Enhanced angiogenesis by recruitment of VEGFR1⁺CXCR4⁺ cells from bone marrow during experimental colitis

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Objective: Angiogenesis is important for ulcer healing. The expression of vascular endothelial growth factor (VEGF) was enhanced in ulcerative colitis (UC). We examined the relationship between VEGF Receptor 1 (R1) signaling and mucosal repair using dextran sodium sulfate (DSS)-induced colitis in a mouse model of UC.

Method: We administered 2.0% DSS solution to wild-type (WT) and VEGFR1 tyrosine kinase (TK) knockout (VEGFR1 TK-/-) mice for 7 days.

Results: Compared to WT mice, VEGFR1 TK⁻/⁻ mice showed shortened colon length, delayed repair, and impaired angiogenesis of inflamed mucosa. Accumulation of VEGFR1⁺epidermal growth factor (EGF)⁺ cells was lower in VEGFR1 TK⁻/⁻ mice. The expression of stromal cell-derived factor 1 (SDF-1) and C-X-C chemokine receptor type 4 (CXCR4) was also suppressed in the VEGFR1 TK⁻/⁻ mice. The number of VEGFR1⁺CXCR4⁺ cells in peripheral blood and the ulcerous area was significantly lower in VEGFR1 TK⁻/⁻ mice. Bone marrow (BM) experiments showed that colon length in WT mice transplanted with VEGFR1 TK⁻/⁻ BM was significantly shorter than that in WT mice transplanted with WT BM. Furthermore, the accumulation of BM-derived VEGFR1⁺EGF⁺ cells and VEGFR1⁺CXCR4⁺ cells in inflamed mucosa were diminished in WT mice transplanted with VEGFR1 TK⁻/⁻ BM.

Conclusion: VEGFR1 signaling plays an important role in angiogenesis and mucosal repair by the recruitment and accumulation of VEGFR1⁺CXCR4⁺ cells in inflamed mucosa from BM via the SDF-1/CXCR4 axis.

Key words: DSS-induced colitis, VEGFR1, CXCR4, mice

Abbreviations: UC, ulcerative colitis; IBD, inflammatory bowel disease; VEGF, vascular endothelial growth factor; TK, tyrosine kinase; VEGFR1, VEGF receptor-1; VEGFR2, VEGF receptor-2; BM, bone marrow; VEGFR1 TK⁻/⁻, VEGFR1 TK knockout; SDF-1, stromal cell-derived factor 1; CXCR4, C-X-C chemokine receptor type 4; DSS, dextran sodium sulfate; PBS, phosphate buffer solution; H&E, hematoxylin & eosin; PCR, polymerase chain reaction; EGF, epidermal growth factor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; WT, wild-type
Introduction

UC is a nonspecific IBD that forms erosions and ulcers in the colon mucosa and exhibits clinical symptoms such as diarrhea and bloody stools. IBD including UC is a worldwide disease whose incidence is increasing in newly industrialized countries. Recent findings showed that angiogenesis plays a critical role in the development of IBD. Angiogenesis is a phenomenon that new vessels are formed from pre-existing microvasculature. In UC, angiogenesis is triggered by inflammation but also abnormal immune response. VEGF-A is known as one of the most potent angiogenesis stimulators. Several studies showed that VEGF has a significant role in bowel inflammation. VEGF mainly binds to 2 types of TK receptors; VEGFR1 and VEGFR2. VEGFR2 has a lower binding affinity to VEGF than VEGFR1 but acts directly on angiogenesis. VEGFR2-deficient mice die in the fetal phase due to angiogenesis failure. Scaldaferrri et al. reported that VEGF-A and VEGFR2 levels were increased in intestinal mucosal samples of IBD patients and mice with experimental colitis. Contrastingly, VEGFR1-deficient mice die from excessive angiogenesis. Interestingly, VEGFR1 TK domain-deficient mice show normal angiogenesis that suggests that VEGFR1 is a negative regulator of angiogenesis during fetal life. VEGFR1 helps the recruitment of BM-derived cells into peripheral blood by the release of SDF-1 in an ischemic hind limb model. These mobilized BM cells express CXCR4 to the ischemic muscle site. We previously reported that VEGFR1 TK signaling is involved in the repair of gastric ulcers and the recovery of blood flow via the recruitment of VEGFR1 positive BM derived cells. Though the expression of the VEGF was enhanced in activated UC, the precise mechanism, especially of the VEGFR expression in mucosal healing of UC, is not yet well understood. In the present study, we show that VEGFR1 TK signaling facilitates the inflamed colonic mucosa to promote mucosal healing. These results suggested that VEGFR1 TK signaling is the key regulator of mobilization of BM-derived VEGFR1+CXCR4+ cells to the ulcerative colitis area.

