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Review

Recent advances in the study of regulatory T cells in gastric cancer

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ABSTRACT

Gastric cancer (GC), which features a complex pathogenesis and mechanism, remains refractory. FOXP3$^+$ regulatory T cells (Tregs), which have been implicated in the progression of gastric cancer, play an immunosuppressive role in the tumor microenvironment. However, the prognostic value of Treg infiltration is still controversial in GC patients. Recently, the association of Tregs with the clinicopathological characteristics of GC patients, the prognostic value of Tregs alone or its combination with other factors to GC patients, the role of Tregs in GC tumor microenvironment, clinical applications and Tregs-targeted therapies for GC patients have become hot issues. In this review, we are going to discuss these scientific researches which focused on these topics.

1. Introduction

Gastric cancer (GC) is the third-leading cause of death for cancer worldwide after lung cancer and liver cancer. It is estimated that there are 961,000 new cases of GC diagnosed annually. GC is particularly prevalent in China, with an estimated 470,000 new cases and over 300,000 GC-related deaths each year. In China, GC ranks second and third in the incidence and mortality of the various malignant tumors [1–3]. Furthermore, GC therapy in China remains unsatisfactory. According to global cancer survival data for the years 2000–2014, the 5-year survival rate of Chinese patients with GC was only 35.9%, which was far lower than the 5-year survival rate of GC patients in Japan and Korea during the same period (60.3% and 68.9%, respectively) [4].

Tumor infiltrative forkhead box P3-positive (FOXP3$^+$) regulatory T cells (Tregs) are an important factor in the immune microenvironment of GC. Tregs are immunosuppressive lymphocytes who exert negative immunoregulatory effects by regulating the active immune function of effector T cells (Teffs). Tregs are closely associated with the progress of a variety of immune-related diseases, including autoimmune diseases, inflammatory reactions, infectious diseases, transplant rejection, allergic diseases, and tumors [5]. FOXP3 is a transcription factor that is specifically expressed by natural Tregs. Its expression and stability are crucial for Tregs to regulate effector T cells' function, and so far it has proven to be the most reliable molecular marker of FOXP3$^+$ Tregs [6,7]. FOXP3$^+$ Tregs inhibit a variety of immune cells including CD4$^+$ T cells, CD8$^+$ T cells, monocytes, and macrophages. The massive accumulation of FOXP3$^+$ Tregs inhibits the immune effective function of effector T cells in tumor microenvironment. Many molecules are closely related to the mechanism of immunosuppression mediated by FOXP3$^+$ Tregs. These molecules include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), CD25, interleukin (IL)-10, transforming growth factor-beta (TGF-β), glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR), and others [8,9].

A great deal of current studies have evaluated FOXP3$^+$Tregs in precancerous GC lesions, concerning the association between Tregs infiltrated in the GC microenvironment and the prognosis of GC patients, the impact on clinicopathological parameters of GC, the influence on prognosis in combination with other related factors, the mechanism underlying the infiltration of FOXP3$^+$Tregs in the GC microenvironment, and Tregs-targeted therapies.

1.1. Generation of Tregs and their functions in tumor microenvironment

Based on their origins, Tregs are generally categorized into two groups: Natural Tregs (nTregs) and induced regulatory T cells (iTregs). The nTregs differentiate from the thymus. For this reason, nTregs are also known as thymus-derived Tregs (tTregs). Under certain conditions, some FOXP3$^+$Treg cells differentiate from conventional T cells (Tconv cells) in the periphery. Therefore, they are termed as induced Treg (iTreg) cells. iTregs are also known as peripherally derived Tregs (pTregs). Many factors (e.g. TGF-β, dendritic cells expressing indole amine 2,3-dioxygenase (IDO), or retinoic acid) can convert peripheral CD4$^+$ naive T cells into iTregs [10–12]. Furthermore, researchers have reported that based on the expression of FOXP3 and CD45RA, Tregs can

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be classified into three types: naive Tregs ($FOXP3^{lo}CD45RA^{+}$), effector Tregs ($FOXP3^{hi}CD45RA^{-}$), and non-Tregs ($FOXP3^{lo}CD45RA^{-}$). Naive Tregs are only weakly suppressive, while effector Tregs, which differentiate from naive Tregs after antigenic stimulation, possess strong suppressive activity and stable function. Non-Tregs cannot exert an inhibitory effect but can secrete pro-inflammatory cytokines [13].

