New Cell Therapy Using Bone Marrow-Derived Stem Cells/Endothelial Progenitor Cells to Accelerate Neovascularization in Healing of Experimental Ulcerative Colitis

Xiaoming Deng[1,4], Sandor Szabo[1,4,5], Longchuan Chen[1], Brankica Paunovic[1], Tetyana Khomenko[1,4], Ganna Tolstanova[1,4,*], Andrzej S. Tarnawski[2,6], Michael K. Jones[3], and Zsuzsanna Sandor[2,6]

*[1]Diagnostic & Molecular Medicine HCG, [2]Internal Medicine HCG, and [3]Department of Research, VA Long Beach Healthcare System; and Departments of [4]Pathology, [5]Pharmacology & [6]Medicine, University of California-Irvine, Long Beach, CA, USA, *Present address: Department of Pharmaco-Physiology, Kiev National Shevchenko University, Kiev, Ukraine*

Abstract: Inflammatory bowel disease (IBD): ulcerative colitis (UC) and Crohn disease (CD) are characterized by recurrent inflammation and ulceration of intestinal and/or colonic mucosa and an inappropriate and delayed healing. Current therapies with, e.g., anti-TNFα antibody (infliximab) and other anti-inflammatory drugs (e.g., mesalamine) do not induce sustained remission, complete healing or prevent recurrence of UC. Although the pathogenesis of UC is not fully understood, pathologic angiogenesis has been postulated as a critical pathogenic component in UC. Recent studies demonstrated that the poor healing, chronic inflammation in colon of UC could be the result of microvascular dysfunction and endothelial barrier defect, resulting in sustained tissue hypoperfusion and ischemia in the colon. Previously, regeneration of injured endothelium and neovascularization were believed to rely solely on the migration and proliferation of neighboring endothelial cells from existing blood vessels. However, accumulating evidence shows that additional mechanisms may exist, and may be mediated by the circulating pool of bone marrow-derived endothelial progenitor cells (BMD-EPC). Furthermore, stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 have been demonstrated to play an important role in the "homing" of BMD-EPC to injured sites and neovascularization in tissue repair. Recent studies by others and us showed reduced BMD-EPC levels in the circulation of IBD patients and rats with experimental UC. However, the potential therapeutic effect of BMD-EPC on neovascularization and colonic mucosal repair in UC has not been elucidated. In this review, we discussed the possibility that impaired contribution of BMD-EPC (i.e., decreased release of BMD-EPC from bone marrow to circulation and/or blocked/impaired homing of BMD-EPC to colonic lesions) may be a critical component of mechanisms in the incomplete/delayed healing of UC, and may offer a novel form of cell therapy for IBD.

Keywords: Bone marrow-derived endothelial progenitor cells, angiogenesis, vasculogenesis, neovascularization, wound/ulcer healing, ulcerative colitis, inflammatory bowel disease.

INTRODUCTION

In 1960, seminal studies by James Till and Ernest McCulloch demonstrated that bone marrow contains a subset of cells which upon transplantation into the spleens of lethally irradiated recipient animals can form macroscopic colonies, termed colony-forming unit spleen (CFU-S), which contain differentiated progeny of multiple blood lineages [1]. The subset of these colonies could reform CFU-S when transplanted into secondary hosts [2]. This work led to the recognition that stem cells are responsible for the maintenance and functional integrity of tissues under steady-state conditions and their repair following injury. These processes are accomplished through self-renewal, differentiation and extensive proliferation, controlled by internal genetic events and conditions extrinsic to stem cells [3]. The finding that adult mammalian stem cells differentiate across the boundaries of tissue lineage has generated much enthusiasm for their potential therapeutic use in targeted tissue regeneration and potentially in wound/ulcer healing. Although progenitor cells that reside within injured tissue normally drive tissue regeneration, recent data indicate that precursor cells originating from the bone marrow replenish these stem-like progenitor cells. Following injury, bone marrow-derived cells engraft as differentiated cells into multiple tissues, enhancing healing [4] by three distinct mechanisms. First, due to the cells' pluripotent nature, they are able to differentiate (in response to tissue-specific signals) into cell types comprising the tissue they have engrafted. Second, these cells may have the capacity to express and secrete several types of growth factors that increase the survival and proliferation of endogenous cells populating the engrafted tissue. Third, these cells can fuse with endogenous cells of the engrafted tissue, increasing their capacity to survive and proliferate. In addition to bone marrow, other sources of adult stem cells with pluripotent or multipotent differentiation capacity have been proposed, including the skin [5], adipose tissue [6], and liver [7]. In this review, we focus on the potential therapeutic role of bone marrow-derived stem cells/endothelial progenitor cells (BMD-EPC) in UC healing.

