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Different Features of the Regulatory T Cells in Gastrointestinal Tract Cancers and Disorders

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Abstract: Regulatory T cells (Treg) located in tissues have a critical role in controllinghomeostasis and immune responses also modulate non-immunological processes.The T cell receptor repertoires of non-lymphoid tissue Tregs are distinct from Tregs in lymphoid organs.Less information is available about various ways to change the program of transcription in tissue-resident subsets of Treg cellsfor adapting to very different fields. However, recent progress in our understanding of Treg cells that reside in two important sites, the gut and adipose tissue, may provide some clues. Gastrointestinal is the largest reservoir for tissue-resident Treg cells in the body. Adequate number and performance of intestinal Treg cells are essential for maintaining normal intestinal immune homeostasis.Treg in the GI tract have shown conflicting features. Therefore, in this study, phenotypic and functional characteristics of Treg cells are discussed in normal conditions, chronic inflammatory bowel disease (IBD) and colorectal cancer (CRC), as well as Treg cell therapeutic strategies in the treatment of these diseases.

Keywords: Regulatory T cells (Treg), inflammatory bowel disease (IBD).

Background

Regulatory T cells are involved in the immune system that plays an important role in various cancers, autoimmune diseases and infectious diseases.(1, 2)These cells can be formed in the thymus followed by self antigen identification, in the gut followed by interaction with antigen-specific cell subsets or in vitro in response to cytokines such as TGF-β.Two main subsets of $CD4^+$ regulatory T cells (Treg) include: thymus-derived *naturalregulatory T cells* (tTreg or nTreg) with suppression activities which are essential for establishing and maintaining immune homeostasis in steady state; *induced Treg cells* (iTreg) that arise from naive T cells followed by self antigen identification outside of the thymus, such as interleukin-10 (IL-10)-secreting T regulatory type 1 (Tr1) cells that do not express Foxp3 and CD25 markers or at the very least,Th3 cells that secrete high level of TGF-β which they express CD25 and FOXP3 markers and IL-35-secreting iTr35cells that do not express FOXP3(3-5).

Other lymphocyte subsets with regulatory function include: inducible $CD8^+$, $CD3^+CD4^-CD8^-$Treg,$CD4^+V\alpha14^+$ *(*NKTreg) and γδ T cells. FOXP3 is considered as a key transcription factor controlling evolution and function of T regulatory cell.(6) However, in human, expression of Foxp3 alone is not sufficient to identify Treg cells.Besides subsets of cells T CD4 + CD25hi, FOXP3 is expressed by some of T CD4 + cells with low levels of CD25 or without CD25.FOXP3 expression may be induced in human T cells that are without Treg function(7).

Treg cells migrate to inflammatory site and draining lymph nodes during the immune response for suppressive action.These mechanisms include inhibitory cytokine production such as IL-10, TGF-β and IL-35; inducing functional cell death by taking and completing the cytokines such as IL-2 or production of granzyme B, creating localized metabolic disorder in target cells; and ultimately inhibition of dendritic cell function(8).

Immune responses in various tissues are affected by Treg FOXP3 + cells.For example, in the intestine, Treg cells have a key role in maintaining tissue homeostasis by inhibiting excessive activation of DC cells and effector T cells. Treg cells modulate the response to commensal microbes in the lamina propria tissue(9). Treg cells have been shown that similarly to autoimmunity in the central nervous system (CNS) can have beneficial effects by inhibiting (or shortening the bindings) inflammatory loop of T cells and APCs(10).Such a mechanism could have the opposite results in tumor that the Treg cells can inhibit the immune response against the tumor, preventing tumor clearance.Treg cells maintain a delicate balance in the course of infection; inhibition of immune response leads to the inability to pathogen clearance, while uncontrolled immune response results in tissue destruction by unwanted immune response.Under special circumstances, in FOXP3+ Tregs can occur loss of Foxp3 expression and suppression functions(11).The key causes of this loss of FOXP3 expression include inflammatory environments with high levels of cytokines that are involved in the induction of effector T cells, such as IL-6 and IFN-γ(12).

Regulatory T cells in gastrointestinal (GI)

Treg cells in addition to peripheral blood and secondary lymphoid tissues can also be found in non-lymphoid tissues such as the skin, lung, liver, intestine, adipose tissue and placenta in non-inflammatory conditions(13).Overall, the analysis of Treg cells in these tissues has shown the characteristics of programmed effector Treg cell along with other detection features such as specific combinations of

molecules implantation, transcription factors, mechanisms of immunoregulatory and TCR pool, indicating considerable expertise of Treg cells in these environments.Colonic Tregs are an unusual population, which has provoked some contradictory observations.TCRs expressed by colonic Treg cells show clear reactions against microbial antigens, which seems to be important triggers for proliferation and differentiation of these cells(14, 15).Accordingly, many studies have demonstrated a reduction in the frequency of colonic Treg cells in germ-free mice and colonization of GF miceby a group of clostridium bacteria leads to differentiation or proliferation of colonic Treg cells (16, 17).