T cell receptor (TCR) signal is indispensable in the process of Treg development, such that blockage of TCR leads to suppressed Treg development [14]. In addition, Treg development requires co-stimulatory molecules such as CD28 and GITR. Another indispensable factor for Treg development is IL-2. Tregs remain non-responsive to high-dose IL-2, solid-coated or soluble anti-CD3 monoclonal antibodies (mAb), or a combination of anti-CD3 mAb and anti-CD28 mAb. Only when a TCR signal and a high concentration of exogenous IL-2 are simultaneously present can a Treg be activated; however, the proliferation is still weaker than that of $CD4^{+}CD25^{-}$T cells [15,16].

In tumor microenvironment, Tregs participate in homeostatic regulation and tumor immune escape. In terms of mechanism, relative report exhibit that Tregs prefer the fatty acid energy supply pathway, and apt to use the metabolites of tumors to obtain energy [17]. Thus, tumor cells proliferate in an immunosuppressive circumstance in which Tregs are enriched, but effector T cells and dendritic cells (DCs) lose their function accordingly. Apart from metabolic causes, there are still many other triggers of Treg aggregation in the tumor microenvironment. Tumor cells and infiltrating macrophages can secrete a variety of chemokines to recruit Tregs [18]. Furthermore, DCs and some suppressors including IL-10, TGF-β, and IDO, which are abundant in the tumor microenvironment, are able to convert Teffs into $CD4^{+}CD25^{+}FOXP3^{+}$ Tregs and amplify thymic-derived Tregs simultaneously.

The best-known function of Tregs is that of suppressing, including $CD4^{+}$ T cells, $CD8^{+}$ T cells, antigen-presenting cell (APC), monocytes, and macrophages. Multiple potential mechanisms have been identified for Tregs suppressive function on the proliferation and activation of Teff cells in vitro and in vivo. These could be grouped into following different modes: (1) Modulation of APC activity through Treg engagement of co-stimulatory receptors on the surface of APC, leading to weaken or abrogated signals from APC to naive/effector cells; (2) Treg could secrete inhibitory cytokines like IL-10,IL-35 and TGF-β, suppressing the activity of effector cells and APC; (3) under certain circumstances, Tregs could have a direct cytotoxic effect through the production of perforin/granzyme and induce apoptosis in effector cells; (4) Tregs may also cause metabolic disruption to prevent naive/effector cell proliferation; (5) Tregs could also compete with effectors cells for APC signals or cytokines, such as IL-2; (6) Tregs in tumors undergo death under oxidative stress, and a sudden death state quickly convert ATP to adenosine, which then binds to receptors on the T cell surface and affects T cell function [19,20].

1.2. Association of Tregs with precancerous lesions related to GC

The discovery of Helicobacter pylori (HP) revolutionized the traditional theory by which several human gastrointestinal diseases develop. It is now believed that HP is not only the major pathogenic factor for several non-malignant gastric diseases such as active chronic gastritis, chronic atrophic gastritis, dyspepsia, and peptic ulcer, but more importantly, HP has been identified as the major etiological factor for malignant diseases mainly GC.

$FOXP3^{+}$ Tregs are expressed at significantly higher levels in HP-infected patients than in non-infected patients [21]. The number of $FOXP3^{+}$ Tregs is significantly elevated in gastritis and GC in HP-infected patients [22]. In children with Helicobacter pylori gastritis, Th17/Treg balance toward a Treg-biased response favors the persistence of bacteria, causing chronic active gastritis, which suggest a negative correlation between the Th17 cells/$FOXP3^{+}$ Treg ratio and the HP density. Moreover, there is a significant increase in the number of Tregs in the gastric mucosa in HP-infected children with gastritis, accompanied by an improvement in gastric inflammation. Furthermore, we can also observed that gastric microbiota can be remodelled by HP, with the up-regulated expression of FOXP3, TGF-β, and IL-10 [23–25]. In other studies, the observation of increased infiltration of $CD25^{+}PD1^{+}$ Tregs in the stomach in HP infection suggests the occurrence of immunosuppression [26]. Thus, Tregs in the mucosa may suppress the immune response and lead to the persistence of HP infection [27]. Consistent with this, the eradication of HP leads to the down-regulation of Tregs and the mRNA level of FOXP3 [28].