Materials and Methods

Animals

Male C57Bl/6 mice (7−10 weeks old, 20−25 g) were obtained from Clea Japan (Tokyo). We started the experiment 1 week after the mice were brought in to help them adapt to the environment. VEGFR1 TK+ mice with a C57Bl/6 hybrid background were created as described previously. All mice were maintained at constant humidity (60% ± 5%) and temperature (22°C ± 1°C) on a 12-hour light/dark cycle. The mice could eat freely throughout the experimental period. All experiments were carried out according to the guidelines for animal experimentation of Kitasato University School of Medicine.

The DSS-induced colitis model

Mice were fed 2.0% (w/v) DSS (MP Biomedicals, Illkirch, France) dissolved in tap water ad libitum for 7 days; mice were given normal water from Day 7.

Evaluation of the colon length

The colon length was measured on Days 0, 7, and 14 from the ileocecal junction to the anal verge. The colon was incised longitudinally and the stool removed with a PBS on a wet acrylic board so as not to stretch the colon for the measurement.

Histological analysis

Small sections of the distal colon were fixed with 10% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), dehydrated in a graded ethanol series, and embedded in paraffin. Sections (3 μm) of the paraffin-embedded tissue were mounted on glass slides and either stained with H&E or processed for immunohistochemistry. For immunostaining, the sections were heated at 100°C for 20 minutes in Histo VT One (Nacalai Tesque, Yokohama). The sections were then treated with 0.03% H2O2 to inactivate the endogenous myeloperoxidase. After inactivation, the sections were incubated for 60 minutes at room temperature in Protein Block Serum-Free (DAKO, Carpinteria, CA, USA) and then incubated overnight at 4°C with rabbit anti-CD31 antibody (1:100; Abcam, Cambridge, UK). Immunoreactive signals were detected with 3,3’-diaminobenzine. Images were captured with a light microscope. The length of the ulcers in the entire histological section was measured using ImageJ software (US National Institutes of Health, Bethesda, MD, USA).

Immunofluorescence analysis

Tissue samples from the distal colon were fixed for 4 hours at 4°C with 4% neutral buffered paraformaldehyde. After fixation, the samples were soaked in 30% sucrose/0.1 M phosphate buffer (pH 7.2) for 24 hours. After embedding with O.C.T. compound (Sakura Finetek Japan, Tokyo), 4 μm-thick cryostat sections were cut, washed
in PBS, and incubated for 1 hour at room temperature in Protein Block Serum-Free (DAKO). After blocking, the sections were incubated overnight at 4°C with the following primary antibodies: (a) rabbit anti-VEGFR1 (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), (b) rat anti-EGF (1:100, BD Biosciences, Franklin Lakes, NJ, USA), and (c) rat anti-CXCR4 (1:100, eBioscience, San Jose, CA, USA). After washing 3 times in PBS, sections were incubated for 1 hour at room temperature with a mixture of the following secondary antibodies: Alexa Fluor 594-conjugated donkey anti-rat IgG (1:1500, Molecular Probes, Eugene, OR, USA), Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:1500, Molecular Probes), and Alexa Fluor 350-conjugated goat anti-rabbit IgG (1:1500, Molecular Probes). Images were captured with an all-in-one fluorescence microscope (BZ-X710; Keyence, Osaka).