Treg induction in the intestinal mucosa:

Abundant iTreg cells are in the intestine, but not in the secondary lymphoid tissues, and this reminds that iTreg cells evolve in response to intestinal antigens derived from commensal bacteria or food(18).Now, it is clear that the development of Treg cells outside the thymus occurs in the gut.In germ-free micehave been shown that in normal circumstances, the small intestinal iTreg cells are induced by food antigens and whereascolonic iTregs are induced by commensal bacteria (19, 20).

Intestinal dendritic cells can induce antigen-specific Treg cells, which are mainly involved in controlling the immune response against the dietary antigens and commensal microbes.Retinoic acid (RA) is a major factor in the induction of Treg as well as homing tendency of activated cells in the mucosa; also RA is not able to induce alone the expression of FOXP3, but upregulate expression in combination with TGF-β(21). RA, mediating orientation of Treg cells to the gut,is a strong induction for CCR9 and α4β7 expression(22).

iTreg produced by $CD103^+$ dendritic cells and stromal cells specific to the mesenteric lymph nodes have a key role in inducing and maintaining oral tolerance(23).Several subsets of Treg cells such as, $CD4^+$ $FOXP3^+$ iTreg cells, IL-10-producing Tr1 cells and TGF-β-producing Th3 cells have been shown in oral tolerance(24).

Among subsets of Tregs, the role of FOXP3 Treg cells (nTregs and iTregs) in oral tolerance is the best studied. Inhibition of differentiation of iTreg by preventing the induction of TGF-β- dependent FOXP3 showed that iTreg cells are essential to inhibit the Th2 type diseases in mucosal sites (25). In addition, the lack of iTreg cells alters the composition of the intestinal microbiota. Therefore, this study suggests that tTreg cells are primarily responsible for the control of autoimmune response, whereas the main role of iTreg cells would be to prevent immune responses against commensal and dietary antigens. This idea is further supported by another study analyzing the TCR repertoire of colonic Treg cells.The comparison of the TCR repertoire of Tregs from this site with other locations revealed that gut antigens form the Treg TCR repertoire in the intestine(26). In fact, a large proportion of colonic Treg cells are specific for bacterial antigens, indicating that iTregs compose a major proportion of the gut Treg cells. However, another recent researchshowed that the TCR repertoires of intestinal and thymic Treg cells are highly similar, suggesting that tTreg cells also contribute significantly to the intestinal Treg pool(14). Therefore, both iTregs and tTreg cells are likely to contribute to the maintenance of intestinal homeostasis, and it remains to be cleared which population plays an important role.The cells of iTreg and nTreg have synergistic function because of having different TCR repertoire without redundancy(27). BothiTregs and nTregs express CD25 and the key Treg lineage transcription factor, so the distinction between these two populations in the peripheral organs remains challenging. Now, Helios and Nrp-1 are two proposed markers for this discrimination. However, these markers are not adequate as diagnostic markers of Treg subsets. Transcription factor Helios has been shown to be expressed at higher level on tTreg cells that can detect them from iTregs(28).But, previous researches have demonstrated that Helios could also be expressed by iTreg cells in vivo (29). In addition, a part of human tTreg cells do not express Helios (30). Nrp-1 is expressed by tTreg cells and lack of its expression in iTreg cells gives distinctionbetween these two populations under non-inflammatory conditions(31). However, the expression of Nrp-1 may be induced in activatedeffector T cells in humans(32). Therefore, the lack of clear markers has caused to be limited our understanding of the relative contribution of tTreg and iTreg cells in pathology, especially in humans.

Controlling the intestinal regulatory T-cells homeostasis by dietary antigens and commensal microbes

The intestinal mucosa is constantly exposed to a various range of foreign antigens, such as dietary antigens, metabolites, and components of the commensal microbiota(33).

The presence of distinct microbial species increases specialized host immune responses by differentiation of appropriate Tregand effector T cells. Colonization with segmented filamentous bacteria (SFB) leads to effector T cells accumulation within the small intestine, especially Th17, and to a lower degree Th1 cells (34). Also, populations of regulatory T-cell are affected by microbial colonization that can result in accumulation of $FOXP3^+$ Treg cells populations and induction of IL-10 production.Experiments using germ-free mice have shown that commensal bacteria are essential for the evolution of normal colonic Treg cells. Studies indicated that *Bacteroides fragilis* and clostridia species are important for intestinal regulatory T-cells homeostasis(19, 35).The most recently,17 clostridial species were identified in the human microbiota, whichinduce gut-homing and proliferation of Treg cells.In addition, it was found that these 17 species provide a relatively high level of short chain fatty acids (SCFAs) that are the products of bacterial decomposition from plant fiber(36).It was shown that the SCFA such as propionate, butyrate and acetate are able to restore the number of Treg cells in micetreated with antibiotics or germ-free mice and increase the number of these cells in specific pathogen-free mice(37).These effects are caused partly by SCFA receptors, free fatty acid receptor 2 (known as GPR43), which is expressed at high levels by colonic Treg cells, but not in circulating Treg cells(38).