In terms of the mechanisms, in vivo amplification of Tregs in HP infection is mainly due to the stimulation of DCs by HP, which leads to the production of IL-1β [29]. TGF-β produced by gastric epithelial cells after HP infection enhances the development of Tregs [30]. One study reported that the expression of the B7-H1 immunoglobulin-like immunosuppressive molecule in the gastric mucosal epithelium after exposure to HP can up-regulate the development of $FOXP3^{+}$ Tregs [25]. Other researchers reported that HP not only increases the number of Tregs infiltrating the gastric mucosa but also changes their characteristics, possibly through CCL20/CCR6-mediated migration of Tregs [31].

Robinson et al. incubated gastric biopsy cell suspensions with a HP lysate antigen. The secretion of IL-10 was greatly increased in stimulated cells compared to unstimulated cells. In addition, patients with peptic ulcer exhibited greatly reduced secretion of IL-10, which correspondingly down-regulated the activity of Tregs, suggesting that Tregs with reduced secretion of IL-10 can easily mediate cell destruction and the inflammatory changes that lead to ulceration [32]. Other results have supported the fact that HP-infected GC patients exhibit increases in the infiltration of Tregs in the local mucosa and in the proportion of Tregs in peripheral blood mononuclear cells (PBMCs). Interestingly, Th1/Th2-derived cytokines were found to have declined continuously from asymptomatic gastritis to atrophic gastritis, intestinal metaplasia, and intraepithelial neoplasia, accompanied by the rise of Tregs, which led to a corresponding increase of $CagA^{+}$ HP in chronic inflammation [33].

The collective results summarized in this section indicate that Tregs have a crucial, indicative function for precancerous GC lesions, and it also play an important role in the development and progression of GC.

1.3. Correlation between Tregs and GC clinical pathology

The increased infiltration of $FOXP3^{+}$ Tregs in the course of HP-associated gastritis and GC has been amply demonstrated by latest studies. Analyses of PBMCs and clinical tumor samples of GC patients have documented the increases in the proportion of Tregs in PBMCs and the proportion of Tregs infiltrating the tumors compared with normal tissues or healthy controls. The sole exception is the description of the lower absolute number of Tregs in the peripheral blood of 33 GC patients than in normal subjects, with no significant change noted in 37 colon cancer patients [34]. In the other study, the expression levels of $FOXP3^{+}$Tregs are closely relative to the occurrence and development of GC, which provides theoretical bases and evidence for the early diagnosis, prognosis evaluation and immunotherapy of GC [35]. Tregs are also reported to be conspicuously increased in gastroesophageal cancer and are closely associated with the degree of pathological changes [36]. Moreover, the degree of infiltration by Tregs has been positively correlated with gastritis, peptic ulcer, and GC; it has been negatively correlated with the degree of intestinal metaplasia in patients with gastritis and peptic ulcer [37]. Other researchers described that compared with the control group, both Tregs and $CD14^{+}HLA^{-}DR^{-}$myeloid-derived suppressor cells (MDSCs) were remarkably higher in digestive ulcers and GC, which indicated that Tregs and MDSCs may be vital in the progression of digestive ulcers and GC [38]. Simultaneously, there were also some reports about the correlations between Tregs and GC-draining lymph nodes. In one study, a significant increase in the proportion of Tregs has been reported in

tumor-draining lymph nodes in patients with GC [39]. In addition, highly expressed FOXP3$^+$ Tregs in sentinel lymph nodes in GC could predict the metastasis to downstream lymph nodes [40].

1.4. Predictive value of Tregs infiltration for prognosis of GC patients

Predicting the prognosis of GC patients with the infiltration of Treg in the tumor microenvironment is still controversial. Poor infiltration by CD83(+) DC and abundant infiltration by Tregs can independently suggest poor prognosis in the microenvironment of gastric cancer [41]. Besides, a high FOXP3/CD4 ratio has been associated with poor prognosis in gastric cardia cancer [42]. Moreover, an increase of FOXP3$^+$ Treg infiltration in GC tissues and a high proportion of Treg/CD8$^+$ T cells both predict poor prognosis [43]. One study also pointed out that the intratumoral infiltration of FOXP3$^+$ Tregs suggests a poor prognosis in patients after radical gastrectomy [44]. Another study, which analysed blood samples from 105 gastric cancer patients following neoadjuvant chemotherapy, showed that CD3$^+$ CD8$^+$ T lymphocytes increased after chemotherapy. The researchers also described a significantly superior prognosis for patients with decreased FOXP3$^+$ Tregs when the expression of CD3$^+$ CD8$^+$ cells was high and the expression of FOXP3$^+$Tregs was low [45]. The determination of Tregs using the surface biomarker CD4$^+$ CD25$^+$ CD127$^{(low/-)}$ has been espoused with the authors opining that Tregs can lead to the progression of GC by inhibiting the proliferation of effector T cells [46].

