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New molecular mechanisms of the unexpectedly complex role of VEGF in ulcerative colitis

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ABSTRACT

The effects of VEGF on endothelial cells are mediated by different intracellular signaling cascades (e.g., Erk1/2, Akt, Src). VEGF plays a recently recognized role in ulcerative colitis (UC) pathogenesis, mostly by increasing vascular permeability and promoting the infiltration of inflammatory cells. We hypothesized that the excessive activation of signal transduction pathways, which is responsible for VEGF/VEGFR-2-mediated endothelial permeability (Src, Akt), is a new element in the pathogenesis of chronic UC. We demonstrated increased expression of pro-angiogenic growth factor VEGF and its receptor VEGFR-2 in colonic tissue during acute 6% iodoacetamide-induced UC in rats and chronic spontaneously developed UC in IL-10 knockout mice (IL-10 KO). Development of acute 6% iodoacetamide-induced UC in rats was accompanied by activation of Erk1/2 and Src kinase, while expression of total proteins Erk1/2 and Src was unchanged. During chronic colitis phosphorylation (i.e., activation) of Erk1/2 was significantly decreased in IL-10 KO mice vs. wild-type mice. Levels of total Erk1/2 proteins were unchanged, but the expression of total Src protein as well as its phosphorylated form was significantly increased in IL-10 KO vs. wild-type mice. There were no changes in total Akt proteins, while levels of activated Akt (pAkt) were slightly increased in IL-10 KO vs. wild-type mice. We conclude that VEGF/VEGFR-2-associated signal transduction pathways, that mediate increased vascular permeability (Src, Akt), might play a central role in perpetuation of chronic experimental UC.

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1. Introduction

Angiogenesis, hence the pro-angiogenic growth factors (e.g., VEGF, PDGF, bFGF) are the mandatory elements of wound/ulcer healing that provide a basis for the complete restoration of the epithelial layer. Our studies on the role of angiogenic growth factors in pathogenesis of duodenal ulcers and experimental ulcerative colitis (UC) revealed a beneficial effect of bFGF and PDGF treatment for duodenal ulcers and UC healing [1–3] that was confirmed by others [4,5]. We predicted a similar therapeutic role for VEGF – yet it worked only for duodenal ulcers [3]. To our surprise, inhibition of VEGF activity by neutralizing anti-VEGF antibody accelerated experimental UC healing [6]. VEGF, unlike other pro-angiogenic growth factors, not only stimulates endothelial cell proliferation and migration, i.e., angiogenesis, but also enhances endothelial permeability [7], with subsequent facilitation of inflammatory cells migration, inflammatory process perpetuation. We [6] and others [8] suggested that VEGF-mediated effect on endothelial permeability might be a predominant element in the pathogenesis of UC.

Although VEGF functions on endothelial cells are mediated by different intracellular signaling cascades (e.g., Erk1/2, Akt and Src), VEGFR-2 is the principal receptor transmitting VEGF actions on the vascular endothelium [9–11]. Namely, VEGF/VEGFR-2-mediated activation of Src, and to a less extent the phosphatidylinositol 3′-OH-kinase/Akt pathways leads to increased endothelial permeability [12,13]. Activation of Erk1/2 pathway results in endothelial cells proliferation [9]. Activation of Akt pathway is important for VEGF-mediated endothelial cells survival [14] and migration [15].

No data are available on the activation of different VEGF/VEGFR-2-mediated intracellular signaling cascades in the acute and chronic stages of UC, despite their importance in the development of target-specific therapeutic approaches to modulate VEGF signaling.

We hypothesized that the pathogenesis of chronic UC is associated with excessive activation of VEGF/VEGFR-2-mediated signal transduction pathways, resulting in enhanced vascular endothelial permeability.

Abbreviations: KO, knockout; UC, ulcerative colitis.

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2. Materials and methods

2.1. Animals

Male IL-10 KO mice with a C57BL/6J background and wild-type C57BL/6J mice of 7 weeks age were obtained from Jackson Laboratory (Bar Harbor, ME). Female Sprague–Dawley rats (170–200 g) were obtained from Harlan Sprague–Dawley (San Diego, CA). The animals were housed in the animal research facility at the VA Medical Center in Long Beach, CA, under pathogen-free environmental conditions. All animals had unlimited access to Purina chow and tap water. These studies were approved by the Subcommittee for Animal Studies of the R&D Committee of the VA Medical Center in Long Beach, CA.

2.2. Iodoacetamide-induced ulcerative colitis model

Experimental ulcerative colitis was induced in rats by the SH alkylator iodoacetamide [16]. Briefly, 0.1 ml of 6% iodoacetamide (Sigma, St. Louis, MO) dissolved in 1% methylcellulose (Sigma, St. Louis, MO) or the vehicle 1% methylcellulose was given once by enema (7 cm from anus) with rubber catheter Nelaton S-8 (Rüsch, Germany) fitted to 1-ml syringe. Rats were euthanized 30 min, 1, 2 and 6 h after intracolonic administration of iodoacetamide.