Recent findings have shown that metabolic status of the host and multiple metabolites of nutrients can affect hemostasis.Vitamins are essential organic compounds that are synthesized in the body or obtained from dietary sources(39).A variety of immunological disorders can occur as a result of vitamin deficiency(40). Vitamin A as one of the most important factors can regulate the intestinal immune cell function(41).Vitamin A is metabolized to RA In the intestine, which has pleiotropic effects on intestinal immune cells and regulates lymphocyte homing to the gut, intestinal IgA production, development of specific DC subsets, and $Foxp3^+$ Treg cell differentiation(42-44). The extent of intestinal immune cell functions affected by RA suggests that vitamin A metabolism is important for immune homeostasis. In fact, the

disruption of RA signaling leads to reduced intestinal $Foxp3^+$ pTreg cell development in vivo(45), and vitamin A deficiency is associated with the induction of colitis(46).Also, vitamin D is a precursor for calcitriol (1, 25 dihydroxy-vitamin D), which maintains calcium and phosphate balance, regulates bone formation, and it has been proved to increase Treg cell differentiation and suppress immune responses(47). In the gut, vitamin D and vitamin D receptor signaling contributes to improve chronic colitis in mousemodels(48). In IBD, low level of vitamin D is reported that associated with higher severity of disease (49, 50). Therefore, vitamin A and D additivesmay have therapeutic effects inIBD diseaseviainduction of intestinal Treg cells.

Treg function in the gut:

It has been well demonstrated that Treg cells play animportant role in the regulation of intestinal immune responses to ensure the protection of host against pathogenic microorganisms and the lack of immune-related pathologies. In the lamina propria of the gut, Treg cells maintain intestinal homeostasis through negative regulation of effector T cells and play a pivotal role in intestinal inflammation suppression by production of IL-10 and TGF-β as well as increased expression of CTLA4(51). Earlier studies has highlighted an immunoregulatory role of IL-10 by showing that the use of antibodies against the receptor IL-10 or transfer of $CD4^+$ T cells deficient in IL-10 to $Rag1^{-/-}$mice caused acute colitis (52, 53). Subsequent studies by searching for the mechanism of IL-10 regulatory activity in the transfer colitis model indicated that $FOXP3^+$ cells deficient in the IL-10 receptor subunit β (IL-10Rβ) unable to protect recipient mice from colitis and missed their ability to express FOXP3, showing that IL-10R signaling in regulatory cells is significanttomaintain of their function(54).The role of IL-10 in regulatory T cell function does not reject a role of TGF-β. The role of TGF-β in studies showing that protection from colitis by Treg cells when CD4+ CD25+ cells with defects in TGF-β into recipient mice were transplanted, did not occur and by the observation that systemic use of anti-TGF-β antibody blocked the ability of $CD4^+CD25^+$ T cells to attenuate colitis(55, 56). Also, TGF-β expressed on the surface of $CD4^+CD25^+$ T cells in relationship with LAP mediated $CD4^+CD25^+$ T cell suppression in vitro and $CD4^+LAP^+$, but not $CD4^+LAP^-$; T cells protected recipient mice from colitis(57).

IL-35 is an immunoregulatory cytokine that is secreted together with IL-10, TGF-β by FOXP3 Treg cells; especially in the presence of effector T cells, IL-35 is produced in considerable level whereby effector T cell proliferation is suppressed. Overexpression of IL-35 has been linked with increased induction of GI cancers (58-60). These findings suggest that IL-35 plays a role in suppressing immunity against tumors.

Treg in Chronic intestinal inflammation: IBD and GI tumors

Tregs in IBD

Inflammatory bowel disease (IBD) is chronic autoimmune disorder that can involve the small intestine or colon, and basically consists of two types of ulcerative colitis (UC) and Crohn's disease (CD). Colorectal cancer risk is increased in patients with IBD. It is believed that IBD arise from a complex interaction of environmental factors, genetic susceptibility, impaired epithelial defense barrier and lack ofregulation of intestinal immune system(61). Although the exact mechanism involved is not yet clear, in recent years significant progress has been achieved in understanding the immunopathogenesis of IBD, resulting in new therapeutic and targeted strategies.

