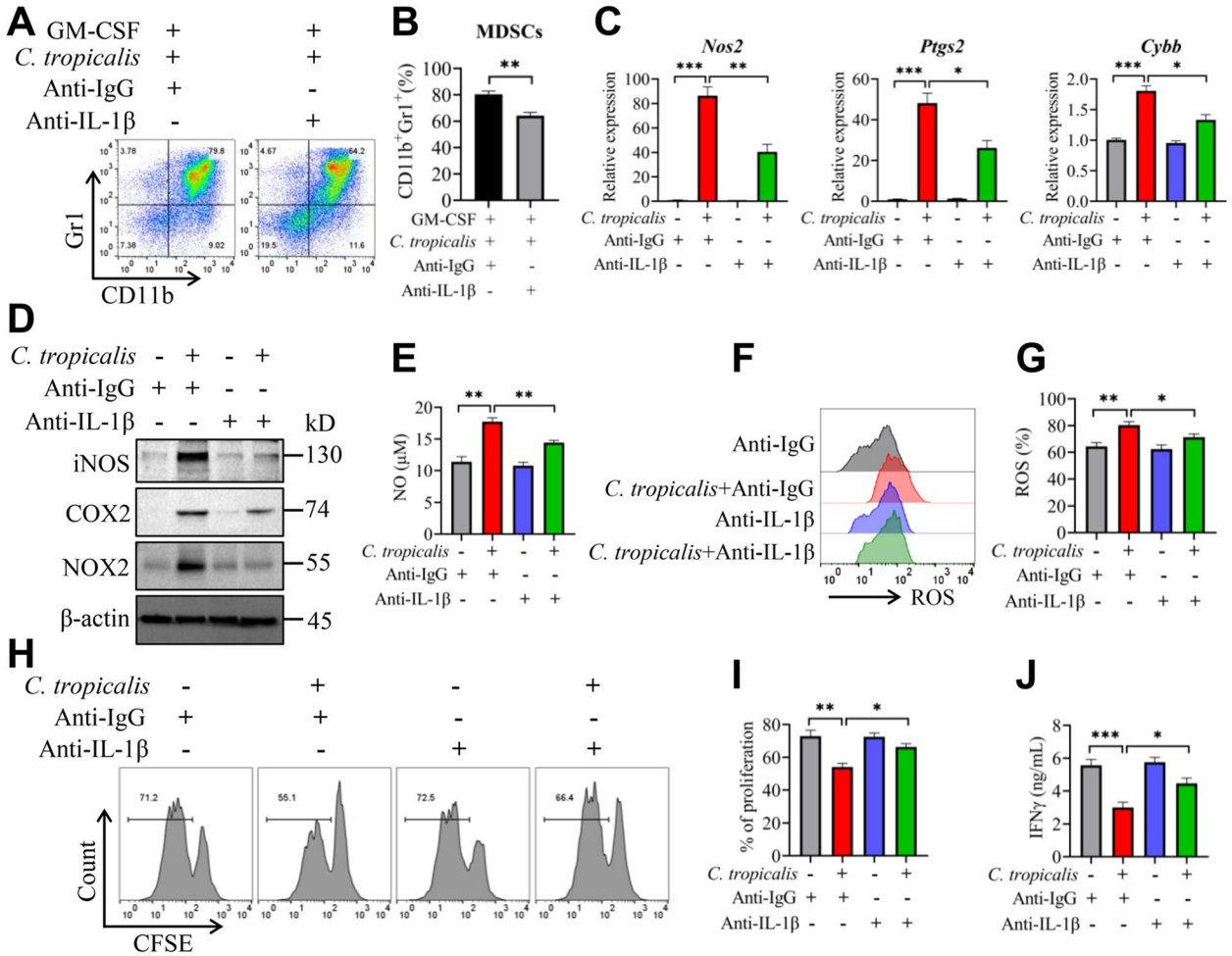

Fig. 4 Blocking IL-1β reverses the immunosuppressive function of MDSCs enhanced by *C. tropicalis*. (**A** and **B**) GM-CSF (40 ng/ml) and *C. tropicalis* (MOI = 1) were added to the BM cells culture system, along with Anti-IL-1β antibody (1 μg/ml) or isotype Anti-IgG antibody (1 μg/ml). After 4 days of culture, the percentage of MDSCs ($CD11b^+Gr1^+$) was determined by flow cytometry. (**C** and **D**) Anti-IL-1β antibody (1 μg/ml) or isotype Anti-IgG antibody (1 μg/ml) were added to the medium cocultured with MDSCs and *C. tropicalis* (MOI = 1). After 6 h or 48 h of culture, the mRNA and protein expression of iNOS, COX2 and NOX2 were detected by qPCR and Western blot, respectively. (**E-G**) Anti-IL-1β antibody (1 μg/ml) or isotype Anti-IgG antibody (1 μg/ml) were added to the medium cocultured with MDSCs and *C. tropicalis* (MOI = 5). After 48 h of culture, the concentration of NO in the cell culture supernatant was detected (**E**), and the production of ROS in MDSCs was analyzed by flow cytometry (**F** and **G**). (**H** and **I**) The immunosuppressive effect of MDSCs on $CD8^+$ T cell proliferation was analyzed by flow cytometry. (**J**) Anti-IL-1β antibody (1 μg/ml) or isotype Anti-IgG antibody (1 μg/ml) were added to the medium cocultured with MDSCs and *C. tropicalis* (MOI = 1). After 24 h of culture, these MDSCs were then cocultured with $CD8.^+$ T cells in 96-well plates in a ratio of 1:1 for 48 h. The concentration of IFNγ in the cell culture supernatant was detected by ELISA. Data are presented as mean ± SEM, $n = 3$. Each panel displays at least three biological replicates from a representative experiment. Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple comparisons test for post-hoc test and the unpaired Student's *t*-test. ns (not significant), $P > 0.05$; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

immunosuppressant function of MDSCs by activating NLRP3 inflammasome.

Neutralization of IL-1β secreted by MDSCs alleviates the progression of *C. tropicalis*-associated colon *cancer*

To further verify whether IL-1β secreted by MDSCs induced by *C. tropicalis* is involved in the progression of colon cancer, we established a mouse colon cancer xenograft model by using MC38 mouse colon cancer cells. In the process of model construction, we first treated mice with Anti-Gr1 antibody to deplete MDSCs, and then injected mice intravenously with MDSCs pretreated with either *C. tropicalis* or Anti-IL-1β antibody (Fig. 6A). We discovered that adoptive transfer of *C. tropicalis*-pretreated MDSCs significantly promoted tumor growth and increased tumor weight and volume (Fig. 6B-D). However, Anti-IL-1β antibody markedly suppressed the promoting effect of *C.*

