Modified Shenlingbaizhu decoction reduces intestinal adenoma formation in adenomatous polyposis coli multiple intestinal neoplasia mice by suppression of hypoxia-inducible factor 1α-induced CD4+CD25+forkhead box P3 regulatory T cells

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Abstract

OBJECTIVE: To test the hypothesis that modified Shenlingbaizhu decoction (MSD) attenuates the formation of intestinal adenomas by regulating activation of CD4+CD25+forkhead box P3 (FoxP3) regulatory T cells (Tregs) by downregulation of hypoxia-inducible factor 1α (HIF-1α).

METHODS: Chemical fingerprints of ginsenoside Rb1, Ginsenoside Rc, paeoniflorin, and dioscin in standard extractions were used as material bases of MSD. Adenomatous polyposis coli multiple intestinal neoplasia (ApcMin/+ ) mice, which harbor a mutation in adenomatous polyposis coli, were used to host intestinal adenomas. Peripheral blood and spleen Tregs were analyzed by flow cytometry. Protein expression was analyzed by immunohistochemistry and Western blotting.

RESULTS: The number and size of intestinal adenomas were significantly reduced by MSD treatment. Mucosal thickening and the spleen size were also substantially decreased by MSD. The carcinogenesis process in ApcMin/+ mice resembled that of human colorectal cancer. Molecular markers of neoplasms, such as β-catenin, cyclooxygenase-2, proliferating cell nuclear antigen, and p53, were substan-

RESEARCH ARTICLE

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Mor-associated Tregs and their influence on the prognosis of solid tumors.

CD4+CD25+FoxP3+ Tregs reduce host antitumor responses, and the presence of Tregs has been associated with poor prognoses of several types of solid tumors. However, the function of tumor-associated Tregs and their influence on the prognosis of CRC are still unclear.

Shenlingbaizhu San is a classic formula of Traditional Chinese Medicine, which was first described in "The Prescriptions of the Bureau of Taiping People’s Welfare Pharmacy" during the Song dynasty. It is effective in improving the Karnofsky performance scale score and increasing CD4+, CD8+, and natural killer T cells in postoperative CRC patients with spleen deficiency syndrome. Modified Shenlingbaizhu decoction (MSD), a well-known formula of Nanfang Hospital, has been used for the treatment of CRC for more than 10 years. A recent study showed that MSD ameliorates CRC by improving inflammatory and immunosuppressive microenvironments in CRC patients.

The adenomatous polyposis coli multiple intestinal neoplasia (ApcaMIN/) mouse harbors a mutation in the adenomatous polyposis coli gene, which is similar or even identical to the mutation found in individuals with familial adenomatous polyposis and 70% of all sporadic CRC cases. In this study, we investigated whether MSD suppressed the incidence of spontaneous intestinal polyposis in ApcaMIN mice and regulated tumor-infiltrating Tregs.

MATERIALS AND METHODS

Preparation of MSD

MSD consists of eleven commonly used herbs: Baizhu (Rhizoma Atractylodis Macrocephalae), Wuzhaolong (Cayratia japonica), Fuling (Poria), Huotanmu (Herba Polygoni Chinensis), Yiyiren (Senen Coicis), Roucongrong (Herba Cistanche Deserticolaec), Renshen (Radix Ginseng), Chuunpi (Toonaninensi Roem), Liougewang (Radix Wikstroemae), Chongjou (Rhiza Paridis Chonglou), Ezhu (Rhiza Carccumae Pharaohisi), and Baishao (Radix Paeoniae Alba). The raw herbs for MSD were purchased from the Affiliated Nan Fang Hospital of Southern Medical University. The herbs were identified as genuine regional drugs by Prof. Chen Xingxing, an expert of Chinese medicine identification, Southern Medical University. The specimens were deposited in the storage cabinet of the Chinese Medical Material Department of the School of Traditional Chinese Medicine, Southern Medical University. They were mixed at a ratio of 20:10:10:10:5:5:5:4:4:4:4 (dry weight). Aqueous extracts of MSD were prepared at 80 °C in 10 volumes of distilled water (mL) by stirring for 1 h. Then, the extract was centrifuged at 1500 × g at room temperature. To obtain a semisolid MSD solution, the supernatant was collected and subjected to condensation under reduced pressure at 70 °C. Quality control of MSD was performed by high performance liquid chromatography. MSD was stored in 0.9% saline at a final concentration of 0.2 g/mL. The solution was stored in aliquots at –20 °C.