**Real-time PCR analysis**

Transcripts encoding CD31, EGF, SDF-1, CXCR4, VEGFR1, GFP, and GAPDH were quantified by real-time PCR as described previously. Total RNA was extracted from distal colon tissue with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Single-stranded cDNA was generated from 1 μg of total RNA via reverse transcription with ReverTra Ace (Toyobo, NY, USA). Quantitative PCR was carried out with SYBR Premix Ex Taq (Takara Bio, Shiga). Real-time PCR primers were designed using the Primer3 software using data from GenBank. We performed real-time PCR with the following primers: for mouse CD31, sense primer 5′-GCACTATTAAGGTGGCGATG-3′ and antisense primer 5′-CAGAGCCACAGTATGAGGAC-3′; for mouse EGF, sense primer 5′-ATGGGAAACAATGTCACGAAC-3′ and antisense primer 5′-CATCTCTCCCAAGCAGTACAAC-3′; for mouse SDF-1, sense primer 5′-CAGAGCCAACGTAAGCA-3′ and antisense primer 5′-AGGTACTCTTGGATCCAC-3′; for mouse CXCR4, sense primer 5′-CTCTGAAGAAGTGGGGTCTGG-3′ and antisense primer 5′-AAGTAGATGGTGGGCAGGAAG-3′; for mouse VEGFR1, sense primer 5′-GATGAAGTTCCCCTGGATGAG-3′ and antisense primer 5′-TTGAACCACTTTCCAAAGC-3′; for mouse GFP, sense primer 5′-ACTACAAGAGGGTGTTGAAGC-3′ and antisense primer 5′-AAGGTGGAAGAGTGGGAGTTG-3′.

**Flow cytometry**

Flow cytometric analyses were performed as described previously. Peripheral blood was collected from tail vein on Days 0 and 10. The cells were labeled with phycoerythrin-labeled anti-VEGFR1 antibody (BD Pharmingen, Franklin Lakes, NJ, USA) and fluorescein isothiocyanate-labeled anti-CXCR4 isotype control antibody (BD Pharmingen) in the presence of the anti-FcR monoclonal antibody 2.4G2 (BD Biosciences). Labeled cells after washing were analyzed with a FACS Calibur flow cytometer (BD Biosciences). The percentage of VEGFR1+CXCR4+ cells was calculated using the flow cytometry results.

**BM transplantations**

BM transplantation was performed as described previously. Donor BM cells from GFP+/-VEGFR1 TK-/- mice and their GFP+/-WT counterparts were harvested using the same method. Donor BM cells (2 × 10⁶ cells/200 μl PBS) were transplanted via the tail vein into irradiated WT mice.

**Statistical analysis**

Data are expressed as the mean ± SD. All statistical analyses were performed using GraphPad Prism software, version 6 (GraphPad Software, La Jolla, CA, USA). Comparisons between 2 groups were carried out using Student’s t-test. Survival was analyzed using the Kaplan-Meier method and Log rank tests. P values of <0.05 were considered statistically significant.

**Results**

**VEGFR1 TK-/- mice had a shorter colon.**

To examine whether or not VEGFR1 TK signaling is important for in healing of UC, WT and VEGFR1 TK-/- mice were treated with DSS to induce colitis. The colon length of VEGFR1 TK-/- mice on Day 14 was significantly shorter than that of WT mice (WT, 69.2 ± 1.12 mm vs. VEGFR1 TK-/-, 62.6 ± 1.33 mm, respectively; P < 0.05, n = 4−10 per group; Figure 1A,B). Representative images of inflamed mucosal lesions stained with H&E on Days 0, 7, and 14 are shown in Figure 1C. Ulcers and infiltration of inflammatory cells were observed in both WT and VEGFR1 TK-/- mice on Day 7. But regeneration of ducts and epithelium were observed in WT mice but not in VEGFR1 TK-/- mice on Day 14.

We defined an ulcer as an area of mucous membrane not covered by epithelium for the measurements and
Figure 1. Healing of DSS-induced colitis was delayed in VEGFR1 TK⁻/⁻ mice.

A. Typical appearance of DSS-induced colitis in WT and VEGFR1 TK⁺/⁺ mice on Day 14. B. The time course changes of colon length in VEGFR1 TK⁺/⁺ mice and WT mice. Data are expressed as the mean ± SD (n = 4−10/group). *P < 0.05 vs. WT. C. H&E staining of inflamed colonic mucosa from WT and VEGFR1 TK⁺/⁺ mice on Days 0, 7, and 14. Bar = 100 μm. D. The ratio of ulcer length in VEGFR1 TK⁺/⁺ mice and WT mice on Days 7 and 14. Data are expressed as the mean ± SD (n = 4−5/group). *P < 0.05 vs. WT.
Figure 2. The effect of VEGFR1 TK signaling in angiogenesis and ulcer healing