Conversely, some studies have described that Treg infiltration predicts favorable prognosis in patients with GC. One study detected the levels of Tregs and MDSCs (Myeloid-derived suppressor cells) in 28 GC patients by multicolour flow cytometry; high levels of Treg infiltration indicated a good prognosis, while high levels of MDSC infiltration suggested poor prognosis [47]. Other researchers presented data indicating that association of intratumoral CD68$^+$ macrophages and Tregs is a promising independent predictor for survival in advanced gastric cancer, suggesting that a combination of concomitant stimulation of intratumoral macrophages and Tregs may be an effective strategy for predicting the prognosis of the advanced GC patients after radical resection [48]. In gastric cardia cancer, stroma infiltration by Tregs may inhibit GC progression caused by long-term inflammation, which suggests a good prognosis [49]. Others also suggested that in the microsatellite instability-high GC population, the high prevalence of CD8$^+$ T cells and FOXP3$^+$ Treg cells predicts good prognosis, with both cell types considered to be independent prognostic factors for these patients [50]. Besides, the high expressions of epithelial-infiltrating CD8$^+$ T cells and FOXP3$^+$ Tregs in the GC microenvironment might also predict good prognosis, the authors suggested that the spatial distance between Tregs and CD8$^+$T cells determines their effect on prognosis, with an intervening distance of 30–110 μm predicting favorable prognosis [51]. The collective findings from these recent studies support the view that infiltration by FOXP3$^+$ Tregs is significantly increased in HP-associated gastritis and GC patients. The correlation between the degree of infiltration and prognosis is still controversial. It may be related to the location of the tumor, the pathological pattern, and whether chemotherapy is provided, among many other factors.

To evaluate the prognosis of GC patients as predicted by Treg infiltration, many studies have attempted to elucidate the synergistic infiltration of other Treg-associated factors with Tregs, with the goal of more accurately predicting the prognosis of GC patients. Several current studies have focused on Treg subsets, with the rational that the infiltration of certain Treg subpopulations may be valuable in predicting the prognosis of GC patients.

In GC patients, the expression of B7-H1(PD-L1) is positively correlated with the infiltration by Tregs, while highly expressed B7-H1 and Tregs are able to independently act as poor prognostic factors for GC patients [52,53]. In the GC microenvironment, the expression of HLA-G (human leukocyte antigen) is positively correlated with the expression of Treg, and negatively correlated with the expression of CD8$^+$ T cells. HLA-G expression might induce the production of Tregs and inhibit the killing function of CD8$^+$ T cells, and a high expression often predicts poor prognosis [54]. A prior study also indicated that in the GC microenvironment, the up-regulation of HLA-G increases tumor infiltration by Tregs and predicts poor prognosis [55]. Other authors suggested that high expressions of CCR7 and FOXP3$^+$Treg can synergistically predict poor prognosis in GC patients [56]. Besides, the expression of tumor-derived HLA-I was positively correlated with the depth of tumor infiltration in 141 cases of GC, but was not directly related to the infiltration by Tregs. The combined use of Treg infiltration and HLA-I was reportedly more accurate in predicting the prognosis of GC patients [57]. In another study, a decrease in FOXP3$^+$ Tregs was evident in GC patients after neoadjuvant chemotherapy, which was accompanied by up-regulation of DC. The down-regulation of Tregs and the increased proportion of DC indicate a good prognosis after neoadjuvant chemotherapy [58].

All in all, the use of Treg infiltration to gauge the prognosis of gastric cancer remains controversial. More recently, different subsets of Tregs have been investigated, some special subsets could mediate immunosuppression specially. In the GC microenvironment, tumor-derived tumor necrosis factor-alpha (TNF-α) can induce the CD45RA$^-$CCR7$^-$Treg subpopulation and inhibit the killing function of cytotoxic CD8$^+$ T cells by IL-10 secretion. Infiltration by this subpopulation of Tregs is associated with poor prognosis in GC patients [59]. Other authors proposed that ICOS$^+$ Treg cells play a major inhibitory role in the GC microenvironment. ICOS$^+$ Treg cells are mainly found in patients with advanced GC and are also closely related to HP infection [60].