IBD mouse models that mimic features of human pathology have been helpful in better understanding and explaining the immunopathology of IBD. They show that chronic inflammation may be result in excessive inflammatory responses or defects in negative regulatory routes(62). In colitis models (T cell transfer model and IL-10 knockout model), role of Treg cells and related cytokines has been shown in IBD(63). Patients with IBD have reduced Treg cells in the peripheral blood compared to healthy individuals and increased in inflamed intestinal mucosa.Disease severity has a different effect on the number of Treg cells in peripheral blood and mucosa (64, 65), or these cells have less ability to suppress proliferation of autologous T cell (66). There aredocuments that Treg cells of peripheral blood and gut of IBD patients are more susceptible to apoptosis compared to non-inflamed colon tissues, and a decrease in Treg cell apoptosis along with an increase in the number of these cells and reduction in disease activity can be observed in patients treated by anti-TNF (67). It can be said that there is no complete evidence that Treg cells are functional or not.In many studies, based on in vitro suppression assays, Treg cells in patients with IBD had functional roles and it was shown that Treg cells in both peripheral blood (68) and mucosa of patients with Crohn's disease and ulcerative colitis (69, 70).The reason for the creation of a deleterious immune response, despite the presence of functional Treg cells in inflamed mucosa, can be effector T cells resistance to immunosuppressive effects of TGF-β. The upregulation of Smad7 (an inhibitor of TGF-β signalling) in the intestinal mucosa of IBD patients show resistance of cells to inhibition of Treg cells (71).

However, other studies have reported different results, indicating that IBDs could be due to functional defects of Treg cells (66, 72). It is impossible to determine whether these defects are initially responsible for the disease development in those patients or is secondary to the excessive inflammation triggered by other mechanisms.In IBD patients study, deficient suppressive function of Treg due to mutation in the FOXP3 gene has been reported in vitro(72), showing that a Treg defect may be a key cofactor for disease development.Although the genetic defect of Treg cells may not happen in most cases as an underlying cause of IBD, Treg deficiency associated with other genetic or environmental factors may be involved in the development or severity of the disease.

Accoringto the correlation between the gut commensalflora and Treg cells it seems that in IBDpatients treatment by specified commensal bacteria may help to accurate dysbiosis and rectify the development of Treg cells along inflammation of the intestine(73).

Increasing the number of peripheral blood T cells co-expressing FOXP3 and IL-17 is observed in patients with CD and UC (66). These $FOXP3^+$ $IL\text{-}17^+$ T cells express the transcription factor RORγt, showing an intermediate phenotypebetween the Th17 and Treg subsets. In a study, $FOXP3^+$ $IL\text{-}17^+$ T cells have been detected in the mucosa of patients with CD, but not in UC (74), whereas another study reported their presence in patients with UC (75). Significant reduction in the ability of Treg cells in suppressing autologous T cell proliferation was associated with increased $IL\text{-}17^+$ T cells among $FOXP3^+$ Treg cells, while other studies have concluded

that mucosal $FOXP3^+$ $IL\text{-}17^+$ T cells have the suppressive ability in patients with UC. Interestingly, other studies examining the mucosal $FOXP3^+$ $IL\text{-}17^+$ T cells functional capacities showed that these cells increased production of inflammatory cytokines IL-6 and IL-1 by colonic tissue cultures in an IL-17-dependent pathway(75). Thus, the capacities of pro-inflammatory by Treg cells simultaneously with the loss of their suppressive activity may be involved in the uncontrolled inflammation *in vivo*.An important point for understanding Treg function in IBD pathogenesis is the lack of reliable markers for identifying Tregs. The classical CD25 and Foxp3 markers that are applied in many works could also be expressed by activated effector T cells. So to achieve certain results, review of the studies will be required to identify Treg using a more refined phenotype.

The role of inhibitory cytokines in IBD

TGF-β:TGF-β is a pleiotropic cytokine released by some different cells in the gut thathave a significant tolerogenic effect by promoting regulatory T cell differentiation(76). TGF-βregulates epithelial cell migration and plays an important role in tissue remodeling in the gut(77). TGF-β1 is produced in large quantities in the gut(78).The active form of TGF-β binding to its receptor activates Smad2/3 proteins, which in the formation of a complex with Smad4 and its inclusion intothe nucleus acts as a transcription factor.Smad7 by competitive binding to the receptor for TGF-β inhibits the phosphorylation of Smad2/3 induced by TGF-β1, which is considered as a negative regulator of the TGF-β signaling pathway(79).However, Smad7 can react with other intracellular proteins and control cellular function through TGF-β1 independent pathways(80).TGF-β1 is highly expressed in inflamed mucosa of patients with IBD, but it is unable to activate Smad-associated intracellular signaling and suppress inflammatory responses.It has been shown that IBD-related inflammation is along with increased levels of Smad7(71). So that the silencing of the Smad7 by specific antisense oligonucleotide restores TGF-β1 functionand this inhibits inflammatory cytokine production, and improves colitis in mice(81).

