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Gastric Cancer Cell-Derived Kynurenines Hyperactive Regulatory T Cells to Promote Chemoresistance via the *IL-10/STAT3/BCL2* Signaling Pathway

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Chemotherapy resistance is frequently observed in gastric cancer patients and is associated with poor prognosis; tryptophan (Trp) catabolism has been recognized as a key metabolic regulator of many types of cancer progression. Regulatory T cells (Tregs) and Trp metabolite kynurenine (Kyn) were analyzed using tumor tissues. Chemotherapy resistance induced by IL-10 or Treg was detected by flow cytometry assay. The activation of *STAT3/BCL2* signaling pathways in gastric cells cocultured by Treg was illustrated by western blotting. Patients' Treg and human gastric cancer organoid model were established to examine the anticancer effects of STAT3 inhibitor. We found that a higher level of IL-10 secreted by Kyn-induced Tregs was responsible for the 5-fluorouracil-induced resistance of gastric cancer cell lines. STAT3 and BCL2 knockout significantly abrogated Treg supernatant- or IL-10-induced chemoresistance in SGC7901 and BGC823 cell lines. Furthermore, STAT3 inhibitor significantly reduced the organoid and clonogenicity of organoids cocultured with Treg. Our data suggested that tumor-derived Kyn may hyperactivate Tregs and induce chemoresistance through the *IL-10/STAT3/BCL2* signaling pathway.

Keywords: gastric cancer, kynurenine, chemoresistance, Treg, *STAT3*

Introduction

Gastric cancer remains the third leading cause of cancer-related deaths worldwide, and to date, the treatment of gastric cancer patients mainly involves surgical resection and chemotherapy, including driamycin, 5-fluorouracil (5-Fu), and other targeted therapy drugs (Fitzmaurice *et al.*, 2019; Wei *et al.*, 2020). However, chemotherapy resistance is frequently observed and accounts for treatment failure as well as a poor survival rate in gastric cancer patients (Biagioni *et al.*, 2019; Chen *et al.*, 2020). Therefore, studies on the molecular mechanisms of chemoresistance in gastric cancer would help provide novel therapeutic targets and prognostic biomarkers for gastric cancer.

The tumor microenvironment is closely associated with chemoresistance in many cancers (Yeldag *et al.*, 2018). Recent studies have shown that alterations in energy metabolism in the tumor microenvironment not only support aberrant proliferation of tumor cells but also participate in the regulation of chemotherapy resistance (Reina-Campos *et al.*, 2017; Li *et al.*, 2018b; Wang *et al.*, 2018). Accumulating evidence indicates that tryptophan (Trp) catabolism is a well-established pathway for immune suppression in tumors (Triplett *et al.*, 2018).

The majority of Trp catabolism-mediated immune suppression in tumors occurs through the kynurenine (Kyn) pathway (Opitz *et al.*, 2020). A previous study has shown that the Kyn pathway promotes $CD4^+$ T cell exhaustion in melanoma (Rad Pour *et al.*, 2019), and another study has reported that the interaction of Kyn with aryl hydrocarbon receptor leads to the generation of regulatory T cells (Tregs) (Mezrich *et al.*, 2010; Gutiérrez-Vázquez and Quintana, 2018). Our recent study demonstrated the role of the Kyn pathway in resistance to anti-PD-1 immunotherapy through inducing cytotoxic $CD8^+$ T cell exhaustion in colorectal cancer (Wu and Zhu, 2021).

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The expression of indoleamine 2,3-dioxygenase (IDO), which catalyses Trp into Kyn, has been found to be associated with poor response to neoadjuvant chemotherapy and prognosis, suggesting the role of Trp metabolism in the chemoresistance of tumor cells (Zhao *et al.*, 2020). Recent studies have reported that the Kyn pathway is activated in the serum of gastric cancer patients, and these studies have demonstrated that the expression level of IDO1 is higher in four of seven gastric cancer cell lines and that it promotes cell migration through Kyn (Choi *et al.*, 2016; Xiang *et al.*, 2019). In this study, we sought to determine whether the activated Kyn pathway plays a role in chemotherapy for gastric cancer and the underlying molecular mechanisms. Our study provides new insight into developing novel therapeutic targets for clinical therapy in gastric cancer patients.

Materials and Methods

Patients and ethics

Patients diagnosed with stomach adenocarcinoma at the First Affiliated Hospital of Jinzhou Medical University were enrolled in this study. The study was approved by the ethics committee and informed consent was obtained from all participants (No. 202145). Detailed information is provided in Supplementary Data S1. Tumor samples were obtained from 20 patients with stomach adenocarcinoma who underwent chemotherapy at the First Affiliated Hospital of Jinzhou Medical University (Liaoning, China) between 2019 and 2021. Patients received chemotherapy regimens of cisplatin and 5-Fu. Therapeutic effects were evaluated according to the standard of RECIST (Response Evaluation Criteria in Solid Tumors). Complete response (CR) was defined as disappearance of all lesions in both primary tumor and lymph nodes; partial response (PR) was defined as at least a 30% reduction in the sum of the longest diameter of target lesions; progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameter of target lesions; and stable disease (SD) was defined as neither sufficient shrinkage to qualify as PR nor sufficient increase to qualify as PD. CR and PR were classified as chemosensitive, whereas SD and PD were classified as chemoresistant.