Moreover, a lot of recent researches have indicated the important role of FOXP3$^+$ tumor cells in the course of GC and in immune escape. One study described that FOXP3, a key protein in Treg differentiation and function, is expressed in Tregs and widely expressed in GC tumor tissues and various GC cell lines. The researchers proposed that the expression of the FOXP3 protein in GC tissue may influence tumor differentiation and immune escape, which may provide a new target for the treatment of GC [61]. In another study, the high expression of FOXP3 in tumor cells in the GC microenvironment predicted a good prognosis [62]. In addition, FOXP3$^+$ tumor cells also play an important role in immune escape in signet-ring cell GC [63]. Other studies showed that the histochemical examination of tissue sections from 118 GC patients associated the expression of FOXP3 in the tumor cells can predict good prognosis; the researchers also suggested that good outlook may be related to the expression of Lats2 (large tumor suppressor) and YAP (Yes-associated protein) in the Hippo pathway [64]. Similarly, FOXP3 expression in tumors often predicts a good prognosis in GC, with a correlation evident between FOXP3 and P21. The researchers opined that this mechanism may be related to the ability of FOXP3 to increase the expression of tumor suppressor protein P21 [65].

The foregoing results indicate the value of subdividing the Treg subpopulations that infiltrated in the GC microenvironment using relevant biomarkers to accurately predict the prognosis of GC patients. Furthermore, immunotherapies that target certain subsets of Tregs may produce better outcomes.

1.5. Possible mechanisms of Treg infiltration in the GC microenvironment

The related mechanisms of FOXP3$^+$ Treg infiltration in the GC microenvironment have been explored in many basic research. The changes of Tregs infiltrating in the microenvironment of GC can be roughly divided into: increasing recruitment and inducing differentiation. Regarding the aggregation of Tregs in this microenvironment, Treg infiltration in Epstein Barr virus-positive GC is more than that in Epstein Barr virus-negative GC. The findings could reflect the recruitment of CCL22, which leads to an increase in the proliferative capacity of Tregs, which in turn reduces the proportion of apoptosis and leads to the infiltration and proliferation of Tregs in the Epstein Barr virus-

positive GC microenvironment of gastric cancer [66]. Other researchers suggested that plasmacytoid DC (pDC) can recruit inducible T cell costimulator-positive Tregs in the GC microenvironment, indicating immunosuppression and a poor prognosis in GC patients [67]. Mizukami et al. confirmed that CCL17 and CCL22 can mediate the aggregation of FOXP3$^+$ Tregs in the early microenvironment of GC [68]. Previously, there were also some other researchers indicated that the localization pattern of Tregs is more meaningful for predicting prognosis than the number of FOXP3$^+$ Tregs, and the spread of FOXP3$^+$ Tregs often predicts a poor prognosis [69]. Besides, Enarsson et al. proposed that Tregs play an immunosuppressive role in the immune microenvironment mainly by reducing the recruitment and migration of T cells through intercellular contact [70]. As for the inducing differentiation, CD19$^+$CD24high CD38high Breg cells may transform effector T cells into Tregs by inhibiting the secretion of interferon-gamma from Th cells [71]. In tumor microenvironment, GC cells may affect Treg differentiation by secreting TGF-β, which mediates immunosuppression [72]. Furthermore, most Tregs, expressed in GC tissue may be transformed from TGF-β1, are secreted by GC cells [73]. Other researches reported that GC cells can induce FOXP3$^+$Treg by producing TGF-β [74] and that upon HP infection, gastric epithelial cells can induce differentiation of naïve CD4$^+$ T cells into Tregs by producing TGF-β [30]. Regarding the Treg-related immunosuppressive mechanism, Deng et al. explained that under the hypoxic conditions in the tumor microenvironment, the expression of FOXP3 can be induced by TGF-β1 [75]. By contrast, Kindlund et al. explained that rather than TGF-β, high levels of IL-10 in Tregs in the GC microenvironment are the major factors leading to Treg proliferation [76]. Moreover, in patients with stage III GC after curative resection, indoleamine 2,3-dioxygenase (IDO) expression is correlated with TGF-β expression, and TGF-β expression is associated with FOXP3 expression. Besides, IDO is related with poor prognosis and immuno-tolerance through attenuation of Treg activation in stage III GC [77].