IL-10: IL-10 is an anti-inflammatory cytokine, which is produced by immune and non-immune cells. Secretion of IL-10 is one of the key inhibitory mechanisms in Treg cellsfor creating tolerance to self and environmental antigens, particularly in the colon, lung and skin (82). The important role of IL-10 in intestinal homeostasis have been proved by the use of recombinant IL-10 or intestinal bacteria able to produce IL-10 can moderate intestinal inflammation in mice(83, 84). In an IBD mouse model, IL-10 secretion by Treg cells and other cells controls the activation of proinflammatory macrophages (85, 86).IL-10 can directly inhibit the Th1 and Th1 and Th17 colitogenic T cells(87), and IL-10 signaling in Treg cells is interestingly essential for colitogenic Th17 control(88). Therefore, IL-10 has an important role for Treg activation and suppressive functions.

G. Sarrabayrouse et al. in a recent study detected CD4CD8αα cells asnew subsets of FOXP3- T cells in the blood and human colonic lamina propriawhich have similar regulatory functions to $FOXP3^+$ T cells and secrete IL-10.*F. prausnitzii* bacterium from species of Clostridium of the gut microbiota is the main inducers of Treg cells and, interestingly, the study also showed thatthe bacteria levels were lower in patients with IBD compared to healthy colon mucosa of patients with colon cancer; and as a result, CD4CD8αα cells are reduced in the gut and the blood of these patients (89).

IL-35: IL-35 is vital for Treg-mediated control of the inflammatory responses in the gut. In fact, in the T cell transfer model of colitis, mice receiving Treg cells from animals deficient in a subunit of the IL-35 receptor were less protected than those transferred with wild-type Treg cells (90). It has been suggested that IL-35 exerts its regulatory effects by inducing the conversion of conventional T cells to induced Treg cells. Indeed, $CD4^+$ T cell activationby IL-35 and TCR signals creates stable iTreg population(3).

Treg cells as a therapeutic tool in the treatment of IBD

In IBD disease, some therapies which were not designed to specifically target Treg cells exert beneficial effects on the disease and a simultaneous impact on Treg cells (91, 92). For example, therapeutic response to anti-TNF resulted in a reduction Treg apoptosis inUC, enhance treg suppression(67, 93)and increase the levels of TGF-β and IL-10 in responder patients(94). Treg cells transfer can treat intestinal pathology of mice(95), and this raisedthe use of Treg cells as a therapeutic method for IBD disease. Currently, administration of Treg cellshas been used in patients with Crohn's disease in phase I and IIa clinical trial. A reduction of CD disease activity was observed in 40% of the patients (96).Encouraging results of this study has led to the development of a larger, ongoing, placebo-controlled clinical trial to assess the effects of Treg cell therapy in patients with Crohn's disease who are resistant to conventional treatments.In order to more effective Treg cells infused in controlling inflammation in patients with IBD, considering the purity of the cells Treg, the ability of homing, antigenic specificity and survival of Treg cells is likely to be strong to develop therapeutic regime.

Treg in gastrointestinal tumors

Treg cells in gastric cancer (GC):

Gastric cancer is the third most common cause of cancer-related mortality in the world (97). Unhealthy diet, smoking and above all infection with the bacterium *Helicobacter pylori* are the main risk factors for Gastric cancer (98). Treg cells play important roles in tumor escape in gastric cancer (99, 100). In the study of patients with gastric cancer, increasing the number of Treg cells has been shown in tumor tissue and peripheral blood(101, 102). The reason for the increased Treg cells into tumor is not only an increase in called Treg cells, but is also induction of FOXP3 expression in tumor site by tumor factors (103). For example, in patients with gastric,colon and lung cancers, one of the mechanisms that induce expression of FOXP3, expression levels of cyclooxygenase 2 (COX-2) by tumor cells has been shown to be mediated production of prostaglandin E2 (PGE2)(104-106).$CD4^+$ $CD25^+$ T cells treated with PGE2 lead to FOXP3 expression and induction of suppressive function of these cells (107).About the prognostic effect of Treg cells in gastric cancer, several studies have reported conflicting results; some studies have shown that Treg cells are protective, whereas in some other studies, Treg cells in TIL or in the peripheral blood of patients with GC are able to suppress effector T cells because of promoting tumor growth (102, 108). Several studies have shown that FOXP3 is expressed in varianttumor cells(100, 109, 110). But the function of FOXP3, in tumor cells is diverse and controversial. Several studies represent that the FOXP3 gene functions as both a tumor suppressor gene for breast, prostate and non-small cell

lung cancer and an oncogenic gene for gastric cancer and hepatocellular carcinoma (111-114).

