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Tumor-Derived GM-CSF Promotes Inflammatory Colon Carcinogenesis via Stimulating Epithelial Release of VEGF

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Abstract

Chronic inflammation is a major driving force for the development of colitis-associated cancer (CAC). Elevated production of granulocyte macrophage colony-stimulating factor (GM-CSF) has been observed in mucosa of patients with inflammatory bowel disease. Its actions in the progression from colitis to cancer, however, remain poorly understood. Herein, we demonstrated that colonic epithelial cells (CEC) were a major cellular source of GM-CSF and its production was significantly augmented when CAC model was established by administration of azoxymethane and dextran sulfate sodium. Furthermore, we showed that GM-CSF was a driver for VEGF release by CEC in autocrine and/or paracrine manners through the extracellular signal-regulated kinase (ERK)-dependent pathway. Blocking GM-CSF activity in vivo significantly decreased epithelial release of VEGF, thereby abrogating CAC formation. In vitro treatment of transformed CEC with recombinant GM-CSF dramatically augmented its invasive potentials, largely in VEGF-dependent fashion. Furthermore, commensal microbiota-derived lipopolysaccharides were identified as a trigger for GM-CSF expression in CEC, as antibiotics treatment or Toll-like receptor 4 ablation considerably impaired its epithelial expression. Overall, these findings may have important implications for the understanding of mechanisms underlying CAC pathogenesis and the therapeutic potentials of regimens targeting GM-CSF or VEGF in clinic. Cancer Res; 74(3); 716–26. ©2013 AACR.

Introduction

Chronic inflammation is thought to be a major driving force for the initiation and progression of cancer in many tissues (1). For example, in the patients suffering from ulcerative colitis, one of the two major forms of inflammatory bowel diseases (IBD) in human, the risk for colorectal cancer is elevated, which is 10-fold greater than that in the general Western population (2, 3). The risk of developing cancer increases strongly with the duration, extent, and severity of inflammatory disease (4). This process is well mimicked by a widely-utilized mouse model of colitis-associated colon cancer (CAC), which is induced by administration of azoxymethane (AOM) followed by repeated oral administration of dextran sulfate sodium (DSS). The molecular mechanisms underlying this neoplastic transformation are poorly understood, but a series of proinflammatory mediators produced by immune and nonimmune cells are reported to contribute to tumor growth in chronic intestinal inflammatory settings (5).

New blood vessel formation (angiogenesis) is a fundamental event in the process of tumor growth and metastatic dissemination (6). VEGF has been regarded as major mediator of this process, which is mainly mediated through VEGFR2, a receptor tyrosine kinase that is expressed at elevated level by endothelial cells (7). Intriguingly, in addition to tumor growth, increasing evidence supports the involvement of angiogenesis and VEGF in the pathogenesis of chronic inflammatory disorders such as rheumatoid arthritis, psoriasis, and IBD (8). Of note, a recent study has shown that angiogenesis and VEGF signaling might pose an important link between inflammation and tumor development (9). Intestinal epithelial cells from patients with CAC harbored activated VEGFR2 and responded to VEGF stimulation with augmented VEGFR2-mediated proliferation through the STAT3-dependent pathway. Blockade of VEGF function with soluble decoy receptors suppressed tumor development, inhibited tumor angiogenesis, and blocked tumor cell proliferation in CAC model (9). The cellular source responsible for VEGF production and the details in the regulation of VEGF secretion, however, is largely unknown in the progression of colitis to cancer.

Granulocyte macrophage colony-stimulating factor (GM-CSF) is a hematopoietic cytokine, whose levels are...
significantly elevated in the inflamed tissues of patients with IBD and rodents subjected to experimental colitis (10, 11). In accordance with this, GM-CSF− mice were more susceptible to DSS-induced colitis, as shown by clinical and histologic parameter and by augmented pro-inflammatory cytokine production (12). However, GM-CSF administration in DSS-treated mice reduced colitis severity, which was associated with accelerated hyperproliferation of intestinal epithelium and mucosal healing following GM-CSF treatment (13). However, the precise role of GM-CSF in the development of CAC is not addressed to date. In this study, we aim to investigate the role of GM-CSF signaling in CAC model using AOM and DSS. Furthermore, whether GM-CSF is a driver for VEGF release in CAC milieu is addressed.