Animals

ApcaMIN male mice on a C57BL/6 background (Jackson Laboratories) were bred with female C57BL/6 mice at Southern Medical University. Specific-pathogen-free C57BL/6J mice were obtained from the Laboratory...
Animal Center of Southern Medical University. Offspring at weaning were genotyped as heterozygotes for the Apc gene by real-time polymerase chain reaction (RT-PCR) using tail DNA. Six C57BL/6 mice (three males and three females), weighing (20±2) g at 4 weeks of age, were used as the wild-type control. Mice were maintained in a 12:12 h light-dark cycle in a low stress environment and provided food and water ad libitum. All procedures involving laboratory animals were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Southern Medical University. Protocols were approved by the Animal Care Ethics Committee of Southern Medical University (Approval No. 2011-0015).

**Experimental procedures**

Male and female Apc<sup>min</sup> mice were randomly assigned to either MSD or placebo treatments by a random number table. MSD (11.21 g/kg) or equivalent normal saline were administered by gavage using a tube twice a day. MSD administration began at 4 weeks of age and continued for 14 weeks. The mice were sacrificed by cervical dislocation at week 18, and colons from the ileocelecal junction to the anal verge were collected. After measuring their length and weight, the colons were cut open longitudinally along the main axis. The number, size, and location of pre-neoplastic and neoplastic lesions (dysplasia and carcinoma, respectively) in the colons were recorded based on gross examination. Then, the colons were cut into pieces at about 1 cm intervals and stored at −80°C for immunohistochemistry, RT-PCR, and protein analyses.

**Flow cytometric analysis of Tregs**

Peripheral blood cells and splenocytes were stained with a FITC-conjugated anti-mouse CD4 monoclonal antibody (mAb) (BD, Franklin, NJ, USA), anti-mouse CD25 mAb (BD, Franklin, NJ, USA), and PE-Cy5-conjugated anti-mouse/rat FoxP3 mAb (BD, Franklin, NJ, USA). The cells were fixed with 1% paraformaldehyde/PBS and analyzed using a FACSCalibur flow cytometer and CellQuest software (BD, Franklin, NJ, USA).

**Histopathological assessment**

For histopathological examination, formalin fixed, paraffin-embedded colon tissues were cut into serial sections (5 μm thick) and stained with hematoxylin and eosin. Histological alterations were assessed, such as dysplasia and carcinoma. Assessment of dysplasia and adenocarcinoma was based on the criteria in the Mouse Models of Intestinal Cancers consensus report.  

**Immunohistochemistry**

Paraffin-embedded colon sections were dewaxed, rehydrated, and pre-treated with hydrogen peroxidase in PBS. Heat-induced antigen retrieval was performed. Sections were incubated with anti-proliferating cell nuclear antigen (PCNA) (clone PC10, 1:300 dilution; Thermo scientific, MA, USA), anti-β-catenin (clone 6B3, 1:100 dilution; Cell Signaling Technology, Boston, USA), anti-p53 (clone CM5, 1:100 dilution; Cell Signaling Technology), anti-COX-2 (1:100 dilution, Thermo scientific, Waltham, MA, USA), or anti-HIF-1α (1:100 dilution, Santa Cruz, Dallas, TX, USA) antibodies. After incubation with a horseradish peroxidase (HRP)-conjugated secondary antibody and tyramide amplification followed by streptavidin-HRP, positive signals were visualized by a DAB kit. Double staining was carried out using a Polink RRt DouSP kit (ZSGB-BIO ORIGENE, Shanghai, China). CD4 (1:100 dilution; Abcam, Cambridge, MA, USA), CD25 (1:20 dilution; Abcam, Cambridge, MA, USA), and FoxP3 (1:200 dilution; Abcam, Cambridge, MA, USA). Sections were examined at x400 magnification and analyzed using NIS-Elements software. The positive content (PC) was calculated by the following formula: PC = mean optical density × positive area.