The tumor samples included 10 chemoresistant gastric cancer (GC) and 10 chemosensitive GC tissues. All samples were collected from patients with informed consent, and all related procedures were performed with the approval of the internal review and ethics boards of the indicated hospitals.

Cell culture and reagents

SGC7901 and BGC723 gastric cancer cell lines were purchased from the National Infrastructure of Cell Line Resource (Beijing, China). Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cell lines were tested for mycoplasma routinely every 3 months using a mycoProbe mycoplasma detection kit (R&D Systems). Kyn and puromycin were purchased from MCE.

Generation of BCL2 or STAT3 knockout using CRISPR-Cas9

SGC7901 and BGC723 gastric cancer cell lines were transfected with sgRNA against *BCL2* or *STAT3* cloned in PX459 (Addgene). In brief, 2×10^5 SGC7901 and BGC723 gastric cancer cells were seeded in six-well plates for 24 h. SGC7901 and BGC723 cells were transfected with 5 μg of PX459 using Lipofectamine 8000 (Beyotime, China) in Gibco Opti-MEM reduced serum medium (Thermo Fisher Scientific). After 48 h, puromycin (2 μg/mL) was used for selection for 14 days, and cells were maintained in puromycin (0.5 μg/mL).

The following primers were used:
SGGFP: 5′-CACCGGGGCGAGGAGCTGTTCACCG-3′;
BCL2-SG1: 5′-CATTATAAGCTGTCGCAGAG-3′;
BCL2-SG2: 5′-TGGCGCACGCTGGGAGAACA-3′;
STAT3-SG1: 5′-AGCTACAGCAGCTTGACACA-3′; and
STAT3-SG2: 5′-ATCTTGACTCTCAATCCAAG-3′;

CCK-8 assay

In brief, 3000 cells were cultured into 96-well plates and incubated overnight at 37°C. The cells were then treated with indicated chemotherapeutic agents, other treatment for 48 h. Cell viability was assessed using the CCK-8 assay (Solarbio, China). After adding 100 μL CCK-8 solution, cells were then incubated for 2 h at 37°C. An automatic microplate reader was used for detection of the OD values at the wavelength of 450 nm.

Apoptosis assay

Cell apoptosis was evaluated with an APC Annexin V Apoptosis Detection Kit (BD). Apoptosis was measured by flow cytometry using an APC Annexin V Apoptosis Detection Kit (BioLegend).

Enzyme-linked immunosorbent assay

For tissue enzyme-linked immunosorbent assay (ELISA), tissues were collected from patients, 0.5 g of tumor tissue and 1.5 mL lysis buffer into homogenizer tube (preloaded with glass beads) on ice. After tissue homogenization, the protein suspension was centrifuged for 15 min at 12,000 *g* at 4°C. The supernatant is further used as the ELISA assay.

Tregs were seeded at 1×10^5 cells/mL in X-VIVO15 supplemented with 5% FBS for 72 h. The supernatant of Tregs were harvested for 15 min at 4700 *g* and cell culture supernatants were collected from Kyn-treated CD4 T cells for analysis of IL-10 concentration by ELISA using the Human IL-10 ELISA Kit (R&D Systems), Kyn from tumor tissue and serum was quantified using a KYN ELISA Kit (E4629; BioVision) following the manufacturer's protocol.

Western blot

SGC7901 and BGC723 gastric cells were lysed by RIPA buffer (Beyotime), and protein concentrations were measured by a BCA protein assay kit (Solarbio). Total protein was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose filter membrane (Beyotime). The membranes were blocked and incubated with BCL2 antibody (No. 15071 1:1000; CST), STAT3 antibody (No. 9139 1:1000; CST), and p-STAT3 antibody (No. 9145 1:1000; CST) followed by incubation with a secondary antibody (1:3000; Solarbio). The protein bands were detected with

ECL detection reagent (Thermo Fisher Scientific), and the images were quantified using a Tanon 4600SF system (Tanon, China).

Flow cytometry

Gastric cancer tissues were cut into small pieces, digested with collagenase/DNase I, and filtered through 70 μm cell strainers to produce a single cell suspension.

First, live/dead fixable was used as a live/dead marker to gate out the dead cells. The samples were then surface-labeled with fluorochrome-conjugated antibodies against CD3 and CD4 for 30 min, and the transcription factor fixation/permeabilization buffer (Biolegend) was used for FOXP3 staining.

Samples were analyzed by flow cytometry (BD), and data were analyzed using FlowJo V10 software.

