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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 ($Apc^{Min/+}$) 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 $Apc^{Min/+}$mice resembled that of human colorectal cancer. Molecular markers of neoplasms, such as β-catenin, cyclooxygenase-2, proliferating cell nuclear antigen, and p53, were substantially ameliorated by MSD treatment. Moreover, MSD downregulated peripheral and spleen CD4+CD25+FoxP3+ Tregs and reduced *in situ* expression of CD4, CD25, and FoxP3 in intestinal adenomas. MSD also suppressed HIF-1α expression in the intestinal adenomas, and HIF-1α inhibition decreased expression of FoxP3 in Jurkat T cells under hypoxic conditions.

Conclusion: MSD is a valid prescription to control the formation of intestinal adenomas in $Apc^{Min/+}$ mice. It exerts anti-cancer effects partially through suppression of HIF-1α that induced activation of CD4+CD25+FoxP3+ Tregs *in vivo* and *in vitro*.

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide. A total of 132,700 new CRC cases and 49,700 CRC deaths were projected to occur in the United States in 2015 [1]. Surgery is one of the treatment options. However, 40%–50% of CRC patients still die from the disease within 5 years of diagnosis [2]. It has been found that regulatory T cells (Tregs), myeloid-derived suppressor cells, and various immunosuppressive factors,

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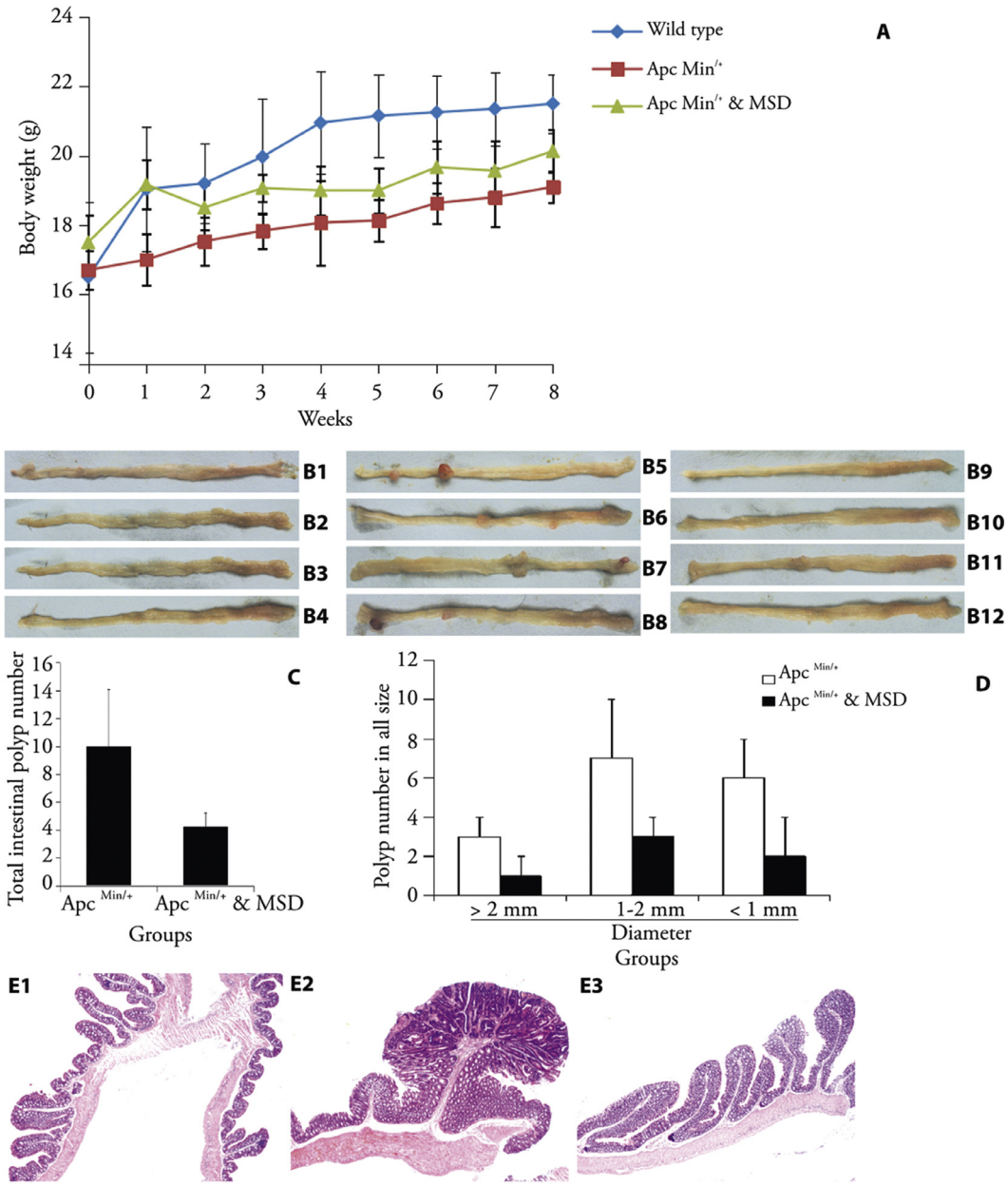

Fig. 1. MSD prevents spontaneous intestinal polyposis formation in $Apc^{Min/+}$ mice.
A: changes in body weight over time in $Apc^{Min/+}$ mice treated with or without MSD compared with wild-type C57BL/6 mice. Statistically significant differences in body weight were observed between drug-treated and $Apc^{Min/+}$ groups. B: microscopic view of the colon in mice. B1, B2, B3, and B4: wild type; B5, B6, B7, and B8: $Apc^{Min/+}$; B9, B10, B11, and B12: $Apc^{Min/+}$& MSD. C: Inhibition of total polyp formation in $Apc^{Min/+}$ 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 $Apc^{Min/+}$ mice compared with control animals. E: most colorectal neoplasms were histologically consistent with tubular adenoma or adenocarcinoma (×40). 1: wild type; 2: $Apc^{Min/+}$; 3: $Apc^{Min/+}$& MSD. Histology was carried out by hematoxylin and eosin staining (×40). MSD: modified Shenlingbaizhu decoction; HIF-1α: hypoxia-inducible factor 1α; $Apc^{Min/+}$: adenomatous polyposis coli multiple intestinal neoplasia. Wild type: treated only with the placebo; $Apc^{Min/+}$: treated with the placebo; $Apc^{Min/+}$& MSD: treated with MSD (11.21 g/kg).

including interleukin-10, transforming growth factor-β, vascular endothelial growth factor, and prostaglandin E_2 (PGE_2), are frequently enriched in the tumor microenvironment and facilitate tumor immune evasion [3]. Antagonizing immunosuppressive mechanisms in the tumor microenvironment is a prerequisite for translation of antitumor immune responses into therapeutic benefits [4].

Tregs exert therapeutic effects during intestinal inflammation. However, suppression of the immune system can be devastating for cancer immunosurveillance [5]. The transcription factor forkhead box P3 (FoxP3) is regarded as a critical developmental and functional factor for CD4+CD25+ Tregs [6,7]. Tregs have been shown to reduce host antitumor responses, and the presence of Tregs has been associated with poor prognoses of several types of solid tumors [8,9]. However, the function of tumor-associated Tregs and their influence on the prognosis of CRC are still unclear [10].

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 [11].

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