A. Expression of mRNA level of CD31 in inflamed mucosa in WT and VEGFR1 TK−/− mice. Data are expressed as the mean ± SD (n = 6−10/group). *P < 0.05 vs. WT. B. Representative immunohistochemical staining of CD31 in inflamed mucosa in WT and VEGFR1 TK−/− mice on Day 14. Bar = 20 μm. C. The number of microvessels in inflamed mucosa in WT and VEGFR1 TK−/− mice on Day 14. Data are expressed as the mean ± SD (n = 6/group). *P = 0.05 vs. WT. D. Expression of the mRNA level of EGF in inflamed mucosa in WT and VEGFR1 TK−/− mice. Data are expressed as the mean ± SD (n = 6−10/group). *P < 0.05 vs. WT. E. The accumulation of VEGFR1+EGF+ cells in the inflamed mucosa in VEGFR1 TK−/− and WT mice on Day 14. Green, VEGFR1+ cells; red, EGF+ cells. Bar = 50 μm. Arrowheads indicate double-positive cells.
Figure 3. Recruitment of VEGFR1^+CXCR4^+ cells into inflamed mucosa in WT mice and VEGFR1 TK^−/− mice

Expression of mRNA levels of SDF-1 (A) and CXCR4 (B) in inflamed colonic mucosa in WT and VEGFR1 TK^−/− mice. Data are expressed as the mean ± SD (n = 4−6/group). *P = 0.05 vs. WT. C. The number of VEGFR1^+CXCR4^+ cells in the peripheral blood in WT and VEGFR1TK^−/− mice on Day 10 measured by two-color flow cytometry. Mobilization of VEGFR1^+CXCR4^+ cells was impaired in VEGFR1 TK^−/− mice on Day 10. Quantification of VEGFR1^+CXCR4^+ cells in peripheral blood on Day 0 (D) and Day 10 (E). Data are expressed as the mean ± SD (n = 3−4 mice/group). *P < 0.05 vs. WT. F. Accumulation of VEGFR1^+CXCR4^+ cells in inflamed colonic mucosa was suppressed in VEGFR1 TK^−/− mice than in WT mice on Day 14. Green, VEGFR1^+ cells; red, CXCR4^+ cells; Bar = 50 μm. Arrowheads indicate double-positive cells. Number of VEGFR1^+ cells (G), CXCR4^+ cells (H), and VEGFR1^+CXCR4^+ cells (I) in inflamed colonic mucosa from WT and VEGFR1 TK^−/− mice on Day 14. Data are expressed as the mean ± SD (n = 4−6/group). *P < 0.05 and **P < 0.01 vs. WT.
VEGFR1 in colitis

Calculations. Compared to WT mice, the ratio of ulcer length was significantly higher in VEGFR1 TK+/+ on Day 14 (WT, 11.2 ± 5.29% vs. VEGFR1 TK-, 47.4 ± 3.87%, respectively; P < 0.05, n = 4 – 5 per group; Figure 1D). These results suggested that the mucosal healing of DSS-induced colitis depended on VEGFR1 TK signaling.

**VEGFR1 TK+ mice suppressed angiogenesis in inflamed colonic mucosa.**

Angiogenesis is important for mucosal healing as reported by Koutroubakis, et al. Based on their study, we confirmed that angiogenesis is involved in the mucosal healing of DSS-induced colitis. We estimated the expression of CD31, a marker of endothelial cells, by real-time PCR and immunohistochemical analysis. Compared to WT mice, the expression of CD31 was significantly suppressed in VEGFR1 TK-/- mice on Day 14 (Figure 2A). Immunohistochemical analysis against CD31 showed that CD31+ vessels were more numerous around granulation tissues in WT mice compared to those in VEGFR1 TK-/- mice (Figure 2B).