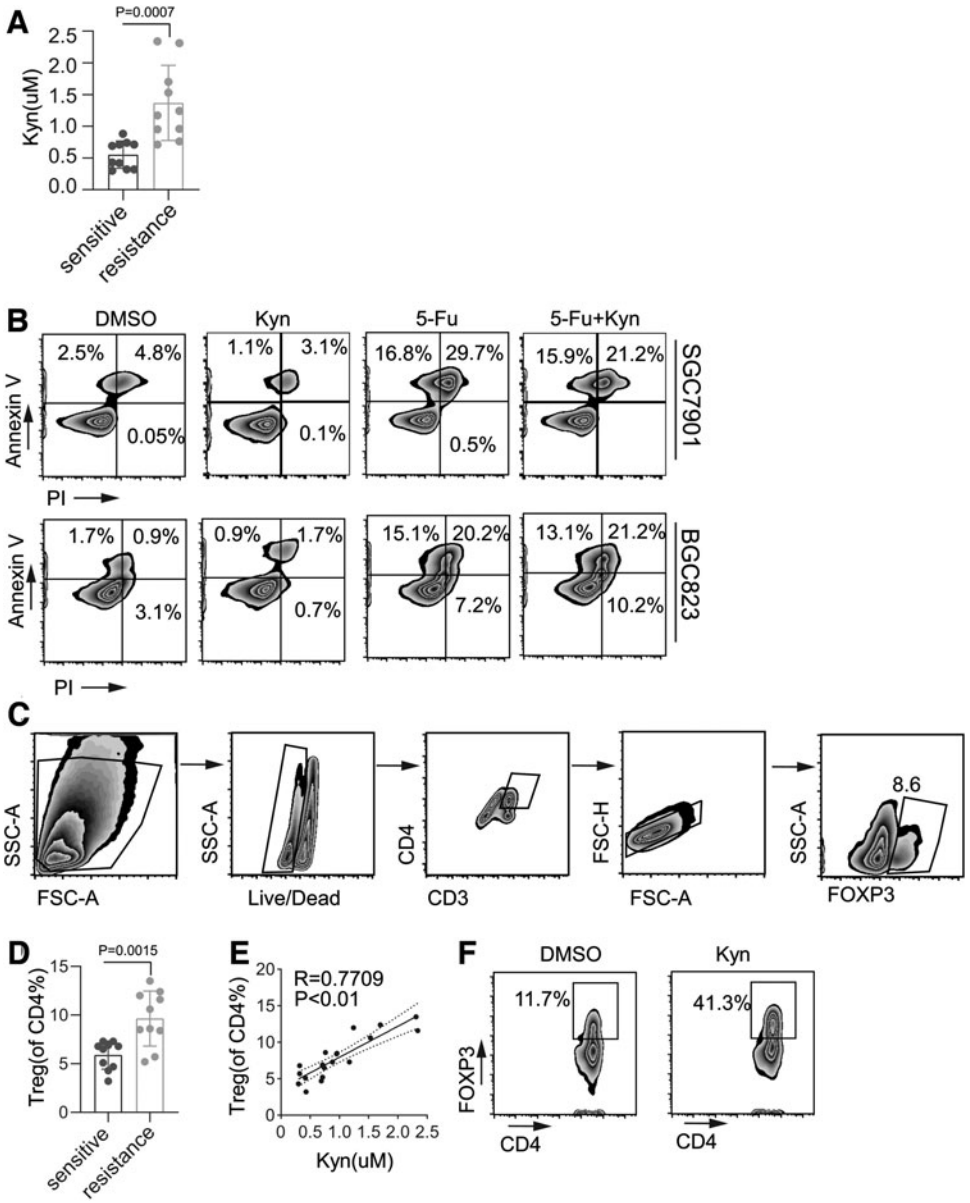

FIG. 1. Serum Kyn levels are increased and associated with Treg proportions in chemotherapy resistant gastric cancer patients. **(A)** ELISA analysis of the secretion of Kyn in tumor cells from chemotherapy-sensitive and chemotherapy-resistant tissues from gastric cancer patients. **(B)** SGC7901 or BGC823 cells were treated with DMSO, Kyn (100 μM), 5-Fu, or a combination of 5-Fu and Kyn for 48 h. Cell apoptosis was analyzed by flow cytometry. **(C)** Gating strategy for Treg analysis by flow cytometry. **(D)** Flow cytometry analysis of the percentage of Tregs in tumor cells from chemotherapy-sensitive and chemotherapy-resistant tumor tissues. **(E)** Correlation study between Tregs and Kyn in tumor cells from chemotherapy-sensitive and chemotherapy-resistant tissues from gastric cancer patients. **(F)** $CD4^+$ T cells was purified *in vitro*. DMSO and Kyn (100 μM) were added on day 0, and $CD4^+$ T cells were gated for Treg analyses on day 3. The percentage of Treg induction was determined by flow cytometry. Data are presented as the mean ± SD of three independent experiments. ELISA, enzyme-linked immunosorbent assay; 5-Fu, 5-fluorouracil; Kyn, kynurenine; SD, standard deviation; Tregs, regulatory T cells.

Treg cell culture

Blood samples were collected from healthy controls after informed consent was provided. All procedures were approved by the institutional review board of the First Affiliated Hospital of Jinzhou Medical University Hospital.

Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll density-gradient centrifugation. Total $CD4^+$ T cells purified with naive $CD4^+$ T cells were enriched by negative selection (EasySep™ naive $CD4^+$ T Cell enrichment kits; STEMCELL, Canada). Isolated human-naive $CD4^+$ T cells cultured in X-VIVO 15 serum-free medium (LONZA) were activated with anti-CD3 antibody (1 μg/mL; Biolegend) and anti-CD28 antibody (1 μg/mL; Biolegend) in a 96-well plate. For Treg culture, T cells were cultured with the addition of IL-2 (100 U/mL; PeproTech), TGF-β1 (PeproTech), and in the presence or absence of Kyn. After 6 days of differentiation, cells were harvested and assayed by flow cytometry.