Moreover, recent data show that Th17 cells might somehow contribute to GC pathogenesis.One gets the impression Th17 cells and Treg balance is important in determining the severity of gastritis caused by *H. pylori*.Both Th17 and Treg cells localize to mucosal surfaces through CCL20 signaling, CCL20-CCR6 axis iscurrently the subject of several studies in patients with IBD (115, 116).Increased expressions of CCL20 in the mucosa of IBD patients and protection from $CCR6^+$ Treg cell-mediated colitis have been shown in the studies (117, 118). There is growing evidence that *H. pylori* infection is negatively associated with IBD (119, 120). Cook KW et al., reported that enhanced Treg cells in peripheral blood express C CR6, and increased expression of Ligand CCR6 (CCL20) in gastric epithelial cells of patients with *H. pylori* infection(121).

Treg cells in pancreatic cancer (PC):

Pancreatic cancer is a devastating form of cancer with a poor prognosis and 5-year survival rate of less than 5%, and most patients develop symptoms of pancreatic cancer in an advanced phase of the disease (122).In animal models, infiltration of Treg cells into pancreatic tumor tissue has been shown even in the early stages of tumor progression and creation of localized immunosuppression (123). Immunohistochemical studies have shown the presence of $FOXP3^+$ Treg cells in pancreatic tumor tissue and their relationship with poor clinical prognosis. Several studies have examined the frequency of Treg cells in peripheral blood of patients with pancreatic cancer (124-127).Some studies reported increased $CD4^+CD25^+$ Treg cells, and some other demonstrated no change in $CD4^+CD25^+FOXP3^+$ Treg cells in the blood of patients with cancer than in healthy controls (127), it seems that this inconsistency in results can be attributed to the defined Treg cells.

More recently, studies have been done on the biology of pancreatic adenocarcinoma and importance of tumor microenvironment in responding to treatment.In fact, one of the histologic features of pancreatic cancer is the dominant desmoplastic reaction, which in addition to pancreatic cancer cells (PCCs), pancreatic stellate cells (PSCs) are present in it.A study shows deleterious effects of PSC cells, which promote immunosuppression in tumor environment through the production of chemokine IP-10, and recruitment of $CXCR3^+$ Treg cells (128); whereas, another study indicates that the PSC fibrosis can inhibit calling Treg cells into pancreatic tumor environment (129). However, in order to find an appropriate treatment strategy to improve the treatment of patients with pancreatic cancer, more clinical and basic researches are required to clarify the interaction between the immune system and PSC cells in these patients.

Treg cells in hepatocellular carcinoma (HCC):

Primary liver cancers include cholangiocarcinomas, hepatoblastomas and hepatocellular carcinomas, which the last type is the most common cancer with a high mortality rate and poor prognosis (130, 131).Evidence suggests that Treg cells are essential factors in the development and prognosis of HCC(132, 133).The available evidence shows that the HCC tumor cells can directly alter the liver microenvironment by Treg cells recruitment (134, 135), or induce Treg cells through TGF-β1 production (136) or up regulation the PD-1 (137), which have been associated with poor patient outcome and recurrence after surgery (138, 139).Increased repressive function and also abnormal functional phenotypes of Treg cells have been reported in patients with HCC. Treg cells in peripheral blood of HCC patients up-regulate CCR6 receptor, which facilitates their migration into the tumor sites (134, 140, 141).

In a study on animal models and patients with HCC, it was found that the Treg cells in HCC had the specifically altered expression of miRNAs affected by FOXP3, which would target important signaling pathways that could affect the functions of Tregs(142).

Treg cells in CRC cancer:

Tumorigenesis in the colon is a complex and multi-step process, which is affected by external and internal factors, such as age, sex, diet, and other lifestyle-related diseases.The disease first appears as an adenomatous polyp, and then resulting in advanced adenoma with severe dysplasia, and eventually leads to invasive cancer. The immune system status in tumor microenvironment is involved on survival of patients with CRC(143).The failure to an effective immune response is thought to be because the tumor microenvironment dominated by immunosuppressive cells and in the meantime Treg cells have attracted special attention because of their ability to inhibit effector T cells, and increase the number of Treg cells enables tumor cells to evade the host immune response(144, 145).

For CRC patients, high number of Treg cells had been indicated in peripheral blood, tumor-draining lymph node, and tumor site (146, 147). On the other hand, since in patients with CRC, carcinoembryonic antigen (CEA), telomerase, HER2/neu, and MUC-1 reactive Treg cells were detected, these Treg cells are specific TAA reactive. Indeed, TAA-specific Treg cells predominantly are in the blood of CRC patients, but are not dectectable in healthy subjects (148).

$FOXP3^+$ Treg cells suppress tumor antigen-specific immune responses in CRC, which may explain ineffective immune response against the tumor (149). Results of a study show that Treg cells may impact on effector T cell trafficking into tumors. Treg-derived adenosine contributes to suppress transendothelial migration of effector T cells into tumors by reducing the ability of monocytes and ICAM to activate the endotheliumin tumor patients. This effect of Treg cells is specifically for cancer patients (150).