Materials and Methods

Mice

Balb/c wild-type (WT) and Toll-like receptor 4 (TLR4)−/− mice were purchased originally from Jackson Laboratory. TLR4−/− mice were backcrossed for 12 generations onto the Balb/c background. Animals were housed in specific pathogen-free conditions with an alternating light/dark cycle. All experiments were performed using 6- to 8-week-old male mice. Care, use, and treatment of mice in this study were in strict agreement with international guidelines for the care and use of laboratory animals and approved by Animal Ethics Committee of Institute of Basic Medical Sciences.

Establishment of CAC model

CAC was induced according to classical protocols as described previously (14), with mild modification (Fig. 1A). On day 1, mice were injected intraperitoneally with AOM (10 mg/kg; Sigma-Aldrich) and maintained on regular diet and water for 5 days. Mice then received water with 2% DSS (MW 36,000–50,000; MP Biochemicals) for 1 week. After this, mice were maintained on regular water for 2 weeks and subjected to three more DSS treatment cycles. On day 35 (two cycles of DSS) or 100 (four cycles of DSS), mice were sacrificed. Macroscopic tumors were counted. The clinical course of disease was followed daily by measurement of body weight and monitoring for signs of rectal bleeding or diarrhea.

Histologic examination and immunohistochemistry

Colons were removed from mice and fixed in 10% neutral-buffered formalin solution and then embedded in paraffin, cut into tissue sections, and stained with hematoxylin and eosin (H&E). Stained sections were examined for evidence of colitis according to the criteria as previously described (15).

Figure 1. Elevated expression of GM-CSF in the CECs of CAC-bearing mice. A, schematic overview of CAC regimen. B, on day 100 after initiation of CAC induction, colonic epithelium and several immune cell subsets in lamina propria were isolated and GM-CSF expression in these cells was examined by quantitative RT-PCR. C, on day 35 (two cycles of DSS) and 100 (four cycles of DSS), colon explants were cultured ex vivo for 24 hours. GM-CSF contents in the supernatants were determined by ELISA. D, samples were collected as described in C. G-CSF and M-CSF contents in the supernatants were determined by ELISA. The data were pooled from four independent experiments. Each group consists of 6 to 8 mice. *, P < 0.01; ***, P < 0.001 versus naïve controls.
The protocol on immunohistochemistry is described in Supplementary Materials and Methods.

**Detection and quantitation of apoptotic cells**
Experiments were carried out as described in Supplementary Materials and Methods.

**Isolation of colonic epithelial cells**
Colon epithelial cells (CEC) were fractionated as described previously (15) with the purity >80%, identified by CK-18 staining.

**Isolation of lamina proria immune cell subsets**
The protocol is described in Supplementary Materials and Methods.

**Immunoblotting**
Standard procedures for immunoblotting are described in Supplementary Materials and Methods.

**Whole colon culture**
Of note, 200–300 mg of colon tissue was washed in cold PBS supplemented with penicillin and streptomycin. These segments were cut into small pieces (0.5 × 0.5 cm) and cultured (three pieces per mouse) in 24-well flat bottom culture plates in serum-free RPMI-1640 medium at 37°C for 24 hours. Supernatants were centrifuged at 13,000 rpm at 4°C for 5 minutes and stored at −80°C until use.

**Cytokine analysis**
Cytokines [GM-CSF, VEGF-A, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF)] were examined in whole colon culture supernatants by ELISA kits obtained from R&D Systems according to the manufacturer’s instructions.