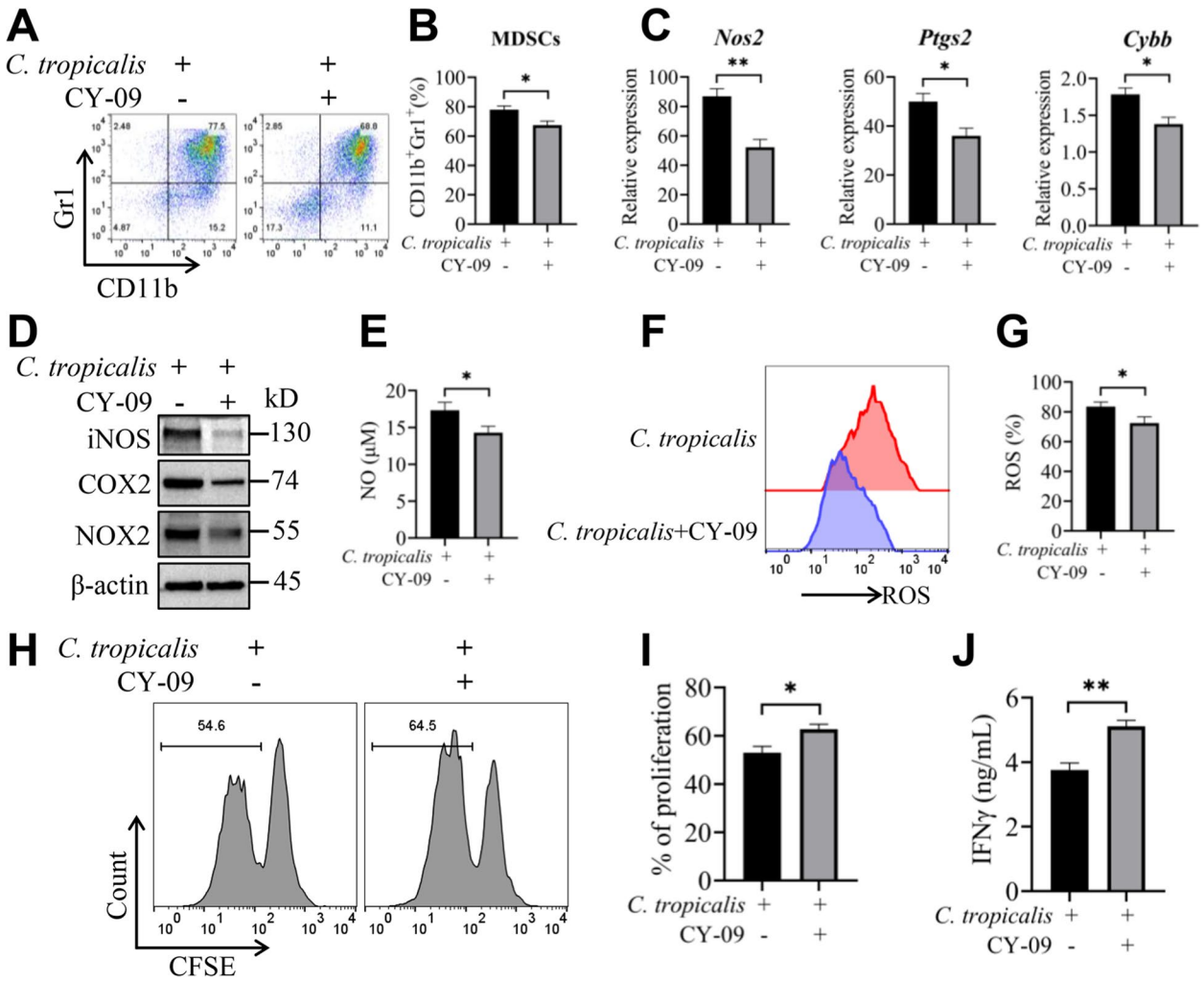

Fig. 5 NLRP3 inflammasome activation mediates the immunosuppressive function of MDSCs enhanced by ***C. tropicalis***. (**A** and **B**) The BM cells were pretreated with or without CY-09 (10 μM). These cells were then treated with GM-CSF (40 ng/ml) and *C. tropicalis* (MOI = 1) for 4 days. The percentage of MDSCs ($CD11b^+Gr1^+$) was measured by flow cytometry. (**C** and **D**) MDSCs were first pretreated with CY-09 (10 μM) and then stimulated with *C. tropicalis* (MOI = 1) for 6 h or 48 h. The mRNA and protein expression of iNOS, COX2 and NOX2 were detected by qPCR and Western blot, respectively. (**E-G**) MDSCs were first pretreated with CY-09 (10 μM) and then stimulated with *C. tropicalis* (MOI = 5) for 48 h. The concentration of NO in the cell culture supernatant was detected (**E**), and the production of ROS in MDSCs was analyzed by flow cytometry (**F** and **G**). (**H** and **I**) The immunosuppressive effect of MDSCs on $CD8^+$ T cell proliferation was analyzed by flow cytometry. (**J**) MDSCs were first pretreated with CY-09 (10 μM) and then stimulated with *C. tropicalis* (MOI = 1) for 24 h. These MDSCs were then cocultured with $CD8.^+$ T cells in 96-well plates in a ratio of 1:1 for 48 h. The concentration of IFNγ in the cell culture supernatant was detected by ELISA. Data are presented as mean ± SEM, $n = 3$. Each panel displays at least three biological replicates from a representative experiment. Statistical analysis was performed using the unpaired Student's *t*-test. ns (not significant), $P > 0.05$; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

tropicalis-pretreated MDSCs on colon cancer (Fig. 6B-D). Histopathological analysis showed that the histological score of tumor tissue was obviously elevated in tumor-bearing mice adoptively transferred by *C. tropicalis*-pretreated MDSCs (Fig. 6E). Moreover, neutralizing IL-1β secreted by MDSCs with Anti-IL-1β antibody significantly reduced the histological score of tumor tissue (Fig. 6E). To evaluate the proliferation of MC38 colon cancer cells, we further detected the expression of PCNA and Ki-67 in tumor tissues by immunohistochemical staining. The results indicated that after the tumor-bearing mice were adoptively transferred by *C. tropicalis*-pretreated MDSCs, the expression of PCNA and Ki-67 in tumor tissue were significantly elevated (Fig. 6F). However, IL-1β neutralization reversed the proliferation of MC38 colon cancer cells enhanced by *C. tropicalis*-pretreated MDSCs (Fig. 6F). In conclusion, these data suggest that targeting IL-1β secreted by