Many studies indicate a various importance of Treg cells in CRC cancer. Aresearch showed that in CRC intratumoral Treg cells suppressed matrix metalloproteases in the presence of IL-17, which were linked to decreased metastases (151). In another work, it was proved that in CRCs with highlevel of microsatellite instability (MSI-H), the density of $FOXP3^+$ Treg cells infiltrating CRCs was significantly higher in parallel with enhanced number of $CD8^+$ T cells and was along with good prognosis (152). Indeed, CRC models show that, at least in early-stages, CRCis along with prolonged pro-inflammatory damages resulting from GI bacteria, and Treg cells are tool in removing the local inflammation that this useful effect of Treg cells gets lost later by converting to a pathogenic phenotype(154).The prognostic effect of Treg in CRCs is controversial. A report indicated that increased peritumoral numbers of $FOXP3^+$Treg cells are due to advanced-stage tumors and weaker overall survival (155). But, the improved survival by increasing the numbers of intratumoral $FOXP3^+$ Treg cells has also been reported in CRC patients(146).

Heterogeneous nature of human intestinal tumor microenvironment may be involved in these paradoxical roles of Treg cells (146, 156).Treg cells subset that develop in human

colorectal cancer differ in their ability to suppress inflammation in comparison to Treg cells which are abundant in healthy individuals. Blatner et al., in 2012reported a subset of Treg cells in human and mice modeling of colorectal cancer, which express FOXP3 and RORγt markers, and these cells have both pro-inflammatory and cell suppressive functions (157).Therefore, these findings suggest that Treg cells are usually very protective by suppressing inflammation in cancer, but changes of these cells to the cells expressing RORγt is deleterious. On the other hand, it has been shown that the activation of Wnt/β-catenin signaling pathway increases RORγt expression in these T cells (158, 159).Thus, the activation of Wnt signaling pathway in inflammation conditions can inhibit the function of Treg cells, which the lack of control in this process can lead to autoimmune responses and even cancer.

The role of inhibitory cytokines in CRC

IL10 is an immunosuppressive cytokine that is upregulated in cancer, and it has been attributed with a role in inhibiting tumor lysis and tumor rejection as well as according some studies has a protective role.The immunosuppressive function of IL10 can be related to its effects on antigen-presenting cells and T cells. IL10 downregulates expression of costimulatory molecules by dendritic cells and induces T-cell anergy in dealing with the antigen (160, 161).The inhibitory impacts of IL10 are not always consistent; for instance IL10 in mice suppresses the proliferation of $CD4^+$ T cells but not necessarily expression of cytokines such as IFNg; or IL10 has various effects on $CD8^+$ T cellsand unexpectedly activates and expands tumor-resident T cells(162, 163). Inhibitory and enhancing effects of IL-10 on T cells depend on the activation status of these cells.The timing of exposure to IL10 seems to be significant, so that inhibitory effects at antigen priming, and stimulatory at recall(164).Recent results show important role of IL10 in IFN-γ –dependent tumor lysis and cytotoxic T-cell differentiation (163, 165, 166).The anti-inflammatory features of IL10, especially in the colon, indicate that this cytokine can suppress some immune responses while enhancing others.

In adenomatous polyps in the small intestine of $APC^{\Delta 468}$mouse model, deficient in IL-10 exacerbate the polyps in the colon and vice versa reduced in the small intestine, suggesting different nature of inflammation in the small intestine and colon.Since polyps is inhibited in the colon but not in small intestine of germ-free mice or treated with antibiotics, this difference could be related to microbes (167, 168).

IL-10 production by Treg cells is essential to control tumorigenesis in intestinal dysplasia derived from inflammation(169).Similarly, in murine model of IBD showed that recombinant IL-10 can improve symptoms (170).However, clinical results in patients with IBD have been disappointing due to dose-limiting systemic toxicity of cytokine. Recently study on the $APC^{min/+}$ mouse model of intestinal polyposis showed that oral administration of IL-10 by effects on IL-17-producing CD4+Foxp3+RORγt+ pathogenic T-regulatory can suppress polyps and improve systemic pathologies and increase the lifespan of mice(171).

TGF-β: TGF-β plays a dual role in human diseases with the influence of context. TGF-β can act as a tumor suppressor and oncogenes (172).Its tumor suppression functions, which are observed in the early stages of cancer and in normal cells, include inhibition of cell proliferation, induction of apoptosis and autophagy regulation. As tumors grow, they alter their response to TGF-β and this factor can be used as a strong promoter of cell motility, invasion, and metastasis as well as maintain tumor stem cell. Also, TGF-βinduces epithelial to mesenchymal transition (EMT) in aggressive and invasive tumors (173). Studies have shown that TGF-β can activate the Smad-independent pathways. TGF-β induces activation of Erk signaling in colorectal cancer and breast cancer cells to promote of adherens junctions and cell migration (174, 175). It has been widely proven that increased expression of TGF-β, mutations or loss of TGF-β receptors or Smad2/4 could lead to the development of colorectal, pancreatic, gastric and prostate tumors (176).Allelic variants of Smad7 (TGFβ signaling pathway inhibitor),characterized by reduced expression of Smad7, is associated with increased risk of colitis-associated colorectal cancer (177, 178).