**Colon carcinoma cell line culture**
Human colon adenocarcinoma cell line HCT116 was purchased from American Type Culture Collection and maintained in McCoy's 5A Medium (GIBCO, Invitrogen) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. CT26, a murine colon cancer cell line, was kindly provided by Dr. Zhihai Qin (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) and cultured in complete RPMI-1640 medium supplemented with 10% FBS, 2 mmol/L glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 50 μmol/L 2-mercaptoethanol. CT26 or HCT116 cells were seeded in 6-well culture plates (1–3 × 10⁶/mL/well) and stimulated with recombinant murine or human GM-CSF, VEGF, interleukin (IL)-1β, IL-6 (Peprotech), or lipopolysaccharide (LPS; Sigma-Aldrich) at indicated concentration for 48 hours. In some settings, neutralizing monoclonal antibody to IL-6 or GM-CSF (1 μg/mL; Biolegend), IL-1 receptor antagonists (IL-1Ra, 2 μg/mL; kindly gifted by Dr. Haiyan Hong at Beijing Proteomics Research Center, Beijing, China) were added to the culture. To determine signaling pathways, cells were preincubated with MAP–ERK kinase (MEK)-1/2 inhibitor (U0126; 1 μmol/L), phosphoinositide 3-kinase (PI3K) inhibitor (wortmannin; 500 nmol/L), and Janus—activated kinase (JAK)–2 inhibitor (AG490; 100 μmol/L) alone or in combination for 30 minutes, followed by GM-CSF stimulation. After incubation, cells were collected for analysis.

**Cell proliferation assays**
Cell growth was assessed by sulforhodamine B (SRB) assay. Cells (5 × 10⁵ cells per well in 100 μL medium) were seeded into 96-well plates in triplicate and exposed to recombinant human GM-CSF (Peprotech) at titrated concentrations. After incubation for indicated time, 50 μL of 30% trichloroacetic acid was added and incubated for 60 minutes at 4°C. After washing and drying the plate, 100 μL of 0.4% SRB was added for 30 minutes. The plates were rinsed with 0.1% acetic acid and air dried, after which 100 μL of Tris base (10 mmol/L) was added, and the plates were shaken for 5 minutes. The SRB value was measured at a wavelength of 590 nm.

**Transwell migration and invasion assays**
In vitro cell migration and invasion assays were performed using Transwell chambers with polyethylene terephthalate membrane (24-well inserts, 8.0 μm; Corning). For the migration assay, 2.5 × 10⁴ cells were added to the top chambers. For the invasion assays, 5 × 10⁴ cells were seeded to the top chambers coated with Matrigel (BD Biosciences). Complete medium alone or with addition of recombinant human GM-CSF at indicated concentrations was added to the bottom wells to stimulate migration or invasion. In some cases, monoclonal antibody to human VEGF (bevacizumab, Roche) or isotype (2 μg/mL) was added into the bottom chamber. After incubation for 16 hours, cells were stained with 0.4% SRB. Quantification was performed by determining SRB value at a wavelength of 590 nm.

**Flow cytometry**
The protocol is described in Supplementary Materials and Methods.

**In vivo neutralization**
Mice subjected to CAC induction received intraperitoneally monoclonal antibody to murine GM-CSF (MP1-22E9) or VEGF (2G11-2A05) at a dose of 0.25 mg/mouse on day −1, 0, 2, 5, and then every 5 days until the animals were sacrificed. The isotype controls were administrated with the same dosage and periodicity. All neutralizing antibodies and isotopes were purchased from Biolegend.

**Commensal depletion**
Six-week-old mice were treated with ampicillin (1 g/L), vancomycin (500 mg/L), neomycin sulfate (1 g/L), and metronidazole (1 g/L) in drinking water for 4 weeks. Stool was collected from antibiotic-treated and -untreated mice and the efficacy of commensal depletion was confirmed by staining of SYBR Green solution as described previously (16). During CAC induction, administration of cocktail of antibiotics was continued until mice were sacrificed. In some settings, flora-depleted mice were injected intraperitoneally with LPS (10 mg/kg).
Quantitative reverse transcriptase PCR

Experiments were carried out as described in Supplementary Materials and Methods. The primers for real-time were listed in Supplementary Table.