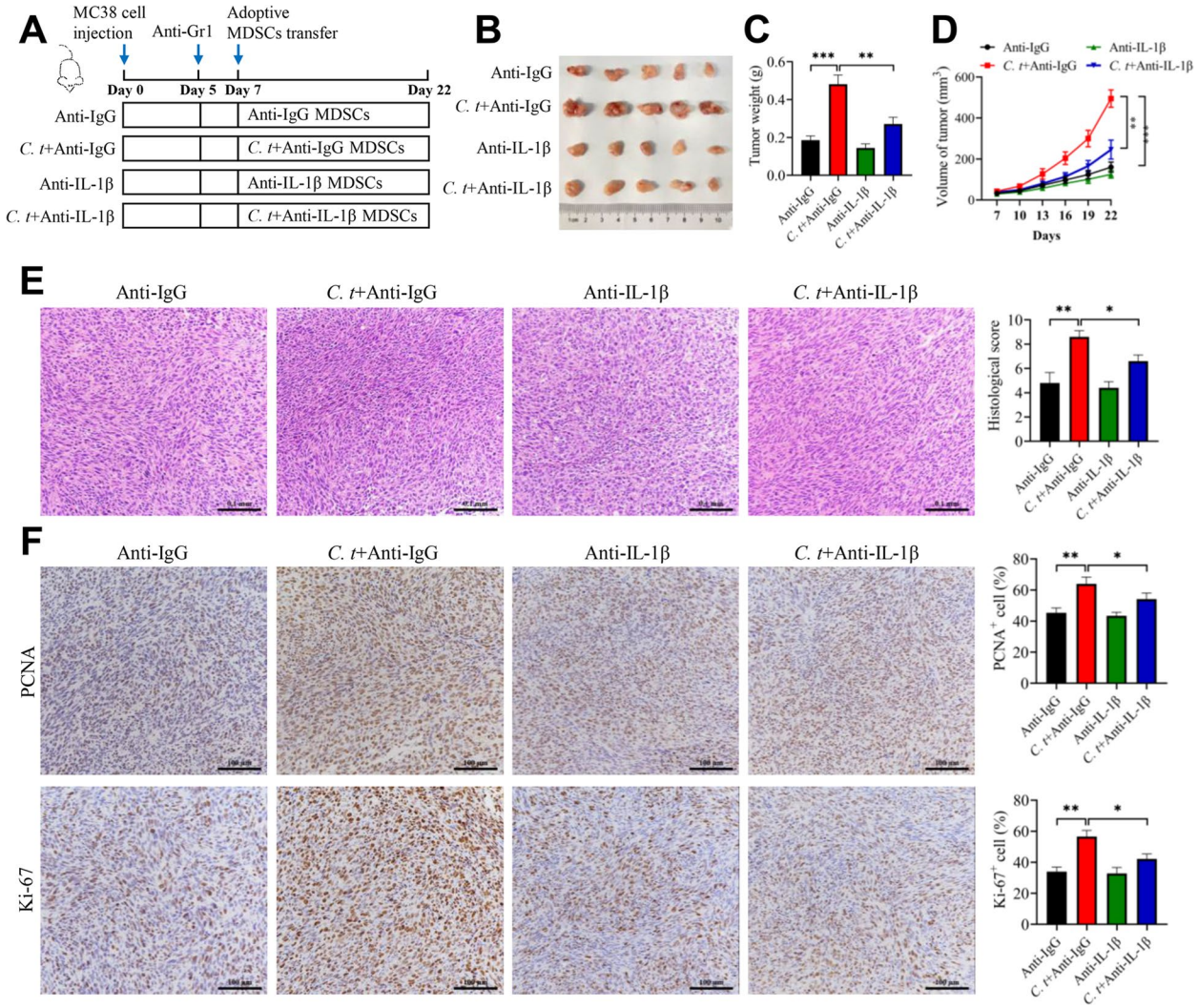

Fig. 6 Neutralizing IL-1β secreted by MDSCs suppresses the development of *C. tropicalis*-associated colon cancer. (**A**) Schematic diagram of establishing a mouse colon cancer xenograft model by using MC38 mouse colon cancer cells. (**B**) The image of tumor in MC38 colon cancer xenograft model. (**C** and **D**) The weight and volume of the tumor were measured. (**E**) Representative histological images of tumor tissues stained by H&E. The pathologist evaluated the histological score. Scale bars, 100 μm. (**F**) The expression of PCNA and Ki-67 in tumor tissues of MC38 colon cancer xenograft model were determined by immunohistochemical staining. The percentage of PCNA-positive and Ki-67-positive tumor cells were assessed. Scale bars, 100 μm. Data are presented as mean ± SEM, $n = 6$. Each panel displays at least six biological replicates from a representative experiment. Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple comparisons test for post-hoc test. ns (not significant), $P > 0.05$; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

MDSCs inhibits the progression of *C. tropicalis*-associated colon cancer.