**Gene and protein expression analyses**

Gene expression was analyzed by quantitative RT-PCR. Total RNA was extracted with TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions. First-strand cDNA synthesis was performed with a cDNA synthesis kit (TaKaRa, Dalian, China). Quantitative RT-PCR was performed using a SYBR Green real-time PCR kit (TaKaRa, Dalian, China). Fold changes were calculated by the ΔΔCt method. All experiments were performed with three biological replicates. Primers for CD4, CD25, and FoxP3 have been described previously.

Protein expression was analyzed by Western blotting. Total protein (50 μg) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After protein transfer to polyvinylidene fluoride microporous membranes (Bio-Rad), the membranes were blocked with 5% nonfat dry milk and incubated sequentially with primary antibodies [anti-PCNA, anti-β-catenin, anti-p53, anti-COX-2, anti-FoxP3 (mouse and human FoxP3, 1:500 dilution; Abcam, Cambridge, MA, USA), anti-HIF-1α (mouse and human HIF-1α, 1:500 dilution; Santa Cruz, Dallas, TX, USA)], followed by incubation with fluorescein-labeled anti-mouse or anti-rabbit IgG (1:1000) and then incubation with an fluorescein alkaline phosphatase-conjugated antibody (1:5000). Immune complexes were detected with an enhanced chemiluminescence reagent. For quantification, signals were densitometrically normalized to β-actin by Quantity One image analysis software.

**Cell culture and transfection**

Jurkat T cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were incubated in RPMI-1640 (Invitrogen, Beijing, China) supplemented with 10% (v/v) fetal bovine serum (Invitrogen, Beijing, China) and 1% (v/v) penicillin-strepto-
mycin (Invitrogen) at 37 °C in a humidified atmosphere with 5% CO₂. Transient transfection of siRNA was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions.

Statistical analysis
Each experiment was repeated at least three times. Data are presented as the mean ± standard deviation (x ± i). All data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data between two groups were compared with two-independent samples tests. Mean values of data from more than three groups were compared by one-way analysis of variance and a multi-comparison followed if significance was found. A value of P < 0.05 was considered as statistically significant.

RESULTS
MSD prevents spontaneous intestinal polyposis formation in ApcMin/+ mice
MSD was well tolerated in mice, and no obvious systemic toxicity was observed during drug treatment as indicated by the general appearance and organ histology. Weight loss was observed in ApcMin/+ mice from week 5 compared with wild-type C57BL/6 mice. MSD increased body weight significantly compared with ApcMin/+ mice (Figure 1A). The mean colon weight was increased and the mean colon length was decreased significantly in ApcMin/+ mice (Table 2) compared with wild-type mice. Such a significant increase in the colon weight to length ratio was the result of apparent mucosal thickening. Mucosal thickening was alleviated by MSD treatment in ApcMin/+ mice.

All intestinal polyps in ApcMin/+ mice were histologically identified as adenomas. However, their number and diameter were significantly reduced in MSD-treated mice (Figure 1B-D). The number of large polyps (>2 mm) was dramatically reduced by MSD treatment. The number of polyps with sizes greater than 2 mm in the MSD-treated group was 66.7% less than that in ApcMin/+ mice without treatment (Figure 1D). This dramatic reduction in polypl number occurred in all sections of the small intestine, which was confirmed histologically (Figure 1E). In addition to developing a large number of intestinal polyps, ApcMin/+ mice developed massive splenomegaly. MSD treatment decreased the size of the spleen in ApcMin/+ mice by 46.7% (P < 0.01, Table 1).

MSD suppresses expression of β-catenin, COX-2, PCNA, and p53 in colon tumors
To determine whether intestinal adenomas phenotypically resembled human CRC, expression of neoplastic markers was assessed by immunohistochemistry. The expression of β-catenin, COX-2, PCNA, and p53 was significantly elevated in intestinal polyps of ApcMin/+ mice. Cells positive for β-catenin, COX-2, and PCNA were decreased 91.6% (P < 0.01), 62.5% (P < 0.01), and 83.3% (P < 0.01) by MSD treatment, respectively. Immunohistochemical analysis revealed a significant decrease of p53 protein expression (CM5 clone: detects both mutant and wild-type forms). Western blot analysis confirmed that MSD significantly decreased β-catenin, COX-2, PCNA, and p53 expression. Thus, the ApcMin/+ neoplastic process resembled human CRC in several aspects of its molecular pathogenesis. MSD effectively ameliorated CRC through downregulation of neoplastic markers.