The number of CD31+ vessels in inflamed mucosa on Day 14 was lower in VEGFR1 TK-/- mice than in WT mice (WT, 19.7 ± 2.11 vessels/high power field (HPF) vs. VEGFR1 TK-/-, 13.3 ± 0.67; P < 0.05, n = 6 per group; Figure 2C). In addition, the expression of EGF, which is known as a stimulator for repairing and regeneration of multiple tissues, was significantly suppressed in VEGFR1 TK-/- compared to WT mice (Figure 2D). Furthermore, double immunofluorescent staining against VEGFR1 and EGF showed that the number of VEGFR1+EGF+ cells in VEGFR1 TK-/- mice was significantly lower than that in WT mice (Figure 2E). These results suggest that VEGFR1 TK signaling facilitates angiogenesis during healing of inflamed colonic mucosa in mice with DSS-induced colitis.

**VEGFR1 TK signaling facilitated recruitment of CXCR4+VEGFR1+ cells to inflamed colonic mucosa in DSS-induced colitis.**

We previously reported that VEGFR1 TK signaling induced the mobilization of VEGFR1+CXCR4+ cells from BM and that they accumulated into the gastric ulcer and ischemic muscles via the SDF-1/CXCR4 axis. Based on these studies, we investigated the relationship between DSS-induced colitis and the SDF-1/CXCR4 axis. The mRNA expression level of SDF-1 (Figure 3A) was significantly decreased in the inflamed colonic mucosa in VEGFR1 TK-/- mice compared to that in WT mice on Day 14. Furthermore, the mRNA expression of CXCR4, specific ligand for SDF-1, was significantly suppressed in the inflamed colonic mucosa in VEGFR1 TK+/+ mice compared to that in WT mice on Day 14 (Figure 3B). We then measured whether VEGFR1+CXCR4+ cells mobilized into peripheral blood and accumulated into the inflamed colonic mucosa or not. The percentage of VEGFR1+CXCR4+ cells in peripheral blood on Day 10 was significantly reduced in VEGFR1 TK-/- mice (WT, 3.80 ± 0.71 vs. VEGFR1 TK+, 1.55 ± 0.31, P < 0.05, n = 4 – 6 per group, Figure 3C-E). Double immunofluorescence staining showed that the accumulation of VEGFR1+CXCR4+ cells to the inflamed mucosa was diminished in VEGFR1 TK-/- mice compared to WT mice (Figure 3F). Furthermore, the number of VEGFR1+ cells in VEGFR1 TK+ mice on Day 14 was lower than that in WT mice (WT, 156.5 ± 6.17 cells/HPF vs. VEGFR1 TK-/-, 99.8 ± 13.1 cells/HPF; P < 0.05, n = 4 – 6 per group; Figure 3G). In addition, the number of CXCR4+ cells in VEGFR1 TK+ mice on Day 14 was lower than that in WT mice (WT, 25.45 ± 1.77 cells/HPF vs. VEGFR1 TK+, 16.8 ± 1.61 cells/HPF; P < 0.05, n = 4 – 6 per group; Figure 3H). The numbers of VEGFR1+CXCR4+ cells were lower in VEGFR1 TK+ mice compared to those in WT mice on Day 14 (WT, 25.0 ± 1.95 cells/HPF vs. VEGFR1 TK+/-, 6.33 ± 2.02 cells/HPF; P < 0.05, n = 3 – 4 per group; Figure 3I). These results suggested that VEGFR1 TK signaling induced the mobilization of VEGFR1+CXCR4+ cells and accumulated into the inflamed colonic mucosa via the SDF-1/CXCR4 axis.

**Transplantation of BM cells from VEGFR1 TK+/- mice delayed the healing of inflamed colonic mucosa.**

BM serves as a source of hematopoietic stem cells that play an important role in regeneration of various organs, such as ischemic muscle, gastric mucosa, and endometrial wall. Therefore, we investigated whether BM-derived cells were recruited into inflamed colonic mucosa to promote mucosal healing or not. To examine the contribution of BM-derived cells expressing VEGFR1 to mucosal healing of the colon in DSS-induced colitis, we generated BM chimeras in which WT mice were transplanted with BM cells from GFP+/+WT chimeric mice (WT→WT mice) and GFP+/+VEGFR1 TK+/- chimeric mice (VEGFR1 TK+/-→WT mice). WT mice transplanted with BM cells of VEGFR1 TK+/- mice were selectively inhibited VEGFR1 TK signaling in BM cells. The colon length of VEGFR1 TK+/-→WT mice on Day 14 was significantly shorter than that of WT→WT mice (WT→WT, 69.4 ± 1.91 mm vs. VEGFR1 TK+/-→WT, 62.7 ± 2.18 mm, respectively; P < 0.05, n = 5 – 7 per group, Figure 4A). The expression mRNA level of CD31
Figure 4. BM-derived VEGFR1\(^{+}\)CXCR4\(^{+}\) cells contributed to the healing of inflamed colonic mucosa.