Statistical analysis

Data are presented as mean ± SD. The Student t test (two tailed) was used to determine the significance, with P < 0.05 considered significant. Statistics were performed using SPSS 10.0 for Macintosh, and graphs were made on Deltagraph (SPSS).

Results

GM-CSF is produced substantially by neoplastic colonic epithelial cells

First, we collected the colon samples of mice suffering from CAC, and isolated the epithelium. Quantitative PCR analysis showed that the neoplastic epithelial cells were one of the major sources for GM-CSF and produced high levels of this cytokine (Fig. 1B), which is paralleled by elevated contents of GM-CSF in the colonic tissues compared with controls (Fig. 1C). Also, as reported by previous studies (17), mononuclear phagocytes (Gr-1lo/CD11b+) residing in lamina propria expressed considerably this cytokine (Fig. 1B). Of note, augmented production of GM-CSF in diseased tissues was observed at the early stage of CAC development (Fig. 1C), in sharp contrast with the patterns of expression of other putative colitis-associated cytokines, for example, G-CSF and M-CSF (Fig. 1D). These results indicate during the progression of intestinal inflammation to tumor, irritated CECs produce large amounts of GM-CSF, which may be a key event linking inflammation and cancer.

GM-CSF drives VEGF expression in malignant colonic epithelial cells

VEGF has been regarded as a critical factor involved in inflammation-associated colorectal cancer (9). Accordingly, we detected its expression in the colons of CAC-bearing mice and found significantly increased production of VEGF at the early and late stage of CAC development (Fig. 2A). The dissection of cell types responsible for VEGF production showed that CECs were one of major cellular sources for VEGF (Fig. 2B). Intriguingly, mononuclear phagocytes residing in lamina propria also expressed this cytokine substantially and the expression was augmented in the CAC-bearing mice (Fig. 2B). Nevertheless, the identity of driver of VEGF secretion is still elusive. Given the same patterns of expression of GM-CSF and VEGF, we hypothesized that GM-CSF was a stimulator for epithelial VEGF expression, as CECs expressed GM-CSFR-α, and the level of this receptor expression was elevated after CAC formation (Supplementary Fig. S1A). To address this issue, GM-CSF was used to stimulate a mouse colon carcinoma cell line who expressed GM-CSFR (Supplementary Fig. S1B). Indeed, GM-CSF could induce considerable expression of VEGF (Fig. 2C), in a dose-dependent manner. Notably, GM-CSF seemed to be a major factor for forcing neoplastic epithelial cells to express VEGF, as IL-6 or IL-1β alone was unable to induce VEGF expression (Fig. 2D), considering their tumor-promoting role in CAC (16, 17), although CT26 virtually expressed the receptor of these cytokines (Supplementary Fig. S2). Therefore, although GM-CSF stimulation also induced IL-6 and IL-1β expression in cancer cells (Supplementary Fig. S3), it seemed to be unlikely that GM-CSF–driven VEGF expression was indirect and via inducing IL-6 or IL-1β expression. This conclusion was further confirmed by the fact that blocking IL-6 or IL-1 activity did not affect GM-CSF–elicited VEGF expression (Fig. 2E).

Neutralization of GM-CSF activity downregulates VEGF production and reduces angiogenesis

We asked whether the effect of anti-GM-CSF on CAC development was associated with reduced VEGF expression and angiogenesis. To this end, VEGF expression in CEC of isotype or GM-CSF antibody-treated mice subjected to AOM–DSS was examined. Indeed, GM-CSF blockade led to significant decrease of VEGF expression in CEC (Fig. 4A). This
was paralleled with reduction in intratumoral vessel density (Fig. 4B). Furthermore, the amounts of phosphorylated STAT3 in CEC were detected. The results showed that the phosphorylation of STAT3 in anti-GM-CSF–treated CEC was pronouncedly inhibited (Fig. 4C). In accord with this, NF-κB activity in epithelium of anti-GM-CSF–treated mice was
restrained, as shown by reduced phosphorylation of the p65 subunit (Fig. 4C).