MSD downregulates peripheral and spleen CD4+ CD25+FoxP3+ Tregs in ApcMin/+ mice
We next examined the effect of MSD on Tregs in ApcMin/+ mice by evaluating the percentage of CD25 + FoxP3+ cells in the CD4+ T cell population of peripheral blood and the spleen. The percentage of CD4 + CD25 + FoxP3 + Tregs in peripheral blood and the spleen was significantly increased in ApcMin/+ mice (Figure 4A, B). MSD significantly decreased the ratio of Tregs compared with ApcMin/+ mice, and no significant difference was found compared with wild-type mice. Interestingly, FoxP3+ Tregs remained as the major cellular source of CD4 + CD25 + T cells in peripheral blood and the spleen during the polyposis process in ApcMin/+ mice (Figure 4C). These results indicated that Tregs played an essential role in carcinogenesis of ApcMin/+ mice, and MSD reduced the formation of intestinal adenomas by regulating the percentage of Tregs in ApcMin/+ mice.

MSD reduces in situ expression of CD4, CD25, and FoxP3 in intestinal adenomas of ApcMin/+ mice
To further investigate whether MSD regulates CD4, CD25, and FoxP3 in Tregs of intestinal adenomas, we performed double immunohistochemical staining for CD4 + CD25 + cells and western blotting of FoxP3. CD4+CD25+ cells were increased in the ApcMin/+ group and significantly decreased by MSD treatment (Figures 5, 6A-C). This result was consistent with the data from flow cytometric analysis of CD4 + CD25 + cells in peripheral blood and the spleen (Figure 4A, B). RT-PCR analysis showed significantly increased expression of FoxP3 and CD25 in ApcMin/+ mice compared with that in wild-type mice. MSD significantly decreased the expression of FoxP3 in ApcMin/+ mice (Figure 6D, E). These results confirmed that MSD suppressed accumulation of tumor-infiltrating Tregs.

MSD suppresses HIF-1α expression in intestinal adenomas of ApcMin/+ mice
Previous studies have shown intense immunoreactivity of HIF-1α, a potent stimulator for polyp formation, in ApcMin/+ mice. The potential effect of MSD on HIF-1α expression was investigated. Both immunohistochemis-
Figure 1 MSD prevents spontaneous intestinal polyposis formation in Apc\textsuperscript{Min}\textsuperscript{−/−} mice. 
A: changes in body weight over time in Apc\textsuperscript{Min}\textsuperscript{−/−} mice treated with or without MSD compared with wild-type C57BL/6 mice. Statistically significant differences in body weight were observed between drug-treated and Apc\textsuperscript{Min}\textsuperscript{−/−} groups. B: microscopic view of the colon in mice. B1, B2, B3, and B4: wild type; B5, B6, B7, and B8: Apc\textsuperscript{Min}\textsuperscript{−/−}; B9, B10, B11, and B12: Apc\textsuperscript{Min}\textsuperscript{−/−} & MSD. C: Inhibition of total polyposis in Apc\textsuperscript{Min}\textsuperscript{−/−} mice by raloxifene and gonadorelin. Data are the mean ± standard deviation of five animals per treatment group. Control and treated groups were significantly different from each other. D: tumor sizes in the small intestine of MSD-treated Apc\textsuperscript{Min}\textsuperscript{−/−} mice compared with control animals. E: most colorectal neoplasms were histologically consistent with tubular adenoma or adenocarcinoma (×40). 1: wild type; 2: Apc\textsuperscript{Min}\textsuperscript{−/−}; 3: Apc\textsuperscript{Min}\textsuperscript{−/−} & MSD. Histology was carried out by hematoxylin and eosin staining (×40), MSD: modified Shenlingbaizhu decoction; HIF-1α: hypoxia-inducible factor 1α; Apc\textsuperscript{Min}\textsuperscript{−/−}: adenomatous polyposis coli multiple intestinal neoplasia. Wild type: treated only with the placebo; Apc\textsuperscript{Min}\textsuperscript{−/−}: treated with the placebo; Apc\textsuperscript{Min}\textsuperscript{−/−} & MSD: treated with MSD (11.21 g/kg).