In addition, some studies have explored the possible mechanisms of Treg infiltration in the microenvironment of GC from different perspectives. IL-35-producing B-cells are related to a range of factors involved in immune regulation and immunosuppression, including Tregs. Therefore, this group of cells plays an important role in the progression of GC [78]. In terms of the specific mechanisms, Yuan et al. pointed out that in the GC microenvironment, tumor-infiltrating Tregs may predict poor prognosis and function by inhibiting the CD4$^+$CD25$^-$T cells, the researchers also described that Tregs exercise this inhibitory function, depending on the production of cyclooxygenase-2 and prostaglandin E2 [79]. There is also a report concerning the increased prevalence of Tregs in gastroesophageal cancer may occur because Tregs are less sensitive to hydrogen peroxide than Tconv cells, which is relatively difficult for them to undergo hydrogen peroxide-mediated apoptosis [80]. In a previous study, they also suggested that Tregs recruited by gastric mucosa can inhibit the effector T cell response caused by HP infection, leading to long-term persistence of inflammation caused by bacteria and the subsequent oncogenesis [81]. Besides, other in vitro findings were consistent with the idea that morphine and ketamine can mediate immunosuppression in GC by up-regulating the ratio of Tregs [82] (Fig. 1).

At present, many studies have focused on explaining the destruction of the different types of T cells' balance in the microenvironment of GC, resulting in the development of tumors. A recent in vivo study reported that GC-mesenchymal stem cells derived from gastric mesenteric cells can affect the balance of Treg/Th17 cells in PBMCs, which may lead to immunosuppression in the tumor microenvironment [83]. Other results suggested that the imbalance between Th17 and Tregs is an important cause of the development of GC. In early pathological changes, the proportion of Th17 is higher, accompanied by an increase in Tregs, as the disease progresses the proportion of Th17 gradually decreases [84]. Peng et al. reported that the proportion of RAR-related orphan receptor gamma t isoform (RORγT) and FOXP3 is increased in PBMCs of GC patients, with the proportion of FOXP3/RORrT increasing as the disease progresses. At the same time, the levels of TGF-β and IL-10 also increase in GC patients, while there is no significant change in IL-17 and IL-23 [85]. Ohtani et al. found that compared with conventional GC patients, the proportion of CXCR3$^+$ T cells/Tregs conspicuously increases in lymphocyte-rich GC patients, indicating that Tregs may play a greater role in conventional GC [86]. Besides, other researchers reported that the imbalance of Th17/Treg in GC is related to the development and progression of the disease [87]. Lin Ret al. suggested that bone marrow-mesenchymal stem cells can mediate an increase in the ratio of IL-10/IFN-γ in HP-induced GC, and correspondingly increase the ratio of Treg/Th17 to create an immunosuppressive microenvironment [88]. In terms of the subtypes of CD4$^+$ T cells in metastatic lymph nodes of GC, Okita et al. also found that the effector T cells in metastatic lymph nodes are significantly reduced compared with non-metastatic lymph nodes, the ratio of Th1/Th2 is also decreased, and the proportion of Tregs increases accordingly [89]. The collective findings indicate that induced by the related factors, recruited from peripheral blood, and the changes in the balance among the various groups of T cells might be crucial for the Tregs infiltration in the GC microenvironment.

1.6. GC immunotherapy targeting Tregs

Treg's immunomodulating property is quite important for the development of tumors. It's specific molecules CTLA-4, GITR, PD-1 are good candidates for Treg depletion or functional modulation. However, tumor-infiltrating Tconv cells and Tregs similarly express immune-checkpoint molecules, including CTLA-4 and PD-1, indicating that antibodies targeting these proteins could affect both cell types [90].

Blocking immune checkpoint especially programmed cell death-1 (PD-1) and its ligand (PD-L1), has proven efficacy in several solid cancers, and seems to be regarded as a potential option in GC treatment. Pembrolizumab is the first anti PD-1 that received FDA approval in GC treatment, which resulted in significant survival improvements in GC patients in the multi-center study, KEYNOTE-059 [91]. However, in Phase III KEYNOTE-061 study, pembrolizumab versus paclitaxel did not demonstrate any benefits in previously treated GC or GEJ cancer [92]. A summary of the phase III clinical studies of anti-PD-L1/PD-1 that have been completed are shown in Table 1 [93,94]. In addition, some reports suggest that PD-L1/PD-1 enhance the Tregs inhibition, others assume that PD-1 inhibition might potentiate the activation and immunosuppressive function of Treg cells. Thus, the effects of PD-1 inhibition on Tregs remain unclear [95,96].