To directly evaluate the association of VEGF expression with CAC development, a monoclonal antibody recognizing VEGF was utilized. We found that blocking VEGF activity did repress tumor growth and attenuate intestinal damage as well as reduce angiogenesis (Fig. 4D–F). In addition, the protumor effect of GM-CSF–VEGF axis during CAC development was partially attributed to its direct regulation of the growth of CEC, as murine CEC virtually expressed VEGFR2 (Supplementary Fig. S2; ref. 9) and anti-GM-CSF treatment reduced VEGFR2 activation in CEC (Fig. 4G). As described above, VEGF blockade resulted in increased apoptosis of CEC (Fig. 4H) and reduced expression of Bcl-XL (Fig. 4I). Furthermore, restricted activation of the STAT3-dependent pathway in anti-VEGF–treated CEC was observed (Fig. 4J). In accordance with this, the proliferation of epithelial cells was scarcely visible in anti-VEGF–treated mice (Supplementary Fig. S4B), which may be due to reduced expression of several cell-cycle genes in CEC (Supplementary Fig. S5B). Taken together, these results suggest that GM-CSF–elicited VEGF expression in CEC may play a crucial role in CAC formation.

Stimulation with exogenous GM-CSF augments the invasive potentials of CEC via inducing VEGF production

Next, we sought to determine the autocrine/paracrine effect of GM-CSF expression on the outgrowth and invasion of malignant colon epithelial cells HCT116, which express GM-CSFR (Supplementary Fig. S1C), we found that stimulation with GM-CSF did not modify the growth of HCT116 (Fig. 5A). The migration and invasion of HCT116, however, was significantly enhanced when exposed to exogenous GM-CSF, in dose-dependent manners (Fig. 5B and C). Furthermore, in agreement with the data shown in Fig. 2D, GM-CSF stimulation did lead to VEGF expression in HCT 116 cell lines (Supplementary Fig. S6A), and this effect was dependent on the activation of the ERK pathway, given that inhibition of this pathway dramatically repressed GM-CSF–driven VEGF expression (Supplementary Fig. S6B and S6C). Therefore, we aimed to determine the relationship between VEGF release and GM-CSF–mediated invasion of HCT116, which virtually expressed the receptors of VEGF (21, 22). Blocking VEGF using neutralizing specific antibody dramatically impaired the potentials of GM-CSF to promote migration and invasion of HCT116 (Fig. 5D and E). These data indicate that GM-CSF could enhance the
Figure 4. Decreased expression of VEGF in CEC following GM-CSF neutralization is involved in CAC tumorigenesis. A, AOM/DSS-administered mice were treated with neutralizing antibody to GM-CSF or isotype (as described in Materials and Methods). On day 100, after initiating CAC induction (see Fig. 1A), CEC was isolated and its VEGF expression was determined by quantitative RT-PCR. B, angiogenesis in tumor tissues was detected by staining with anti-CD31 antibody. Representative images from three independent experiments are shown. Arrows, vessels. Scale bar, 50 μm. Staining density was measured and quantified with ImagePro plus 7.0 Software using images (left). Fifteen to twenty histological fields were randomly selected from each slide for analysis of staining density. C, CEC was isolated from mice treated with anti-GM-CSF or isotype and protein was extracted. Phosphorylation of the NF-κB p65 subunit was examined by immunoblotting. D–J, mice subjected to CAC induction were injected with neutralizing antibody to mVEGF-A or isotype according to the protocol as described in Materials and Methods. D, tumor number was counted. E, histologic examination of colon tissues was performed by H&E staining. Colitis score was shown. Scale bar, 200 μm. F, angiogenesis in tumor tissues was detected by staining with anti-CD31 antibody. Representative images from three independent experiments were shown. Arrows, vessels. Scale bar, 50 μm. Staining density was measured and quantified with ImagePro plus 7.0 software using images (left). Fifteen to twenty histologically fields randomly selected from each slide for analysis of staining density. G, on day 35 after initiation of DSS drinking, CEC of anti-GM-CSF or isotype-treated mice was isolated and VEGFR2 phosphorylation in CEC was determined by immunoblotting. The experiments were repeated three to four times with similar results. H, apoptosis of CEC was detected by TUNEL method. Quantitation of apoptotic cells was shown. Scale bar, 100 μm. I and J, the protein of CEC was extracted and Bcl-XL (I) contents and phosphorylation of STAT3 (J) were detected by immunoblotting. The data were pooled from three independent experiments. Each group consists of 6 to 8 mice. **, P < 0.01; ***. P < 0.001 versus isotype controls.
invasive behavior of transformed CEC by upregulating its expression of VEGF.