Organoid culture and growth

We harvested 10–20 mm gastric tumors from the patient, mechanically disrupted them with a scalpel and enzymatically digested them on a shaker (120 RPMs, 37°C, 1 h) in the presence of a collagenase solution, containing DMEM-F12 (Thermo Fisher Scientific), 2 mg/mL collagenase (Sigma, United Kingdom), 2 mg/mL trypsin (Thermo Fisher Scientific), 5% FBS (Thermo Fisher Scientific), 5 μg/mL insulin (Thermo Fisher Scientific), and 50 μg/mL gentamicin (Solarbio). Gastric cancer organoids were maintained at 37°C as 3D spheroid cultures in Matrigel. The organoids were cultured in human complete medium (advanced DMEM/Ham's F-12 supplemented with penicillin/streptomycin; Thermo Fisher Scientific) and GlutaMAX (Thermo Fisher Scientific). Minimal basal medium supplemented with 1 × B27 (Thermo Fisher Scientific) and 10 μM Y-27632 (Selleck) was used for Matrigel drops. Organoids were passaged by removing the medium and breaking the Matrigel with phosphate-buffered saline. Organoids were then trypsinized for 90 s at 37°C in a water bath with 1 × TrypLE (Thermo Fisher Scientific), and single cells were plated in drops of 10–15 μL. Pictures were taken every 24 h and analyzed. Relative cell growth was quantified. For organoid and Treg coculture, human Tregs from PBMC of gastric cancer patients were isolated by human $CD4^+CD25^+$ Treg Isolation kit (Cat. No. 130-091-041 or 130-091-301; Miltenyi Biotec, Germany) following the manufacturer's instructions.