In addition, CTLA-4 is an inhibitory receptor that could hamper T cell activation and its inhibition by anti-CTLA-4 monoclonal antibody can expectedly lead to T cell activation. Two anti-CTLA-4 mAbs, ipilimumab and tremelimumab, were firstly tested by clinical trials as monotherapy in advanced cancer patients who had not exhibited favorable responses to conventional chemotherapy. In a Phase II study, ipilimumab did not show immune-related progression-free survival (irPFS) improvement compared with best supportive care in GC patients [2.92 months, 95% confidence interval (CI), 1.61–5.16 vs. 4.90 months, 95% CI, 3.45–6.54, HR = 1.44; 80% CI, 1.09–1.91; P = 0.097]. Grade 3/4 treatment-related adverse events occurred in 23% of ipilimumab-treated patients, in whom diarrhea (9%) and fatigue (5%) were most frequent [97]. And another phase II trial investigated tremelimumab as a second-line treatment for patients with metastatic gastric and esophageal adenocarcinomas, 18 patients received tremelimumab. In this trial, most drug-related toxicity was mild, one patient achieved a partial response after eight cycles (25.4 months) and remains well on study at 32.7 months [98]. Besides, current immune checkpoint inhibitors mostly block the signal rather than deplete cells expressing immune checkpoints. However, there are a large controversy about CTLA-4 blockers if they not only block the signal but also deplete Treg cells. Moreover, a recent study showed that ipilimumab and tremelimumab do not reduce Tregs in bladder, prostate and melanoma human cancers,

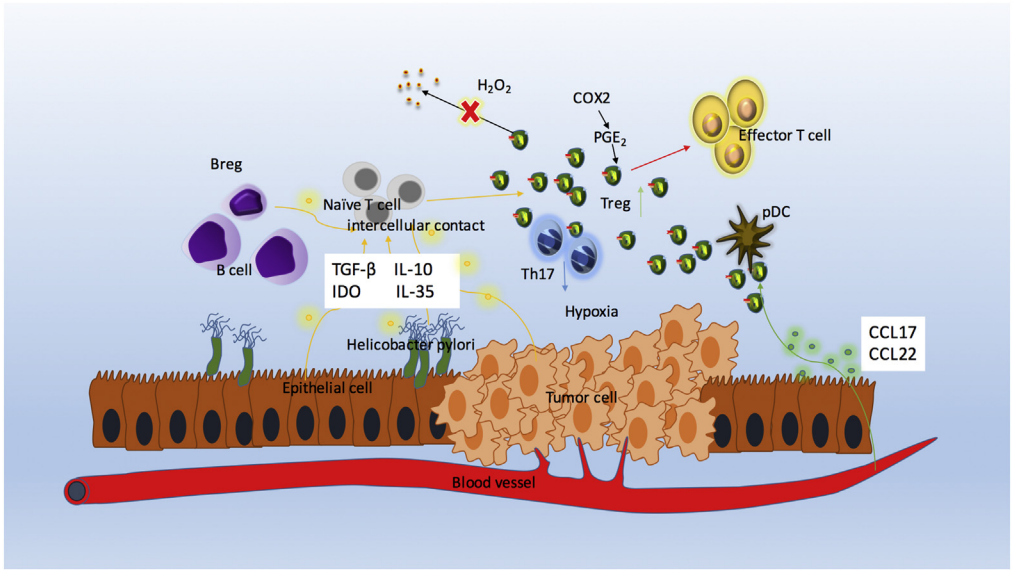

Fig. 1. Variable mechanisms of Tregs infiltration & differentiation in GC tumor microenvironment. The changes of Tregs infiltrating in the microenvironment of GC can be roughly divided into: increasing recruitment and inducing differentiation. Plasmacytoid dendritic cell (pDC) can recruit inducible T cell costimulator-positive Tregs in the GC microenvironment. CCL17 and CCL22 can mediate the aggregation of $FOXP3^+$ Tregs. Tregs infiltration may due to reducing the recruitment and migration of T cells through intercellular contact. Under the hypoxic conditions in the tumor microenvironment, GC tumor cells may affect Treg differentiation by secreting TGF-β, upon HP infection, gastric epithelial cells can induce differentiation of naïve $CD4^+$ T cells into Tregs by producing TGF-β, and other immunosuppressive factors including IL-10, IL-35, IDO. Additionally, Tregs exercise inhibitory function depending on the production of cyclooxygenase-2 and prostaglandin E2, and they are less sensitive to H_2O_2 than Tconv cells, so that it is relatively difficult for them to undergo H_2O_2-mediated apoptosis. Furthermore, imbalance of Th17/Treg in GC is also related to the development and progression of the disease.

however, several other reports described opposite results [99]. In general, the response rate of anti-CTLA-4 mAbs is still disappointing, combining CTLA-4 blockade with antigen-targeted therapy may need further investigation.