**CEC challenged by commensal flora-derived LPS produces GM-CSF**

Commensal translocation is thought to be a key event for initiation of intestinal inflammation and CAC development (23). Thus, we hypothesize that the attack of luminal microbiota is required for GM-CSF expression in CEC. To address this issue, bacteria inhabiting the gut were eradicated by antibiotics treatment followed by AOM/DSS. Indeed, depletion of colonic microorganism partially suppressed GM-CSF expression in the colon (Fig. 6C), and in CEC after CAC induction (Fig. 6D). This suggests commensal flora as a trigger for GM-CSF expression in CEC.

To further dissect this issue, TLR4−/− mice were subjected to CAC induction. As described previously (24), TLR4 knockout protected against CAC development (Fig. 7A). In parallel with macroscopic changes, the absence of TLR4-mediated signaling led to a considerable reduction of GM-CSF expression in CEC after CAC induction (Fig. 7B). As CEC expressed TLR4 (Supplementary Fig. S7A and S7B), we speculated that the outcome was directly due to loss of TLR4 in CEC. To address this, CT26 cells, that virtually expressed TLR4 identified by fluorescence-activated cell sorting analysis (Supplementary Fig. S7C), were stimulated with TLR4 ligand LPS. Consequently, LPS challenge was sufficient to induce GM-CSF and VEGF expression in transformed CEC (Fig. 7C). To further investigate the link of CEC-derived GM-CSF expression to LPS challenge in vivo, we injected LPS into commensal flora-depleted mice. The result showed that LPS administration efficiently triggered GM-CSF expression in CEC (Fig. 7D). Overall, these findings indicate an important role of commensal flora-derived LPS-mediated signaling in promoting GM-CSF expression in CEC during CAC tumorigenesis.

**Discussion**

Recent studies have demonstrated that GM-CSF plays a nonredundant role in facilitating epithelial cell proliferation and ulcer healing in response to epithelial injury during intestinal inflammation (13, 25). Its function, however, on the development of CAC remains unclear. In the present study, we provide evidence for the first time that GM-CSF is a key regulator to promote the development of CAC, and that neoplastic CECs are an important producer for this cytokine. Given that GM-CSF
administration has been proven to be effective for treatment of IBD (13, 26), our data highlight that it may be necessary to rigorously assess these therapeutic regimens.