Table 1 Colon and spleen assessment in mice (\textpm\textit{s})

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (g)</th>
<th>Length (cm)</th>
<th>W/L ratio</th>
<th>Weight (g)</th>
<th>SW/BW ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.253±0.0113</td>
<td>8.602±0.4907</td>
<td>0.0246±0.0020</td>
<td>0.073±0.0273</td>
<td>0.0223±0.0037</td>
</tr>
<tr>
<td>Apc\textsuperscript{Min}\textsuperscript{−/−}</td>
<td>0.344±0.0336*</td>
<td>6.753±0.8739*</td>
<td>0.0509±0.0035*</td>
<td>0.150±0.0533*</td>
<td>0.006±0.0004</td>
</tr>
<tr>
<td>Apc\textsuperscript{Min}\textsuperscript{−/−} &amp; MSD</td>
<td>0.258±0.0305*</td>
<td>8.248±0.6656*</td>
<td>0.0334±0.0076*</td>
<td>0.084±0.0356*</td>
<td>0.013±0.0053</td>
</tr>
</tbody>
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Notes: wild type: treated only with the placebo; Apc\textsuperscript{Min}\textsuperscript{−/−}: treated with the placebo; Apc\textsuperscript{Min}\textsuperscript{−/−} & MSD: treated with MSD (11.21 g/kg). MSD: modified Shenlingbaizhu decoction; HIF-1α: hypoxia-inducible factor 1α; Apc\textsuperscript{Min}\textsuperscript{−/−}: adenomatous polyposis coli multiple intestinal neoplasia. *\textit{P} < 0.05 and **\textit{P} < 0.01, compared with the wild type group; *\textit{P} < 0.05 and **\textit{P} < 0.01, compared with the Apc\textsuperscript{Min}\textsuperscript{−/−} group; *\textit{P} < 0.05 and **\textit{P} < 0.01, compared with the wild type group.
MSD suppressed FoxP3 expression via inhibition of HIF-1α in Jurkat T cells

To determine whether inhibition of HIF-1α suppressed FoxP3 expression under hypoxia, Jurkat T cells were incubated in 1% O2 in the presence or absence of MSD. The expression of HIF-1α was increased in a time-dependent manner by incubation in 1% O2 for 0, 6, 12, and 24 h. Expression of FoxP3 under hypoxia was increased correspondingly with extension of the culture time (Figure 9A, C, D). Next, we pretreated Jurkat T cells with HIF-1α siRNA for 48 h, followed by exposure to MSD for 24 h. The results showed that MSD decreased hypoxia-induced FoxP3. MSD and HIF-1α siRNA exhibited a synergistic effect on down-regulating HIF-1α and FoxP3 (Figure 9B, E, F). Expression of FoxP3 and HIF-1α were positively correlated under hypoxic conditions. Hence, the results indicated that MSD suppressed HIF-1α expression and downregulated FoxP3 in Jurkat T cells.

DISCUSSION

This study demonstrated that administration of MSD significantly suppressed the formation of intestinal adenoma in ApcMin/+ mice. MSD not only reduced the incidence and multiplicity of intestinal neoplasms but also downregulated PCNA, COX-2, β-catenin, and p53. Furthermore, MSD did not show apparent toxicity in animals during long term treatment, suggesting that
MSD treatment might be a potent alternative regimen for intestinal cancers. Among stromal cells infiltrating tumors, Tregs represent one of the most important players mediating immunosuppression. Therefore, radically resected patients show significantly reduced Tregs frequencies. Patients with advanced colon cancer are more likely to have significantly higher proportions of circulating Tregs than patients with Dukes A and B. Our findings demonstrated that infiltrating CD4+CD25+FoxP3+ Tregs were significantly elevated in Apc\textsuperscript{min/+} mice, and numbers of T cells and Tregs were reduced by MSD treatment. Because cancer itself may be able to drive Treg recruitment as a strategy of immunoevasion, targeting Tregs might be a potential therapeutic approach for tumors in the process of evasion.
The numbers of FoxP3+ Tregs are higher in human colon cancers than in surrounding unaffected mucosa. They may contribute to tumor immune escape and disease progression. It has been reported that increased tumor-related expression of FoxP3 in CRC cells is associated with an adverse prognosis. The expression of FoxP3 has been positively correlated with Dukes staging and lymph node metastasis. The current study showed that expression of FoxP3 was positively correlated to the formation and growth of intestinal adenoma in ApcMin mice. Effective Treg depletion may protect mice from carcinogenesis and eradicate a proportion of de novo-established tumors. Our results showed that MSD reduced the expression of FoxP3, which in turn decreased carcinogenesis in the intestines. These data are consistent with a recent report in which FoxP3 expression was decreased by exercise accompa-
Figure 8 MSD suppresses HIF-1α expression in intestinal adenomas of Apc<sup>min</sup> mice
A: western blot assays were used to examine HIF-1α in colon tumors of groups. 1: wild-type; 2: Apc<sup>min</sup> mice; 3: Apc<sup>min</sup> mice. B: analysis of the HIF-1α staining score. C: analysis of the HIF-1α protein expression. Results represent mean ± standard deviation. *P < 0.01, compared with the wild-type group; **P < 0.01, compared with the Apc<sup>min</sup> group. 1: Wild-type; 2: Apc<sup>min</sup> mice; 3: Apc<sup>min</sup> mice. D: colorectal cancer cells (LS174T) and colorectal cancer cells (DLD1) cells were treated for 24 h with 10 mg/mL MSD under hypoxic conditions. Protein levels of HIF-1α were detected by western blot analysis. E: analysis of the HIF-1α protein expression.