Blockade of IL-1β secreted by MDSCs enhances antitumor immunity in *C. tropicalis*-associated colon *cancer*

To elucidate the mechanism by which neutralization of IL-1β secreted by MDSCs alleviates tumor progression, we analyzed the percentage and phenotype of $CD8^+$ T cells in the spleen and tumors of the above colon cancer xenograft model. The results demonstrated that adoptive transfer of *C. tropicalis*-pretreated MDSCs significantly reduced the infiltration of $CD8^+$ T cells in spleen and tumor (Fig. 7A-D). However, blocking IL-1β secreted by MDSCs restored $CD8^+$ T cell infiltration in the spleen and tumors of tumor-bearing mice adoptively transferred by *C. tropicalis*-pretreated MDSCs (Fig. 7A-D). We further detected the expression of CD8α in tumor tissues by Western blot assay. Consistent with the above results, the expression of CD8α was significantly inhibited after adoptive transfer of *C. tropicalis*-pretreated MDSCs in tumor-bearing mice (Fig. 7G). Moreover, Anti-IL-1β antibodies reversed this effect of MDSCs pretreated with

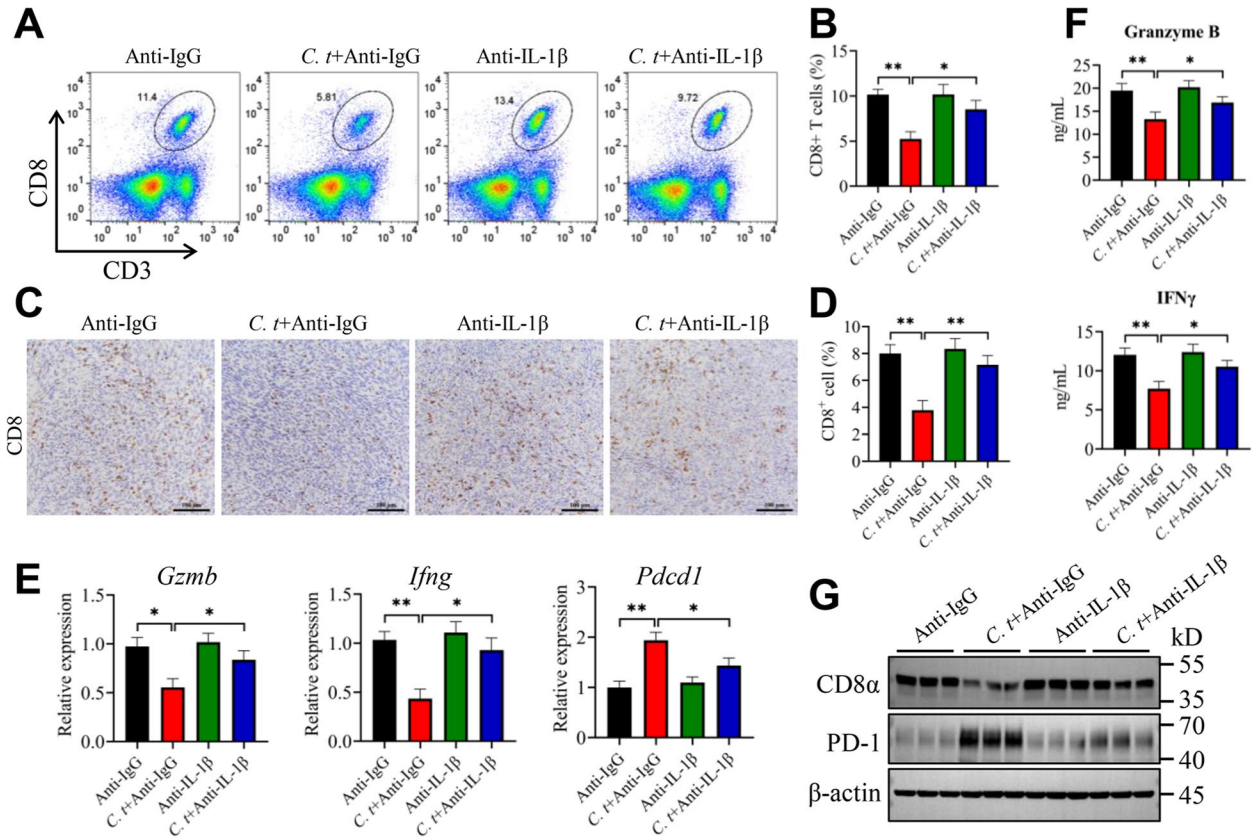

Fig. 7 Blockade of MDSCs-derived IL-1β facilitates antitumor immunity in ***C. tropicalis***-associated colon cancer. (**A** and **B**) The percentage of $CD8^+$ T cells in the spleen of MC38 colon cancer xenograft model was analyzed by flow cytometry. (**C** and **D**) Immunohistochemical staining was used to detect the infiltration of $CD8.^+$ T cells in tumor tissues of MC38 colon cancer xenograft model. Scale bars, 100 μm. **(E)** The mRNA expression of *Gzmb*, *Ifng* and *Pdcd1* in tumor tissues were detected by qPCR. (**F**) The levels of Granzyme B and IFNγ in tumor tissue were measured by ELISA. (**G**) The expression of CD8α and PD-1 in tumor tissues was detected by Western blot. Data are presented as mean ± SEM, $n = 6$. Each panel displays at least six biological replicates from a representative experiment. Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple comparisons test for post-hoc test. ns (not significant), $P > 0.05$; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

C. tropicalis (Fig. 7G). Notably, *C. tropicalis*-pretreated MDSCs also resulted in lower expression level of Granzyme B and IFNγ and higher expression level of PD-1 in tumors of tumor-bearing mouse (Fig. 7E-G). However, this effect was eliminated when IL-1β was blocked with Anti-IL-1β antibody (Fig. 7E-G). This suggested that blockade of IL-1β increased cytotoxic T cell activation in colon cancer. Taken together, these results support that blocking IL-1β by Anti-IL-1β antibody boosts antitumor immunity in *C. tropicalis*-associated colon cancer.

Discussion