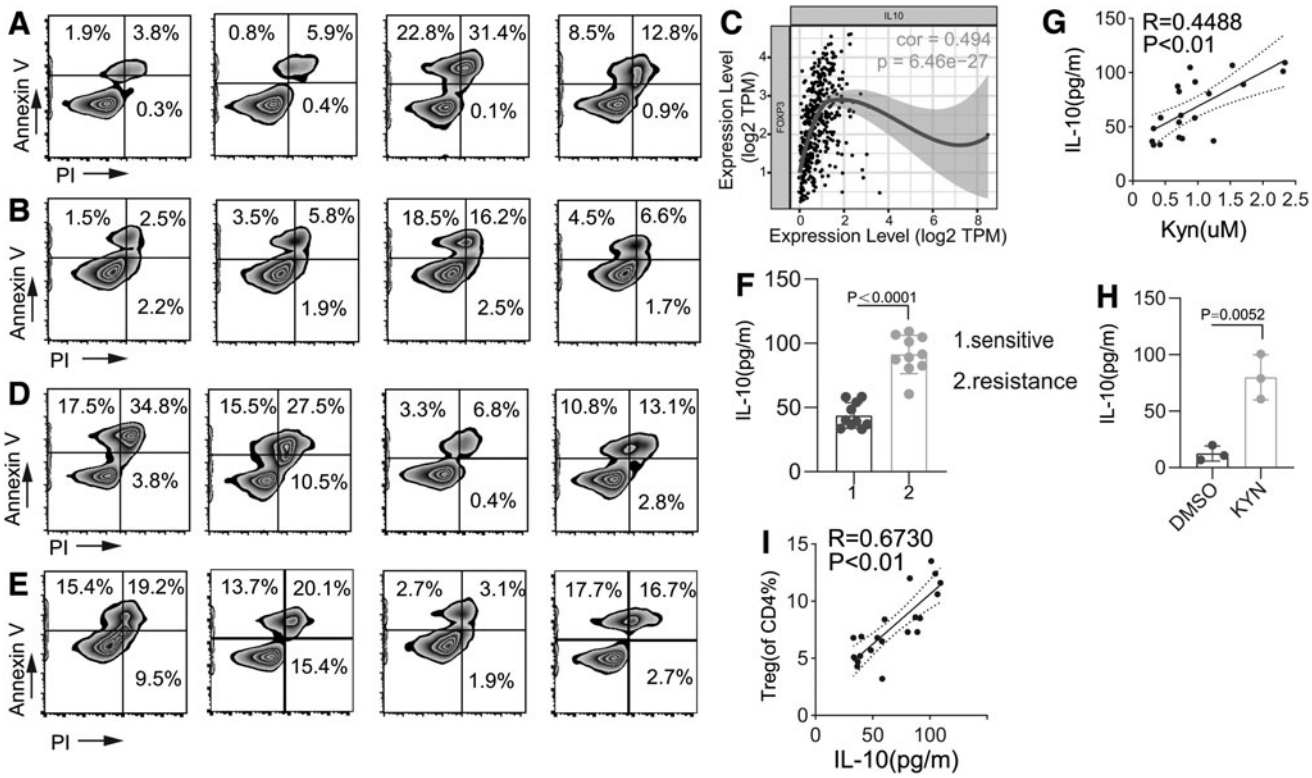

FIG. 2. Treg-derived IL-10 promotes chemoresistance in gastric cancer patients. SGC7901 **(A)** and BGC823 **(B)** cell lines were cultured overnight in NC medium or supernatants from Treg cells and then treated with DMSO or 5-Fu. Cell apoptosis was analyzed by flow cytometry. **(C)** The relationship between *IL-10* and *FOXP3* was analyzed using TCGA dataset. SGC7901 **(D)** and BGC823 **(E)** cell lines were cultured overnight in NC medium or supernatants from Treg cells in the presence of IgG or anti-IL-10 mAb. **(F)** ELISA analysis of the secretion of IL-10 in tumor cells from chemotherapy-sensitive and chemotherapy-resistant tumor tissues. **(G)** Correlation study between IL-10 and Kyn in tumor cells from chemotherapy-sensitive and chemotherapy-resistant tissues from gastric cancer patients. **(H)** Correlation study between IL-10 and Tregs in tumor cells from chemotherapy-sensitive and chemotherapy-resistant tissues. **(I)** ELISA analysis of the secretion of IL-10 by CD4 cells in the presence of DMSO or Kyn. Data are presented as mean ± SD of three independent experiments. TCGA, The Cancer Genome Atlas.

Statistical and bioinformatics analysis

All statistical analyses were conducted using R software. For bioinformatics analysis, all statistical analyses and graphical work were performed in TIMER (Li *et al.*, 2017). For survival analysis, The Cancer Genome Atlas (TCGA) RNA-Seq data from gastric cancer (Fragments Per Kilobase of exon model per Million mapped fragments [FPKM] value) were collected. Kaplan–Meier survival analysis between groups was performed using the ''survival'' R package. Pearson's test was used to describe the correlation between Kyn or IL-10 concentrations and the percentages of $CD4^+$ T cells. Significant differences were considered when $p < 0.05$.

Results

Serum Kyn levels are increased and associated with Treg proportions in chemotherapy-resistant gastric cancer patients

Kyn is a key metabolite for regulating T cell proliferation and survival and tumor progression. First, we compared Kyn expression between the chemotherapy-sensitive and chemotherapy-resistant groups and found higher expression of Kyn in the serum of chemoresistant gastric cancer patients (Fig. 1A). We speculate that Kyn may directly induce chemotherapy resistance in tumor. However, Kyn did not enhance the 5-Fu-induced cell death in the SGC7901 and BGC823 tumor cell lines *in vitro* (Fig. 1B). These results indicated that Kyn may promote chemotherapy resistance by influencing tumor immune microenvironment in gastric cancer patients.

Of interest, we found that the percentage of Tregs was significantly higher in the peripheral blood of the resistance group than in the peripheral blood of the sensitive group (Fig. 1C, D), and the percentage of peripheral Tregs in the resistance group was positively correlated with the serum Kyn level (Fig. 1E). We further purified $CD4^+$ T cells and induced Tregs in the presence of Kyn *in vitro*, and as expected, Kyn facilitated Treg induction *in vitro* (Fig. 1F). Therefore, these findings suggested that Kyn may induce increased Treg numbers in the tumor microenvironment, thus promoting chemotherapy resistance in gastric cancer.

Treg-derived IL-10 promotes chemoresistance in gastric cancer patients

Next, we sought to explore the underlying molecular mechanisms of how Kyn/Treg promotes chemoresistance in gastric cancer patients. SGC7901 and BGC823 cell lines were cultured for 24 h in medium or culture supernatants from Treg cells followed by 5-Fu treatment. The above Treg supernatants were capable of reducing 5-Fu-induced gastric cancer cell apoptosis (Fig. 2A, B), suggesting Treg itself releases a certain factor(s) to induce chemotherapy resistance. Treg cells secrete inhibitory cytokines, including IL-10 and TGF-β, in a contact-independent manner. A previous study has shown that Tregs in gastric cancer mucosa express high levels of IL-10 but low levels of TGF-β (Stewart *et al.*, 2013; Kindlund *et al.*, 2017).

In this study, we found a positive correlation of IL-10 and FOXP3 expression in TCGA gastric cancer tumor database (Fig. 2C). To explore whether Treg-derived IL-10 participates in chemoresistance in gastric cancer, we used an IL-10 mAb to neutralize IL-10 in Treg-derived culture

