Natural regulatory T cells and bifunctional regulatory T cells: potential targets of traditional Chinese medicine in treating colitis-associated colon cancer

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Colon cancer (CRC) is one of the most common malignancies globally, ranking third in terms of new cancer cases and second as a cause of cancer deaths [1]. In recent 30 years, the incidence and mortality rates of CRC in China have been increasing, gradually exceeding the global levels [2]. Due to the fact that most CRC patients are diagnosed at the advanced stage, the treatment of this disease is challenging and often ineffective. Therefore, prevention and early diagnosis of CRC are crucial.

Currently, it has been confirmed that a history of inflammatory bowel disease (IBD), genetics, and unhealthy eating habits are risk factors for CRC. Among them, IBD, as a chronically recurring inflammatory condition, plays a particularly important role in the development of CRC. As early as 1863, Rudolf Virchow, the “father” of modern pathology, recognized the close link between chronic inflammation and cancer development, proposing the famous hypothesis “inflammation is the prime culprit of cancer” [3]. In 1925, Crohn and Rosenberg first reported the association between IBD and CRC [4]. Recent epidemiological studies have found that the incidence of CRC in IBD patients is 60% higher than that in the general population [5]. Furthermore, the incidence of CRC in IBD patients gradually increases with the duration of the disease, with a deterioration rate of 8% after 20 years and 18% after 30 years [6]. To date, research on colitis-associated colon cancer (CAC) is still ongoing. How to treat CAC has become an important question in CRC prevention and early diagnosis.

Discovery of natural regulatory T cells (nTreg) and bifunctional regulatory T cells (biTreg)

During the progression of CAC, there are two simultaneous states in the intestine: immune hyperactivation (inflammation) and immune suppression (low immune surveillance function and tumor immune evasion). As an important immunosuppressive cell, the role of regulatory T cells (Treg) in CAC has been controversial. Does Treg inhibit (“good” role: anti-inflammation) or promote (“bad” role: induction of tumor immune evasion) the progression of the disease? It has long puzzled immunologists until the discovery of nTreg and biTreg.

It is well-known that during the progression of IBD, the relative deficiency of Treg differentiation leads to insufficient secretion of anti-inflammatory factors such as IL-10, further aggravating the inflammatory response [7]. Inducing T cell differentiation towards Treg can suppress intestinal inflammation [6]. However, for a long time, the role of Treg in CAC is controversial. Some studies have reported that promoting Treg differentiation can suppress inflammation and treat CAC (i.e., Treg plays a “good” role) [8, 9]. Other studies have found that extensive infiltration of Treg can promote tumor progression in CAC by suppressing immune surveillance function (i.e., Treg plays a “bad” role) [10, 11]. So, what exact role does Treg play in CAC? It has always been a hot and difficult topic in immunology research.

Inflammatory polyps are the main precursor lesions leading to colon cancer. The adoptive transfer of Tregs from healthy mice but not from tumor bearing mice into polyposis prone mice reduces the number and size of inflammatory polyps [9]. This suggests that Tregs from healthy mice and polyposis prone mice have some different characteristics, which may be the reason for the conflicting results in Treg research on CAC. Further research showed that Tregs in polyposis prone mice differ from those in normal mice. These Tregs were not only lose their anti-inflammatory activity but also promote the inflammatory response, playing a role in accelerating the progression of CAC [9]. What changes have occurred in Tregs during progression of CAC? What causes these changes? Interestingly, “nTreg” and “biTreg”, two different subtypes of Tregs, were discovered in recent years. And their dynamic changes gradually revealed the answer to these questions.

The imbalance of nTreg/biTreg in the colonic immune microenvironment (excessive conversion of nTreg to biTreg leading to a decrease in nTreg and an increase in biTreg) is the key to promoting the progression of CAC

nTreg: nTregs are directly differentiated from naïve CD4+ T cells in the thymus. nTregs express CD25, Foxp3, and produce cytokines such as IL-10, participate in the resolution of inflammation, tissue repair, and immune tolerance [12]. biTreg: in recent years, researchers have discovered a novel subset of Tregs that can simultaneously produce IL-17A and IL-10, referred to as “biTreg” due to their bifunctional nature of immune activation and suppression. Compared to nTregs, biTregs express higher levels of the Th17 cell transcription factor RORγT and lower levels of the Treg transcription factor Foxp3. Therefore, they are also known as RORγT+ Tregs [13]. Under physiological conditions, there is a dynamic balance between nTregs and biTregs in the intestine, maintaining intestinal immune homeostasis. However, during the progression from colitis to CRC, this balance is disrupted and is characterized by decreased nTregs and increased biTregs. The reprogrammed tumor immune microenvironment caused by nTreg/biTreg imbalance exhibits a pro-inflammatory and pro-tumor phenotype.