CRC is directly associated with inflammation, as evidenced by the invasion of multiple types of immune cells and the secretion of pro-inflammatory cytokines. The pro-inflammatory cytokine IL-1β is a pleiotropic cytokine that can be involved in tumor progression and tumor metastasis through various pathways. The mechanism of IL-1β promoting tumor is complex. For example, IL-1β can recruit immunosuppressive cells (TAM, MDSCs, Treg), and promote angiogenesis and endothelial cell activation. Concerning the tumor metastasis, previous studies have indicated that IL-1β promotes tumor metastasis by inducing angiogenesis, inhibiting antitumor immunity and increasing the expression of adhesion molecules [25, 37, 38]. Therefore, the level of IL-1β expression was closely correlated with tumor metastasis. As a result, IL-1β has become a therapeutic target for many tumors, such as CRC, breast cancer, lung cancer, and pancreatic cancers. However, the role of IL-1β in antitumor immunity of *C. tropicalis*-associated CRC is not fully understood. In the present study, we demonstrated for the first time that IL-1β inhibited antitumor immunity by mediating the immunosuppressive function of MDSCs enhanced by *C. tropicalis*, thereby participating in the progression of CRC.

Previous studies by our research group have demonstrated that *C. tropicalis* is significantly enriched in the

intestines of patients with CRC and closely correlated with the development and progression of CRC [19]. Furthermore, the abundance of *C. tropicalis* was also markedly increased in the gut of tumor-bearing *Card9*$^{-/-}$ mice [19] and *C. tropicalis* exacerbated the disease severity of CRC by augmenting the immunosuppressive function of MDSCs [20]. Therefore, *C. tropicalis* plays an essential role in the development and progression of CRC. There is an urgent need to find therapeutic strategies and therapeutic targets for *C. tropicalis*-associated CRC. Our recent findings revealed that *C. tropicalis* facilitated CRC by activating the NLRP3 inflammasome of MDSCs. However, the relationship between IL-1β produced by the NLRP3 inflammasome and the immunosuppressive function of MDSCs enhanced by *C. tropicalis* in the tumor microenvironment of CRC has not been clearly defined. Understanding the relationship between IL-1β produced by the NLRP3 inflammasome and the immunosuppressive function of MDSCs augmented by *C. tropicalis* may provide a potential immunotherapeutic target for the treatment of *C. tropicalis*-associated CRC. In this study, we elucidated for the first time that IL-1β produced by the NLRP3 inflammasome promoted *C. tropicalis*-associated CRC by mediating the immunosuppressive function of MDSCs augmented by *C. tropicalis*. Our present study also indicated for the first time that blocking IL-1β secreted by MDSCs through Anti-IL-1β antibodies alleviated the progression of *C. tropicalis*-associated CRC by enhancing antitumor immunity. Therefore, IL-1β secreted by MDSCs is a promising immunotherapeutic target for clinical treatment of *C. tropicalis*-associated CRC. Therefore, our study has profound clinical implications, providing a promising immunotherapeutic target for clinical treatment of *C. tropicalis*-associated CRC.