A. Colon length was significantly shorter in VEGFR1 TK\(^{-/-}\)→WT mice compared to WT→WT mice on Day 14. Data are expressed as the mean ± SD (n = 5−7/group). *P < 0.05 vs. WT→WT.

B. Expression of mRNA level of CD31 in WT→WT mice and VEGFR1 TK\(^{-/-}\)→WT mice on Day 14. Data are expressed as the mean ± SD (n = 4−5/group). *P < 0.05 vs. WT→WT.

C. Accumulation of BM-derived GFP\(^{+}\)VEGFR1\(^{+}\)EGF\(^{+}\) cells in inflamed colonic mucosa was suppressed in VEGFR1 TK\(^{-/-}\)→WT mice on Day 14. Green, GFP\(^{+}\) cells; Blue, VEGFR1\(^{+}\) cells; red, EGF\(^{+}\) cells. Bar = 50 μm. Arrowheads indicate triple-positive cells.

D. Accumulation of BM-derived GFP\(^{+}\)VEGFR1\(^{+}\)CXCR4\(^{+}\) cells in inflamed colonic mucosa was suppressed in VEGFR1 TK\(^{-/-}\)→WT mice on Day 14. Green, GFP\(^{+}\) cells; Blue, VEGFR1\(^{+}\) cells; red, CXCR4\(^{+}\) cells. Bar = 25 μm. Arrowheads indicate triple-positive cells.

E. Number of GFP\(^{+}\)VEGFR1\(^{+}\)CXCR4\(^{+}\) cells in inflamed colonic mucosa from WT→WT and VEGFR1 TK\(^{-/-}\)→WT mice on Day 14. Values represent the mean ± SD (n = 4/group). **P < 0.01 vs. WT→WT.
growth factors, such as VEGF and SDF-1, regulate proinflammatory responses. Cytokines and various diseases correlate with the angiogenic and destruction. The initiation and progression of these characterized by chronic inflammation that leads to tissue in the healing of inflamed colonic mucosa.

The initiation and progression of these characterized by chronic inflammation that leads to tissue in the healing of inflamed colonic mucosa. The results from VEGFR1 TK-/- mice compared to those in WT→WT mice (Figure 4F). Furthermore, BM-derived GFP+VEGFR1+CXCR4+ cells were significantly reduced in VEGFR1 TK-/-→WT mice compared to those in WT→WT mice (WT→WT, WT→WT). These results indicated that the recruitment of BM-derived VEGFR1+CXCR4+ cells that enhance EGF expression.

Discussion

The objective of the present study was to investigate the role of VEGFR1 TK signaling in healing of UC by using DSS induced colitis. The results from VEGFR1 TK activity demonstrated that VEGFR1 TK signaling promotes mucosal healing and angiogenesis through the enhancement of EGF expression on BM-derived VEGFR1+CXCR4+ cells accumulated into inflamed colonic mucosa via the SDF-1/CXCR4 axis.

Recent studies showed that the expression of VEGFR1 and VEGFR2 was enhanced in certain conditions such as inflammation and tumor growth.20 Angiogenesis is an important factor for prognosis and progress in IBD,4 especially colonic microcirculation plays an important role in the pathology of IBD.22-26 The enhancement of microvessel density in colonic tissue of both UC and Crohn's Disease patients was seen in immunohistochemical analysis, which showed disease activity and expression of VEGF.27 In our preliminary experiments, the expression of VEGF-A, VEGFR1, and VEGFR2 in the colonic inflamed mucosa of DSS-induced colitis was elevated, especially the expression of VEGFR1 was increased soonest (data not shown). We focused on these results to investigate the role of VEGFR1 TK signaling in the healing of inflamed colonic mucosa.