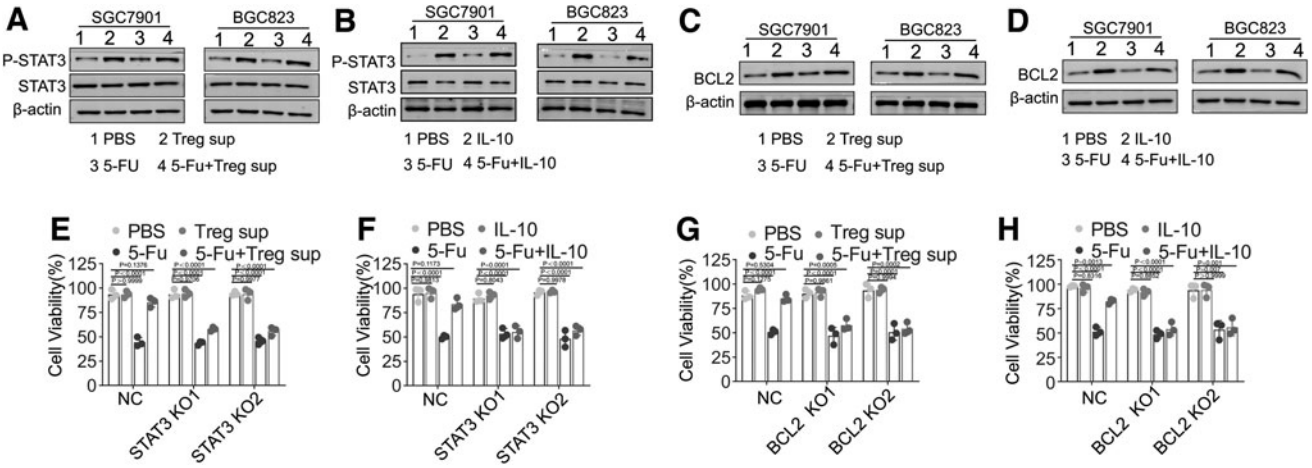

FIG. 3. Tregs promote chemoresistance through the IL-10/STAT3/BCL2 signaling pathway. **(A)** Western blotting assay of p-STAT3 and STAT3 expression in SGC7901 and BGC823 cells treated with PBS (IgG), 5-Fu (50 μg/mL), Treg supernatant, or a combination of 5-Fu and Treg supernatant. **(B)** Western blotting assay of p-STAT3 and STAT3 expression in SGC7901 and BGC823 cells treated with PBS (IgG), 5-Fu, IL-10, or a combination of 5-Fu and IL-10. **(C)** Western blotting assay of BCL2 expression in SGC7901 and BGC823 cells treated with PBS (IgG), 5-Fu, Treg supernatant, or a combination of 5-Fu and Treg supernatant. **(D)** Western blotting assay of BCL2 expression in SGC7901 and BGC823 cells treated with PBS (IgG), 5-Fu, IL-10, or a combination of 5-Fu and IL-10. **(E)** Proliferation of SGC7901-NC, SGC7901-*STAT3* KO1, and SGC7901-*STAT3* KO2 cells treated with PBS, 5-Fu, Treg supernatant, or a combination of 5-Fu and Treg supernatant. **(F)** Proliferation of BGC823-NC, *BGC823*-STAT3 KO1, and BGC823-*STAT3* KO2 cells treated with PBS, 5-Fu, Treg supernatant, or a combination of 5-Fu and Treg supernatant. **(G)** Proliferation of SGC7901-NC, SGC7901-*BCL2* KO1, and SGC7901-*BCL2* KO2 cells treated with PBS, 5-Fu, Treg supernatant, or a combination of 5-Fu and Treg supernatant. **(H)** Proliferation of BGC823-NC, BGC823-*BCL2* KO1, and BGC823-*BCL2* KO2 cells treated with PBS, 5-Fu, Treg supernatant, or a combination of 5-Fu. Data are presented as the means ± SD of three independent experiments. PBS, phosphate-buffered saline. For better viewing of the data, please see the online version.

supernatant. Both SGC7901 and BGC823 cell lines treated with Treg supernatant in the presence of anti-IL-10 mAb significantly abrogated Treg-induced chemoresistance in gastric cancer cells (Fig. 2D, E).

Moreover, serum IL-10 levels were significantly higher in the chemoresistance group and associated with the percentage of peripheral Tregs as well as serum Kyn levels (Fig. 2F–H). Induced Tregs in the presence of Kyn showed higher levels of IL-10 in the culture supernatant *in vitro* (Fig. 2I). The above results indicated that gastric cancer cell-derived Kyn induces Treg IL-10 secretion to promote chemotherapy resistance.

Tregs promote chemoresistance through the IL-10/STAT3/BCL2 signaling pathway

IL-10/STAT3/BCL2 is a well-known signaling pathway that is associated with drug resistance (Gritsko *et al.*, 2006; Yang *et al.*, 2015). Immunoblotting assays showed that SGC7901 and BGC823 cell lines cultured with either Treg supernatant or IL-10 significantly upregulated p-STAT3 and BCL2 expression (Fig. 3A–D). Positive correlations of STAT3 or BCL2 with FOXP3 expression were also detected in TCGA gastric cancer tumor database (Supplementary Fig. S1A, B). To further evaluate whether Treg-derived