In this study, we analyzed the TCGA database and tumor tissues of patients with CRC to show that IL-1β expression was significantly elevated in CRC. And the abundance of *C. tropicalis* was positively correlated with IL-1β expression. Furthermore, high level of IL-1β was strongly associated with recruitment and immunosuppressive function of MDSCs in CRC. Our proteomic analysis then revealed that *C. tropicalis* promoted high level of IL-1β secretion from MDSCs, and IL-1β is the most secreted cytokine from MDSCs stimulated by *C. tropicalis*. This suggests that IL-1β may play a crucial role in the immunosuppressive function of MDSCs induced by *C. tropicalis*. A recent study using single-cell sequencing analysis indicated that IL-1β is the gene most associated with resistance to anti-PD-1 therapy in patients with CRC [29]. Moreover, this study further found that IL-1β was positively correlated with MDSCs by analyzing tumor tissue samples from patients with CRC, whereas IL-1β was negatively correlated with CD8$^+$ T cells. Earlier studies have reported that chemotherapy agents can activate the NLRP3 inflammasome in MDSCs, leading to the production of IL-1β, which reduces antitumor immunity [39]. Therefore, these results all confirm that IL-1β can be involved in the progression of CRC by inhibiting antitumor immunity. In the current study, our proteomic analysis also showed that *C. tropicalis* significantly upregulated Cytokine-cytokine receptor interaction pathway. Therefore, we speculated that the IL-1β secreted by MDSCs is likely to act on MDSCs through its receptor IL-1R, thereby regulating the differentiation and function of MDSCs. Targeting IL-1R has been reported to alleviate many diseases caused by IL-1β. For example, anti-IL-1R antibodies can reduce the severity of colitis and IL-1R antagonists can enhance the antitumor effects of chemotherapy drugs [39, 40]. Thus, this further suggests that IL-1β/ IL-1R signaling may play a crucial role in the progression of CRC.

MDSCs exert immunosuppressive function to inhibit the proliferation and activation of T cells through various molecular mechanisms [41]. In the present study, we found that IL-1β and *C. tropicalis* synergistically facilitated the expansion and immunosuppressive function of MDSCs. Moreover, blocking IL-1β by Anti-IL-1β antibody reversed the immunosuppressive function of MDSCs enhanced by *C. tropicalis*. Consistently, when MDSCs were treated with an inhibitor of NLRP3 inflammasome activation, the immunosuppressive function of MDSCs stimulated by *C. tropicalis* was suppressed. These results are sufficient to indicate that *C. tropicalis* promotes the production of IL-1β by inducing the NLRP3 inflammasome activation, thereby enhancing the immunosuppressant effect of MDSCs on T cells.

In recent years, increasing studies have shown that immunotherapy is the most promising therapeutic strategy for tumor treatment. For example, immune checkpoint blockade treatment has exhibited promising results in patients with CRC. Recent studies have revealed that enhanced antitumor immunity can promote the efficacy of anti-PD-1 in CRC [42, 43]. In this study, our findings suggested that targeted inhibition of IL-1β secreted by MDSCs remarkedly enhanced antitumor immunity and mitigated the progression of *C. tropicalis*-associated CRC. We also elucidated the mechanism by which blockade of IL-1β secreted by MDSCs alleviates *C. tropicalis*-associated CRC. Our results suggested that targeting IL-1β secreted by MDSCs with Anti-IL-1β antibody increased CD8$^+$ T cell infiltration and promoted cytotoxic T cell activation in CRC. Therefore, specifically targeting IL-1β secreted by MDSCs is a promising therapeutic strategy for CRC.