2.3. IL-10 KO mice

IL-10 KO mice spontaneously develop a chronic inflammatory bowel disease, with an incidence of 95% by 10–12 weeks of age [17]. Since IL-10 KO mice on genetic backgrounds C57BL/10 demonstrate less susceptibility to intestinal inflammation among inbred strains of mice we used mice at 18 weeks. Mice were euthanized with CO_2 inhalation, followed by cervical dislocation. During autopsy the entire colon (from anus to cecum) was removed and used for further examinations.

2.4. Western blots

Total proteins (50 or 100 μg) which were extracted from colonic mucosa in a lysis buffer containing protease (Sigma, St. Louis, MO) and phosphatase inhibitors (Thermo Fisher Scientific Inc., Waltham, MA) were processed routinely for Western blots as described previously [6]. Primary antibodies were used against VEGFR-2 (1:200), VEGF, Erk1, Erk2, $pErk^{Tyr204}$, Src, $pSrc^{Tyr416}$ (1:500) (Santa-Cruz Biotech., Santa Cruz, CA), Src, $pSrc^{Tyr416}$, Akt and $pAkt^{Ser473}$ (1:500) (Cell Signaling Technology, Inc., Danvers, MA). The loading controls were performed by using a mouse monoclonal antibody to GAPDH (1:2000; EnCor Biotech., Alachua, FL). Every Western blot was repeated at least twice using proteins from three different mice/group.

2.5. Statistical analysis

Quantitative results are expressed as means ± SD. The statistical significance was determined by the non-parametric Mann–Whitney *U*-test, or Student's *t*-test where appropriate, and p values of <0.05 were considered statistically significant.

3. Results

3.1. Increased expression of VEGF and VEGFR-2 during acute stage of iodoacetamide-induced UC in rats and chronic stage of experimental UC in IL-10 KO mice

Iodoacetamide-induced UC is characterized by fast development of mucosal and submucosal edema as early as 2 h followed by erosions, ulcers and acute inflammation with abundant infiltration of neutrophils 6 h after iodoacetamide enema [16]. Development of iodoacetamide-induced UC was accompanied by significant increases of VEGF and VEGFR-2 expressions in colonic mucosa (Fig. 1A and B).

3.2. Deletion of IL-10 evokes spontaneous development of chronic colitis in IL-10 KO mice

Colonic levels of VEGF protein were significantly higher in IL-10 KO mice vs. wild-type littermates (Fig. 1C). Furthermore we showed significant overexpression of VEGFR-2 in IL-10 KO mice vs. age-matched wild-type littermates (Fig. 1D).

3.3. Different activation of VEGF/VEGFR-2-associated signal pathways in colonic tissue during acute and chronic stages of experimental UC

The binding of VEGF to VEGFR-2 leads to the activation of several angiogenic intracellular signaling pathways, e.g., Erk1/2, Src and Akt that are responsible for mediating different VEGF effects on endothelial cells. In our study, development of acute iodoacetamide-induced UC was accompanied by activation of Erk1/2 and Src kinases in colonic mucosa. Though level of Erk1/2 phosphorylation begins to decrease in 6 h after iodoacetamide enema, the level of pSrc continues to increase (Fig. 2A). The expressions of Erk1/2 and Src total proteins were unchanged (Fig. 2A).

Opposite effect was observed during chronic colitis (Fig. 2B). We found altered activation of VEGF/VEGFR-2-associated key signal pathways in colonic tissue of IL-10 KO vs. wild-type mice. The phosphorylation (i.e., activation) of Erk1/2 was significantly decreased in IL-10 KO mice vs. wild-type mice. Levels of total Erk1/2 proteins were unchanged. Furthermore, the expression of total Src proteins as well as its phosphorylated form was significantly increased in IL-10 KO vs. wild-type mice. Since activation of phosphatidylinositol 3′-OH-kinase/Akt pathway mediates VEGF-induced endothelial permeability along with its role in endothelial cells survival and migration [13–15], we checked Akt activation. We did not find changes in total Akt proteins, while the levels of activated Akt (pAkt) were slightly increased in IL-10 KO vs. wild-type mice.

4. Discussion

In present study we showed for the first time an activation of VEGF/VEGFR-2-associated signaling pathways, which mediates VEGF-dependent vascular permeability (Src and Akt), but not proliferation of endothelial cells (Erk1/2) in colonic tissue during chronic spontaneously developing UC in IL-10 KO mice, while development of acute colitis triggers phosphorylation both Erk1/2 and Src. Taking into account the pivotal role of angiogenesis and VEGF-mediated effects for ulcer healing, selective inhibition of VEGF-mediated vascular permeability might be a new, safe approach in UC treatment, as we demonstrated recently [6].

UC is a chronic inflammatory disease, which is characterized by sustained inflammation and chronic recurrent ulcers. Success of complete wound/ulcer healing is dependent on adequate process of angiogenesis and granulation tissue formation. Pathogenesis of UC is associated with increased angiogenesis which is abnormal, with leaky, immature blood vessels that cannot lead to appropriate ulcer healing. Thus, maintenance of chronic inflammation might be the reason for disease recurrence [18,19].

VEGF is a key pro-angiogenic growth factor in UC pathogenesis. Unlike other pro-angiogenic growth factors (e.g., bFGF, PDGF, HGF) [2,4,5], VEGF upregulation is harmful in UC healing and its inhibition is beneficial [6,8]. We showed that the beneficial effect of