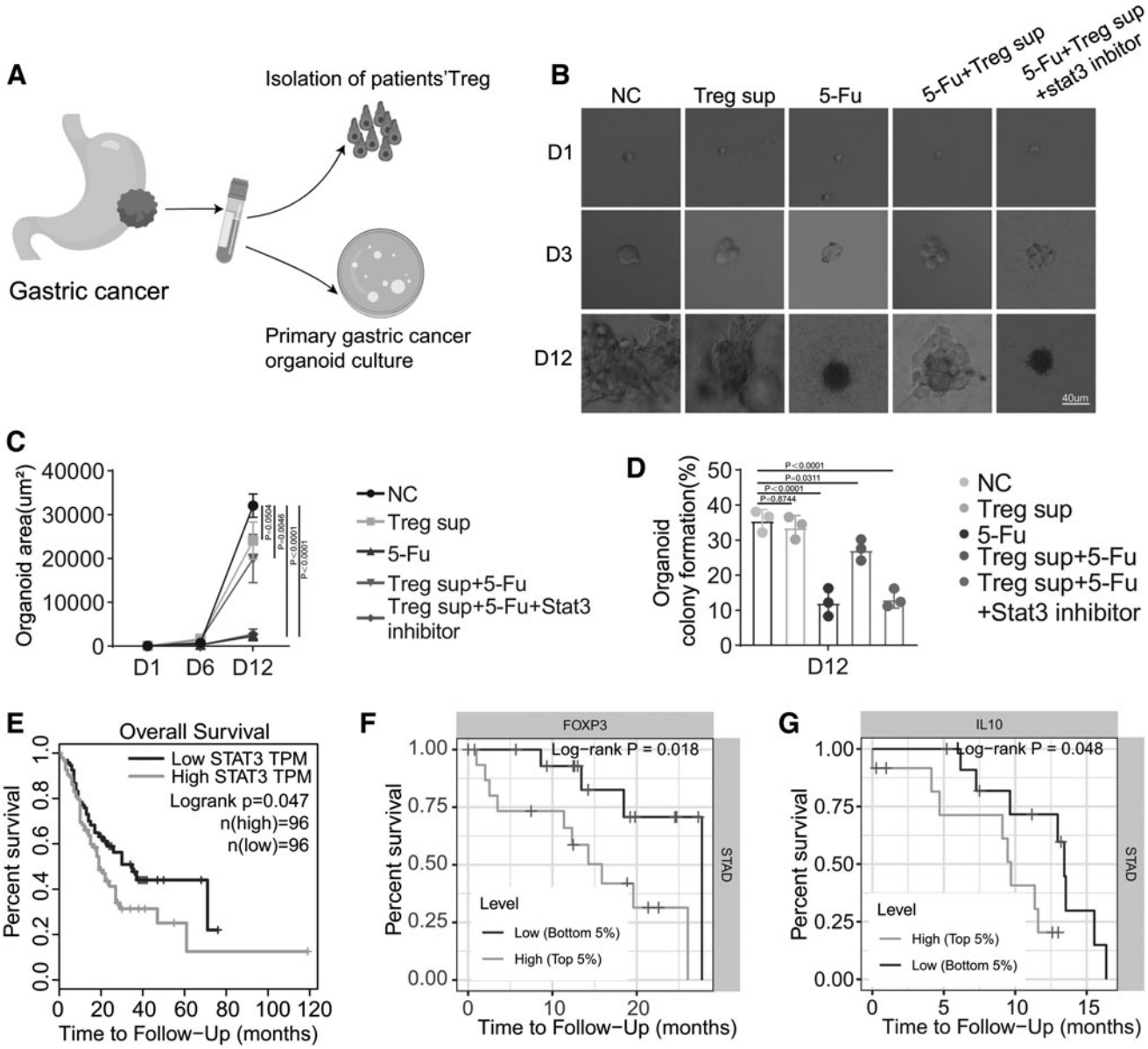

FIG. 4. Tregs promote chemoresistance through the *IL-10/STAT3/BCL2* signaling pathway. **(A)** Workflow of organoid cultures from gastric cancer tissues from patients. **(B, C)** Microphotographs of gastric cancer organoids in the indicated groups on days 1, 3, and 9. The area of the organoid area was calculated. **(D)** Colony formation analysis of gastric cancer organoids in the indicated groups on day 9. **(E)** The relationship between patient overall survival and *STAT3* expression from TCGA dataset was analyzed. **(F)** The relationship between patient overall survival and *FOXP3* expression from TCGA dataset was analyzed. **(G)** The relationship between patient overall survival and *IL-10* expression from TCGA dataset was analyzed. Data are presented as means ± SD of three independent experiments.

IL-10 promotes chemoresistance through the STAT3/BCL2 signaling pathway, we successfully knocked out *STAT3* or *BCL2* expression in SGC7901 and BGC823 cell lines, which was validated by an immunoblot assay (Supplementary Fig. S1C). *STAT3* and *BCL2* knockout significantly abrogated Treg supernatant- or IL-10-induced chemoresistance in SGC7901 and BGC823 cell lines (Fig. 3E–H).

These data suggested that Kyn induces Tregs to produce IL-10 and promote chemoresistance through the *IL-10/STAT3/BCL2* signaling pathway in gastric cancer cells.

To evaluate the Treg-induced drug resistance *in vivo*, we finally established a human gastric cancer organoid model and cultured it with supernatant from blood sample-derived Tregs. The organoid area and organoid colony formation were analyzed to evaluate the effect of Tregs on organoid growth (Fig. 4A). Cultured with supernatant from Tregs reversed 5-Fu-inhibited organoid growth, confirming the role of Tregs in promoting chemoresistance in gastric cancer. However, this effect was abrogated by the STAT3 inhibitor, which significantly reduced the organoid area and clonogenicity of organoids (Fig. 4B–D). Finally, using TCGA tumor database, we found that patients with gastric cancer expressing high levels of *STAT3*, *FOXP3*, and *IL-10* exhibited worse overall survival than patients expressing low levels of these molecules (Fig. 4E–G).