UC is a disease in the IBD group of diseases, characterized by chronic inflammation that leads to tissue destruction. The initiation and progression of these diseases correlate with the angiogenic and proinflammatory responses. Cytokines and various growth factors, such as VEGF and SDF-1, regulate angiogenesis that is indispensable for the healing of ulcers.11 Those VEGF and SDF-1 are also known as inducers of mobilization of BM stem cells to peripheral blood or other organs.28 In murine models of gastric ulcers and ischemic hind limb models, mobilization of BM-derived stem cells expressing CXCR4 and VEGFR1 was found to enhance angiogenesis.10,11

SDF-1 is a chemokine also known as C-X-C motif chemokaine ligand 12 and is expressed in BM-derived stromal cells.29 CXCR4 is a receptor specific for SDF-1. Expression of SDF-1 is observed in almost all organs, and expression of CXCR4 has been reported in lymphocytes, monocytes, vascular endothelial cells, and tumor cells. Tachibana et al.30 demonstrated that CXCR4 and SDF-1 play a critical role in the formation of the gastrointestinal vasculature. The SDF-1/CXCR4 axis in endothelial cells in the ulcer area has been reported to contribute to the development and maturation of endothelial cells during the healing process.31 In the present study, the expressions of SDF-1 and CXCR4 were suppressed in inflamed colonic mucosa in VEGFR1 TK-/- mice compared to those in WT mice (Figure 3A,B).

We have previously reported that VEGFR1 TK signaling facilitated ulcer healing and angiogenesis through the enhancement of EGF expression on VEGFR1+CXCR4+ cells recruited to ulcer granulation tissue.11 The numbers of VEGFR1+CXCR4+ cells in peripheral blood and inflamed colonic mucosa were lower in VEGFR1 TK-/- mice (Figure 3C-I). The SDF-1/CXCR4 axis is important for the process of the colitis healing that depended on VEGFR1 TK signaling.

BM contains stem cells that can induce neovascularization and improve organ functions. CXCR4 is highly expressed by BM-derived endothelial cells and is involved in their mobilization and homing.32 SDF-1 expression is directly regulated by VEGF.33 VEGF stimulates the expression of SDF-1 in perivascular cells and attracts CXCR4+ cells. Blocking VEGFR1 reduces the number of recruited perivascular cells in tumors.34 VEGFR1 signaling in BM cells is important for the recruitment of BM cells at the site of angiogenesis. In our former studies, we reported that the recruitment of BM cells at the ulcer granulation and ischemic sites was...
dependent on VEGFR1TK signaling. The present study showed that BM-derived GFP+VEGFR1+CXCR4+ cells in inflamed colonic mucosa were diminished in WT mice transplanted with VEGFR1 TK−/− BM (Figure 4G,H). This result indicated that VEGFR1 TK signaling in BM cells is a critical determinant for the healing of colitis and the promotion of angiogenesis.

EGF is an important factor in mucosal healing. EGF activates the protein TK in the intracellular domain of the EGFR. TK stimulates cell division and induces cell proliferation. EGFR deficient mice die in the embryonic stage or exhibit developmental disorders of major organs such as the gastrointestinal tract and lung. Re-epithelialization of gastrointestinal mucosa promoted by EGF signaling is an important process for intestinal repair. In the present study, EGF expression was significantly suppressed in the inflamed colonic mucosa of VEGFR1 TK−/− mice. This result indicated that EGF expression is dependent on VEGFR1 TK signaling. Furthermore, EGF+ cells co-localized with BM derived GFP+ cells in inflamed mucosa lesions were also co-localized with VEGFR1+ cells. This result indicated that BM-derived VEGFR1+ cells induced epithelial cells to form in ulcerous lesions. It has previously been reported that monocytes and macrophage express VEGFR1. This suggests that VEGFR1+CXCR4+ cells may originate from monocytes or macrophages. It is well known that CD11b and F4/80 are major markers for macrophages; therefore, in the present study, we did not estimate the expression of those markers. Further studies on the identification and function of VEGFR1+CXCR4+ cells and the relationship between macrophages and angiogenesis in DSS-induced colitis are warranted.

In conclusion, the present study shows that VEGFR1 TK signaling promotes angiogenesis and healing of DSS-induced colitis through the recruitment and accumulation of BM-derived VEGFR1+CXCR4+ cells. Angiogenesis plays an important role in curing IBD including UC. Future treatment options will be targeted to VEGFR1 and its downstream signaling.

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Conflicts of Interest: None

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