nTreg inhibits inflammation in CAC, the numbers of nTreg decrease during the progression of CAC

Comparison of Treg subsets between healthy individuals and colon cancer patients has revealed a significant decrease in the ratio of nTregs to total Tregs in colon cancer patients [12]. Animal experiments have also confirmed that the ratio of nTregs in colonic inflammatory polyps is only one-thirty of that in healthy mouse colonic tissue [9]. Professor Theresa L. Whiteside, a renowned immunologist and former president of the American Association of Immunologists, refers to nTregs as “good Tregs” in tumors. Her numerous studies have confirmed that nTregs can inhibit the occurrence of tumor-related inflammatory reactions and play an active role in the treatment of inflammation-related tumors [11].
Similarly, the transplantation of nTregs can effectively reduce the generation of colonic inflammatory polyps in mice [9]. nTregs are the main source of IL-10 in the intestine, and IL-10 can alleviate intestinal inflammation and inhibit tumor formation by suppressing the activation of various proinflammatory cells [17].

biTreg promotes colonic inflammation and suppresses the anti-tumor activity of CD8αT cells, leading to immune evasion of colon cancer cells, the numbers of biTreg increase in CAC models. Compared to healthy individuals, the number of biTregs in the peripheral blood and tumor tissue of CRC patients is increased [18]. Meanwhile, multiple animal experiments have shown the presence of a large infiltration of biTregs in the intestines of mice with CAC models [10]. Further analysis revealed that these biTregs can both promote the occurrence of intestinal inflammation and exhibit strong immunosuppressive activity, promoting the progression of CAC in multiple ways [10]. On the one hand, biTregs exacerbate colonic inflammation: single-cell sequencing results show that Tregs in colon tumor tissue of CRC patients highly express RORγT and IL-17A [18]. In addition, analysis of biTregs in CAC models revealed that they secrete high levels of the proinflammatory cytokine IL-17A while their ability to produce IL-10 is reduced, resulting in the loss of anti-inflammatory activity and the promotion of colonic inflammation instead [10]. On the other hand, biTregs induce the death of CD8α T cells and suppress the occurrence of anti-tumor immune responses. CD8α T cells are cytotoxic T cells with significant tumor killing function. The decrease in the number of CD8α T cells is closely related to the immune evasion of colon cancer cells. Compared to normal mice, the number of CD8α T cells is significantly reduced after biTreg transplantation in mice [18]. Further analysis revealed that biTregs induce the death of CD8α T cells through TGFβR1, and the use of TGFβR1 inhibitors (LY3200882) prevents biTreg transplantation from inducing CD8α T cell reduction in mice [18].

The excessive conversion of nTregs to biTregs leads to the imbalance of nTreg/biTreg during CAC progression, and inhibiting the conversion of nTregs to biTregs can reshape the immune microenvironment and block the progression of CAC. The current research has found that biTreg is derived from the conversion of nTreg [11]. In the process of colon cancer transformation, the expression of Foxp3 in nTreg decreases while the expression of RORγT increases, prompting its gradual transformation into biTreg. This leads to a decrease in nTreg and an increase in biTreg, thereby promoting inflammation and tumor progression (Figure 1). Multiple animal experiments have confirmed that inhibiting the conversion of nTreg into biTreg can reduce the level of inflammatory factors and the number of inflammatory polyps in the intestines of mice with CAC [10, 12]. Since biTreg expresses the Th17-related transcription factor RORγT at higher levels compared to nTreg, inhibiting RORγT expression in Treg can block the conversion of nTreg into biTreg. Compared to wild-type mice, mice with conditionally knocked-out RORγT in Treg (inhibiting biTreg differentiation) showed reduced intestinal inflammatory responses and a decrease in the number of colonic inflammatory polyps [12].

### Future prospects

Traditional Chinese medicine and its active components have demonstrated broad therapeutic potential in the treatment of CAC. For example, berberine [19], ShaoYao decoction [20], Wumei pill [21], and Huangqin decoction [22, 23] can ameliorate CAC through inhibiting inflammatory response, regulating intestinal flora, and suppressing tumor cell proliferation. The discovery of the dynamic changes between nTreg and biTreg in CAC will help to elucidate the mechanism of action of traditional Chinese medicine in the treatment of CAC.

### References


Author contributions
Ning Wang: Investigation, Writing - Original Draft; Huan Pei and Li-Wei Xing: Investigation; Si Wang: Writing - Original Draft; Jia-Bao Liao: Visualization; Wei-Bo Wen: Conceptualization, Supervision; Yu-Hong Bian and Huan-Tian Cui: Conceptualization, Writing - Review & Editing. All authors agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
CRC, colon cancer; IBD, inflammatory bowel disease; CAC, colitis-associated colon cancer; nTreg, natural regulatory T cells; biTreg, bifunctional regulatory T cells; Treg, regulatory T cells.

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