Discussion

The development of clinical strategies to overcome chemoresistance is a central goal in gastric cancer research. In this study, we found an elevated Kyn level in chemotherapy-resistant gastric cancer patients compared with chemotherapy-sensitive gastric cancer patients. Further mechanistic study showed that Kyn promoted Treg induction and chemotherapy resistance through the *IL-10/STAT3/BCL2* signaling pathway.

Trp catabolism has been implicated in the resistance of anti-PD1 and anti-CTLA-4 immunotherapy. Holmgaard *et al.* (2013) found that in a murine B16 model, the antitumor effect of anti-CTLA-4 therapy is significantly increased in $IDO^{-/-}$ mice. Botticelli *et al.* (2018, 2020) found that higher IDO activity, expressed as the Kyn/Trp ratio, predicts resistance to anti-PD-1 treatment. In a recent study, Nguyen *et al.* (2020) showed that colorectal cancer patients who failed cisplatin chemotherapy possess significantly higher Kyn/Trp ratios than the pretreatment baseline, indicating that Trp catabolism is involved in resistance to cisplatin chemotherapy. Our present study revealed a higher level of serum Kyn in chemotherapy-resistant gastric cancer patients, which significantly increased proportions of Tregs that promote chemotherapy resistance in gastric cancer patients.

The IDO/Kyn pathway has long been recognized as a critical regulator of immune system activity. IFN activates IDO1, whereas corticosteroids activate Trp-2,3-dioxygenase (TDO) (Spranger *et al.*, 2013; Badawy, 2018). Corticosteroids have been used clinically to prevent and treat cancer-related adverse reactions, mainly for pretreatment before administration of certain chemotherapy agents, antiemetic treatment for radiotherapy, and chemotherapy-related vomiting, and anti-inflammatory treatment for inflammatory injuries (Santomasso *et al.*, 2021). Corticosteroids may enhance differentiation of Treg through TDO/KYN to promote chemotherapy resistance, which is worthy of in-depth research in the future.

In this study, all our patients received corticosteroids treatment before chemotherapy, which led to us not knowing whether the tumor immune microenvironment will change in patients who did not receive glucocorticoid treatment. Li. *et al.* (2020) showed that TDO2 promotes the Epithelial-Mesenchymal Transition of hepatocellular carcinoma by activating Kyn-AhR pathway, thereby participating in the metastasis and invasion of HCC. Corticosteroid's treatment may cause liver metastasis, which may change tumor immune microenvironment compared with primary tumor. It is worth future investigation.

Previous studies have shown that Kyn plays a critical role in Treg induction (de Araújo *et al.*, 2017; Nguyen *et al.*, 2020). The Kyn/AhR signaling pathway epigenetically regulates Foxp3 expression and induces the generation and function of Tregs (Singh *et al.*, 2011; Campesato *et al.*, 2020; Qiu *et al.*, 2020). Disruption of the Treg/Th17 balance has been implicated in the pathogenesis of gastric cancer (Li *et al.*, 2013; Wang *et al.*, 2017).

A recent study has found that $TIM3^{+}/LAG3^{+}$ Tregs contribute to KRAS-related chemoresistance and correlate with CD8 T cell exhaustion and ILC2 augmentation (Domvri *et al.*, 2021). This study showed that coculture with Treg supernatant and an anti-IL-10 mAb significantly promoted 5-Fu-induced chemoresistance in gastric cancer cell lines, indicating that Treg-derived IL-10 promotes 5-Fu-induced gastric cancer cell line chemoresistance. Our study revealed a contact-independent manner of Tregs in inducing chemoresistance in cancer patients.

Several studies have demonstrated the role of STAT3 in regulating cancer chemoresistance in many cancers (Li *et al.*, 2018a; Wang *et al.*, 2018). The *BCL2* gene is a well-characterized antiapoptotic gene, and the *STAT3/BCL2* signaling pathway is also implicated in the drug resistance of B-non-Hodgkin's lymphoma and breast cancer (Alas and Bonavida, 2001; Yang *et al.*, 2015). Analysis of TCGA tumor database showed that high levels of *STAT3* exhibited worse overall survival in gastric cancer patients, and *STAT3* or *BCL2* knockout reversed Treg supernatant-induced gastric cancer chemoresistance. Together, these findings suggested the importance of the *IL-10/STAT3/BCL2* signaling pathway in chemotherapy resistance in gastric cancer.

The two limitations of this article are as follows: first, our study is focused on gastric cancer; whether tumoral cell-derived Kyns active Tregs to enhance chemoresistance in other cancer types warrants future investigation. Second, although STAT3 inhibitor did improve the chemosensitivity of tumor cells, the dose and schedule may need to be further optimized to improve efficacy and/or reduce possible toxicity in mouse model or clinical trials.

Conclusion

In summary, we elucidated the effect of Kyn on chemoresistance through Treg activation and the *IL-10/STAT3/BCL2* signaling pathway in gastric cancer patients. Our study provides a new potentially clinical strategy targeting Trp catabolism or the *STAT3* signaling pathway to treat gastric cancer.

Authors' Contributions

Z.W. conceived, designed the study and draft the article. D.W. performed the experiments and conducted the data analysis.

Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1

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