Furthermore, directly or indirectly, several potential strategies to control Treg-mediated immunosuppression in tumor microenvironment have been investigated clinically and/or preclinically. Some of the direct strategies involve targeting of certain molecules specifically expressed by Treg cells, such as CD25, OX40, GITR or ICOS. However, the role of anti-CD25 mAb is now debatable. The results of daclizumab (a humanized IgG1 monoclonal antibody targeting CD25) in combination with DC vaccination for the treatment of metastatic melanoma have been disappointing, due to the combined effects of the depletion of both Tregs and activated Teffs [100]. At present, translational research data, particularly immune monitoring focusing on Tregs, have not been reported for any of these clinical trials involving OX40, GITR or ICOS agonists in GC. In addition, targeting molecules and signals that are crucial for Tregs survival and function, such as TCR and IL-2R signalling, is also another direct approach. Other immunosuppressive cells or factors in the tumor microenvironment that might indirectly influence Treg cell survival and/or activity could also be targeted. For example, tumor-associated macrophages (TAMs) or MDSCs can be targeted to reduce the levels of chemokines that recruit Treg cells, such as CCL17 and CCL22 [101]. Other factors, such as VEGF and TGF-β, which are enriched in tumors and have roles in Treg cell activation, are other potential candidates for indirect inhibition of Treg cell-mediated immunosuppression. In 2018, a report detailed the immune profiles of paired primary tumor samples obtained from patients with gastric cancer before and after treatment with the anti-VEGFR2 mAb ramucirumab, and suggested that the density of effector Tregs, but not $CD8^+$ T cells, in the tumor microenvironment was substantially decreased after VEGFR2 inhibition [102]. Besides, there are also some small molecules for Treg depletion or functional modulation. For example, cyclophosphamide, used at low doses over a long term, has been shown to selectively reduce highly proliferating Tregs, including those in the tumor tissues, and enhance anti-tumor immune responses [103].

In order to distinguish and selectively deplete tumor-infiltrating Treg cells while preserving other Treg cells which are critical for suppressing autoimmunity, one strategy is to target effector Tregs specially. Yet, no Treg cell-targeted therapy has been proven to be quite effective in the clinic, which predominantly ascribes to the difficulties associated with selectively targeting effective Tregs. Since further basic and translational research is warranted, and Treg cell development, maintenance and inhibitory function, especially in the tumor microenvironment, are extensively understood, they could potentially lead to the increase in the efficacy of Tregs-targeted therapies and the reduction of the risk which brings about adverse effects of such treatments. In the future, in terms of such innovative insights and comprehensive immune profiling of the tumor microenvironment, immune precision including Tregs-targeted therapy, which targets at the individual GC

Table 1
Results of Phase III clinical trials of ICIs in patients with GC.

Trial	PD-L1 status	Arm (N)	Primary end point	PFS (months)	OS (months)
ATTRACTION-02	Unselected	Nivolumab (330) Placebo (163)	OS	1.6 1.5 HR = 0.60 (0.49–0.75) P < 0.0001	7.5 5.1 HR = 0.63 (0.49–0.75) P < 0.0001
KEYNOTE-061	Positive	Pembrolizumab (196) PTX (199)	OS PFS	1.5 4.1 HR = 1.27 (1.03–1.57)	9.1 8.3 HR = 0.82 (0.66–1.03) P = 0.0421
JAVELIN Gastric 300	Unselected	Avelumab (185) Physician's choice (PTX or IRI) (186)	OS	1.4 2.7 HR = 1.73 (1.4–2.2) P > 0.99	4.6 5.0 HR = 1.1 (0.9–1.4) P = 0.81

Abbreviations: ICI: immune checkpoint inhibitor; IRI: irinotecan; OS: overall survival; PFS: progression-free survival; PTX: paclitaxel.

patient, might be set up.

2. Conclusions

In summary, further researches are urgently required to unravel the mechanism of the development and progression of GC. More functional subsets and other related molecules of Treg are needed to be explored thoroughly to better evaluate the prognosis of GC patients. In addition, the unsatisfied immunotherapeutic circumstances in GC patients may also be closely associated to Tregs. The development of science and technology and in-depth research in related fields will provide new inspirations for the comprehensive treatment of GC.

Declaration of Competing Interest

The authors have no financial commercial conflicts of interest.

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