In a mouse model, inducing increased Smad7expression in T cells was shown to promote the number of cells $CD4^+$ $T\text{-}bet^+$ $ROR\gamma t^+$ T cells infiltration of tumor, which these cells produce TNF-α and IFN-γ; in indeed, the plastic effects of Smad7 on T cells phenotype lead to protection against colon cancer (179).

IL-35: Immunosuppressive and anti-inflammatory functions of IL-35suppress T CD4 and T CD8 proliferation, as well as inhibit the TH17 function and cell differentiation into TH17 (3).Also, IL-35 possesses the ability to inhibit antibody response and plays a role in infectious tolerance (180, 181). Generally, IL-35 expression has been considered by suppressing immune system associated with tumor progression and poor prognosis(59, 182, 183). In a study, increased expression of IL-35 in colorectal tumor cells and its relationship with tumor metastasis and clinical stages of the disease have been reported (60).However, conflicting results have been reported, for example, ectopic expression of IL-35 is said to suppress cell growth in cancer cells through G1 phase cell cycle arrest with increased apoptosis (184).. But the exact impact of IL-35 is still not entirely clear on tumorigenesis, especially in the development and metastasis of colorectal cancer.

Treg cells as a therapeutic tool in the treatment of CRC

Treg cell-mediated immune suppression is one of the main barriers to succeeded tumor immunotherapy(185).Treg cells may be an attractive therapeutic target. Depletion of Tregs by cytotoxic drugs and Treg modulation in patients with CRC might raise antitumor immune immunotherapy (186).In addition, the study showed that depletion of Treg cells in the peripheral blood of patients with CRC enhances $CD4^+$ T cell responses to TAA antigens (148).However, currently losses or profits of increased Treg cells are more controversial in CRC. Since there are differences between Treg cells and Effector T cells in the repertoires of TAA antigens recognized by these cells, selected sets of TAAs can be used for tumor vaccinations that induce optimal effector T-cell responses but at least Treg activity without the need for remove of Treg cells (187).

One of the new therapeutic approaches for cancer treatment is the immune checkpoint inhibitors.Preliminary studies suggest that the immune checkpoint inhibitors, especially anti-CTLA4, anti-PD-1 and anti-PD-L1 may be effective in the treatment of patients with GI cancer(188-190). In advanced gastric and colonic cancer, different clinical trials using antibodies to CTLA4, PD-1 and PD-L1 alone or in combination are currently ongoing. Primary findings wereelevated immune response by these drugs, while CTLA4 antibodies can increase T cells activated through APC cells in the lymphoid tissues, regulation of signaling pathways associated with PD-1 can lead to more effective function of effector T cells by interfering with tumor-

associated immune suppression (188, 191, 192).Other immune checkpoints such as OX40, TIM 3 and LAG 3 are also in progress in early clinical trials(193-195). Therefore, combination therapies taking into account immune checkpoint inhibitors combined with other therapies such as chemotherapy, radiotherapy, etc. are suitable alternative treatment options, which are currently in early stages of development (196, 197).The combination of oncolytic virus with immune checkpoint inhibitors can increase the treatment efficiency of these inhibitors through induction of tumor infiltration (198, 199).

Concluding remarks

Regulatory T cells are a key factor in suppressing inflammation and maintaining immune tolerance,escaping tumor immune and reversing adoptive immunotherapy in cancer patients.The immunosuppressive function of Treg cells is especially important in the intestine where its mucosa is exposed to a variety of foreign antigens.Treg cell dysfunction is associated with a disruption in intestinal tolerance and imbalance of microbiota that may contribute to pathological inflammatory processes such as IBD and CRC diseases.Studies have reported Treg cells dysfunction in IBD patients and high densities of tumor-infiltrating Treg cells in CRC patients and its relationship with better or worse outcomes for disease. Detailed explanation is not clear for these inconsistent results. Treg cells phenotypic heterogeneity, gene expression andTreg cells functional activitiesmay have contributed to the somewhat contradictory results; it can be noted to other factors such asinsufficient markers or different techniques employed in researches to identify and monitor Treg cells, as well as microbial, nutritional and environmental agents associated with such diseases are important.

According to a heterogenous population of regulatory T cells, identifying the subsets of Treg cells and their specific roles is critical to the discovery of pathologicalTregs, targeting them as opposed to systemic therapies and thus single cell analyses, such as single cell RNA-seq, may help to recognition these cells.

Conflict of Interest

There is no conflict of interest to be declared.

Authors' contributions

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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