The molecular actions of GM-CSF in promoting the progression of colitis to cancer are still unknown. In this report, we provided clues that transformed epithelial cell-derived GM-CSF elicited large release of VEGF by itself in an autocrine and/or paracrine manner, thereby facilitated uncontrolled proliferation of CEC and intratumoral angiogenesis. This regulation of VEGF production and angiogenesis by GM-CSF is in agreement with recent studies in tumor and nontumor models (27, 28). The dissection of signaling pathways involved in this process revealed that the ERK-mediated pathway was required for GM-CSF-elicited VEGF expression. Indeed, ERK signaling is one of the major pathways that is responsible for VEGF expression induced by various stimuli in transformed or nontransformed cells (29, 30). Of note, activation of ERK signaling seems to increase translocation of hypoxia-inducible factor 1α (HIF-1α) from cytoplasm to nuclei, consequently it binds to the VEGF promoter and initiates the transcription of the growth factor (31). Considering elevated contents of HIF-1α are a universal feature in tumor cells, including colon carcinoma cells (22), it is plausible that GM-CSF-elicited VEGF expression in CEC in chronic inflammatory microenvironment is attributed to, at least in part, increased HIF-1α translocation. Of note, mononuclear phagocytes (including macrophage and dendritic cells) residing in lamina propria are also cellular sources of GM-CSF and VEGF in the setting of CAC. So it is likely that mononuclear phagocyte-derived GM-CSF/VEGF also has a role in malignant transformation of CEC during intestinal inflammation and that there is cross-talk between CEC and mononuclear phagocytes. The details need further investigation in the future.

Our data demonstrated that GM-CSF stimulation in malignant colon epithelial cells enhanced their motility. VEGF was a key player in this process, which is in line with previous reports showing that VEGF represents one of proinvasive factor for human colon cancer cells (32). Of note, GM-CSF had no ability to enhance proliferation of CEC in vitro. Thus, in the context of CAC, other proinflammatory factors may be involved in hyperproliferation of epithelium, for example, IL-6. Blockade of GM-CSF in vivo downregulates the expression of these factors, thereby represses CAC formation. In fact, we found that blocking GM-CSF did decrease IL-6 expression in lamina propria mononuclear phagocytes (data not shown). Therefore, GM-CSF has the ability to promote the growth and invasion of malignant CEC via acting on epithelium itself and/or modulating protumor function of stromal cells in the surroundings.

GM-CSF is secreted by a variety of cell types in vitro in response to proinflammatory stimuli (33). In CAC model, DSS administration disrupts the epithelial barrier and led to translocation of luminal microbiota into mucosa that triggers intestinal inflammatory reaction. Insult of invasive microorganisms and their components such as LPS is sufficient and necessary to GM-CSF expression in CEC, in that eradication of commensal flora or TLR4 deficiency resulted in substantial reduction of GM-CSF expression in CEC after CAC induction. Other factors such as TNF-α and IL-1 that have potentials to induce GM-CSF (34), however, may be involved in this process, because these factors play a crucial role in the pathogenesis of CAC (16, 35). Of note, the expression of these proinflammatory mediators in CAC microenvironment is regulated by...
Thus, it is reasonable that commensal microbiota and their components (e.g., LPS) induce GM-CSF expression in CEC directly and indirectly in the milieu of intestinal inflammation.

Our study proposed a schematic model of GM-CSF function on CAC carcinogenesis as following: oral administration of DSS leads to disruption of colonic epithelial barrier and commensal translocation into mucosa. Challenge of microbiota elements such as LPS induces high-level expression of GM-CSF in CEC. GM-CSF has a positive feedback on overexpression of GM-CSF and elicits VEGF release by CEC, the latter promotes hyperploration of CEC and angiogenesis thereby enhances its tumorigenic capacity. Notably, besides an immune-independent effect of GM-CSF on CAC development, its influence on immune cells (e.g., myeloid suppressor cells) in the microenvironment of inflammation is desired to be addressed, as shown by a recent study that tumor-derived GM-CSF potently induced the development of myeloid suppressor cells that suppressed antitumor immune response (37). Indeed, in CAC milieu, we found increased accumulation of Gr-1⁺ myeloid suppressor cells in the lesions and that blocking GM-CSF activity dramatically decreased the number of this population (our unpublished data). Thus, further investigations on the immune-dependent effect (particularly myeloid suppressor cells) of GM-CSF on CAC development are warranted. Overall, our findings may have implications for understanding of mechanisms underlying CAC pathogenesis.

Targeting GM-CSF or VEGF may have therapeutic potentials to the treatment of CAC in clinic.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: Y. Wang, G. Han, G. Liu, C. Hou, R. Guo, Y. Li, G. Chen
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Li, C. Hou
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