Figure 9 MSD suppresses FoxP3 expression via inhibition of HIF-1α in Jurkat T cells
A: western blot analysis was performed to detect HIF-1α and FoxP3 protein levels in Jurkat T cells after incubation in 1% O2 for 0 h (1), 6 h (2), 12 h (3), and 24 h (4). B: Jurkat T cells were transfected with siRNA against HIF-1α or non-targeting scramble siRNA. Then, Jurkat T cells were treated for 24 h with 10 mg/mL MSD. 1: non-targeting scramble siRNA; 2: non-targeting scramble siRNA + MSD; 3: HIF-1α siRNA; 4: HIF-1α siRNA + MSD. C: analysis of the HIF-1α protein expression in Figure A. D: analysis of the FoxP3 protein expression in Figure A. E: analysis of the HIF-1α protein expression in Figure B. F: analysis of the FoxP3 protein expression in Figure B. Results of HIF-1α and FoxP3 protein expression analyses represent mean ± standard deviation.*P < 0.01 and **P < 0.05, compared with the 0 h group; ***P < 0.05 and ****P < 0.01, compared with non-targeting scramble siRNA group. MSD: modified Shenlingbaizhu decoction; FoxP3: forkhead box P3; HIF-1α: hypoxia-inducible factor 1α.
Hypoxia is one of the most common features in the cancer microenvironment, which promotes various pro-metastatic mechanisms. HIF-1α is a transcriptional master regulator that enhances various metastatic mechanisms under hypoxia. It is a major oncogene overexpressed in colon cancers. In the present study, we found that HIF-1α was upregulated by hypoxia in Jurkat T cells and downregulated after treatment with MSD in ApcMsd mice. HIF-1α promoted expression of FoxP3 in Jurkat T cells. We also found that HIF-1α inhibition downregulated the expression of FoxP3. Our study has shown that FoxP3 and HIF-1α expression was positively correlated in Jurkat T cells regardless of hypoxia.

Apc and HIF-1α have an antagonistic connection. Apc-mediated repression of HIF-1α requires wild-type Apc, low levels of β-catenin, and nuclear factor-kB activity. Therefore, Apc mutations in ApcMsd mice produce a carcinogenesis advantage by promoting HIF-1α expression. Hypoxia robustly enhances the expression of COX-2 and PGE2. HIF-1α is an intrinsic molecular cue that promotes FoxP3 expression. Hypoxia enhances Treg abundance in vitro and in vivo. Thus, it is plausible that MSD reduced the HIF-1α level in ApcMsd mice and subsequently suppressed the activation of Tregs and ameliorated the formation of intestinal adenoma.

MSD prevents spontaneous intestinal polyposis formation in ApcMsd mice. The overexpression of HIF-1α in adenoma of ApcMsd mice recruits Tregs to generate an immunosuppressive microenvironment and facilitate escape and growth of intestinal adenoma. In conclusion, MSD decreases Tregs infiltration to suppress the formation of CRC. Further studies will be conducted to investigate how MSD regulates activation and recruitment of Tregs and their underlying mechanism in regulating carcinogenesis.

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