In general, not only MDSCs can secrete IL-1β, but also tumor cells or other immune cells (including

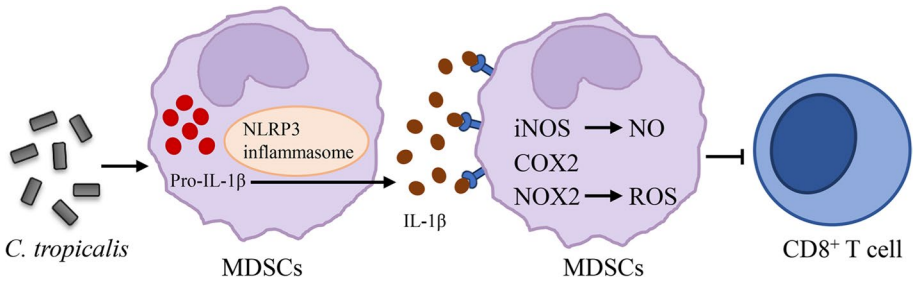

Fig. 8 Diagrammatic depiction of the role of IL-1β in mediating the immunosuppressive function of MDSCs enhanced by *C. tropicalis* to suppress antitumor immunity

macrophages, DCs, etc.) can secrete IL-1β [44, 45]. Our previous studies have suggested that *C. tropicalis* promotes CRC progression by enhancing the expansion and immunosuppressive function of MDSCs [19, 20]. Moreover, our recent study further reported that *C. tropicalis* promoted the development of CRC by inducing the activation of the NLRP3 inflammasome and the production of IL-1β in MDSCs [21]. However, when *C. tropicalis* acted directly on CRC cells, it had no effect on tumor cells. Meanwhile, *C. tropicalis* also had little effect on the function and activation of other immune cells in the tumor microenvironment of CRC. In the present study, our proteomic analysis showed that IL-1β was the most secreted cytokine of MDSCs stimulated by *C. tropicalis*. Therefore, we concluded that *C. tropicalis* promoted CRC mainly by regulating IL-1β secreted by MDSCs and IL-1β secreted by other cells had little effect on CRC promoted by *C. tropicalis*.

Conclusions

In conclusion, this study suggests the role and mechanism of IL-1β in mediating the immunosuppressive function of MDSCs enhanced by *C. tropicalis*. *C. tropicalis* augments the immunosuppressive function of MDSCs by promoting the secretion of IL-1β from MDSCs, thereby facilitating the development of CRC (Fig. 8). Blockade of IL-1β secreted by MDSCs exhibits prospective antitumor efficacy against *C. tropicalis*-associated CRC, highlighting that MDSCs-secreted IL-1β may be an effective immunotherapeutic target for the treatment of CRC.

Abbreviations

CRC	Colorectal cancer
COAD	Colon adenocarcinoma
BM	Bone marrow
MDSCs	Myeloid-derived suppressor cells
IL-1β	Interleukin-1β
GM-CSF	Granulocyte–macrophage colony-stimulating factor
IL-6	Interleukin-6
PCA	Principal component analysis
iNOS	Inducible nitric oxide synthase
NOX2	NADPH oxidase 2
COX2	Cyclooxygenase 2
ROS	Reactive oxygen species
NO	Nitric oxide
CFSE	Carboxyfluorescein succinimidyl ester
IFNγ	Interferon γ
PCNA	Proliferating cell nuclear antigen
PD-1	Programmed cell death protein 1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-024-01771-y.

Additional file 1: Fig. S1. Related to Fig. 1. (A) The expression level of NLRP3 in human CRC tissues and adjacent nontumor tissues was determined by immunofluorescence staining, $n = 50$. Scale bars, 50 μm. (B) The correlation between the abundance of *C. tropicalis* and NLRP3 expression in human CRC tissues. Correlation analysis was performed by Spearman's rank tests.

Additional file 2: Full uncropped membrane blots images of Western Blot.

Acknowledgements

Not applicable

Authors' contributions

TW and AL designed and conceived this work. ZZ, YC and XP carried out the experiments. ZZ, PL and ZR collected, analyzed and interpreted data. YC and SS supervised the study and contributed to the visualization of results. ZZ wrote the original manuscript. ZZ, XW and TW revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data supporting the conclusions of this work are accessible in the Additional file and manuscript. The source data generated for the present study are available on reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

The patients included provided their written informed consent. All studies involving tumor tissue and adjacent nontumor tissue samples from patients with CRC were performed in accordance with the approval of the Ethics Committee of Medical School, Nanjing University. All mouse studies were approved by the Institutional Animal Care and Use Committee (IACUC-D2202134) of Nanjing University.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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