*Address correspondence to this author at the VA Medical Center (05/113) 5901 East 7th Street Long Beach, CA 90822, USA; Tel: 562-826-8000; Ext. 3470; Fax: 562-826-5768; E-mail: zsuzsanna.sandor@va.gov

BMD-EPC

Two groups independently demonstrated in 1997 and 1998 that purified CD34+ hematopoietic progenitor cells from adult bone marrow can differentiate ex-vivo into cells having an endothelial phenotype [8,9]. These cells named "endothelial progenitor cells" express various endothelial markers and are incorporated into neovessels at sites of ischemic hypoxia and injury. Although each of these early investigations utilized cells that were not truly pluripotent stem cells, but rather lineage-committed progenitor cells, subsequent studies utilizing uncommitted, immature stem cells have verified the concept that bone marrow-derived stem cells (BMD-SC) can differentiate into endothelial cells. Several independent studies demonstrated that BMD-SC and BMD-EPC can engraft sites of injury and significantly contribute to and enhance neovascularization and healing [10-13], making BMD-SC and BMD-EPC a potential therapeutic modality for ulcer/wound and tissue injury healing. A major focus has been on BMD-SC and BMD-EPC ability to form new vessels in injured tissues, and another has been on their ability to repair endothelial damage and restore both monolayer integrity and endothelial function in denuded vessels. Several studies have attempted to improve BMD-EPC isolation and purification, optimize techniques for cell expansion both *in vivo* and *in vitro*, and identify factors to enhance stem/progenitor cell mobilization to and from the bone marrow and direct their "homing" to the desired sites of engraftment [14-20]. These studies involved elucidation of the chemokine/growth factor receptor signaling pathways

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that regulate BMD-EPC self-renewal, niche retention, mobilization, colonization and differentiation. Stromal-derived growth factor-1 (SDF-1), which acts through its CXCR4 receptor to modulate several diverse stem cell functions including proliferation, niche retention, homing and colonization, is the most fully characterized factor acting on BMD-EPC [21]. BMD-EPC are mobilized and released out of the bone marrow in response to peripheral tissue hypoxia and/or trauma, through increased production and release of BMD-EPC-activation factors, such as hypoxia-inducible factor-1 α (HIF-1α), vascular endothelial growth factor (VEGF), or SDF-1 with a concentration gradient greater than that in the bone marrow [22-24]. These growth factors attract BMD-EPC to the site of injury.

After release from the bone marrow, BMD-EPC follow cytokine gradients to activated tissues, and then act in one of three ways: paracrine, integration or new-vessel formation. When reduction of SDF-1 levels in the bone marrow niche occurs, increased numbers of hemotopoietic stem cells are released into the peripheral circulation [25]. After release of BMD-EPC into the peripheral circulation, SDF-1 continues to play a crucial role in directing them to the site of injury (Fig. **1**). The chemotactic response stimulated by SDF-1 causes changes in cell adhesion and cell secretion, in turn guiding them to migrate across the basement membrane of the endothelium towards a high SDF-1 concentration gradient. Several other chemokines and growth factors such as granulocyte colony-stimulating factor, granulocyte monocyte-colony stimulating factor, stem cell factor, erythropoietin, basic fibroblast growth factor and VEGF have also been demonstrated to modulate/direct stem cell function [26,27]. Growth factors in particular can trigger commitment towards a given cell type. For example, VEGF can induce a non-committed stem cell or partially committed progenitor cell towards an endothelial phenotype. Combinations of chemokines and growth factors can produce a synergistic enhancement of stem cell proliferation and migration [28].

NEOVASCULARIZATION IN WOUND/ULCER HEALING

Growing evidence indicates that new blood vessel formation (neovascularization) in wound/ulcer healing occurs by two events: A) Sprouting angiogenesis - formation of new blood vessels from pre-existing vessels. B) Mobilization of BMD-EPC and other progenitor/stem cells from bone marrow to circulation and their homing to wound/ulcer area for angiogenesis and vasculogenesis, respectively (Fig. **1**). Namely, angiogenesis is the formation of new blood vessels, either from existing endothelial cells of the wound/ulcer vascular network that are migrating to and proliferating in the wound substrate [29] or through the process of intussusceptions [30], where the capillary wall extends into the lumen to split a single vessel in two. Vasculogenesis is the generation of new blood vessels by recruitment of undifferentiated BMD-EPC to the

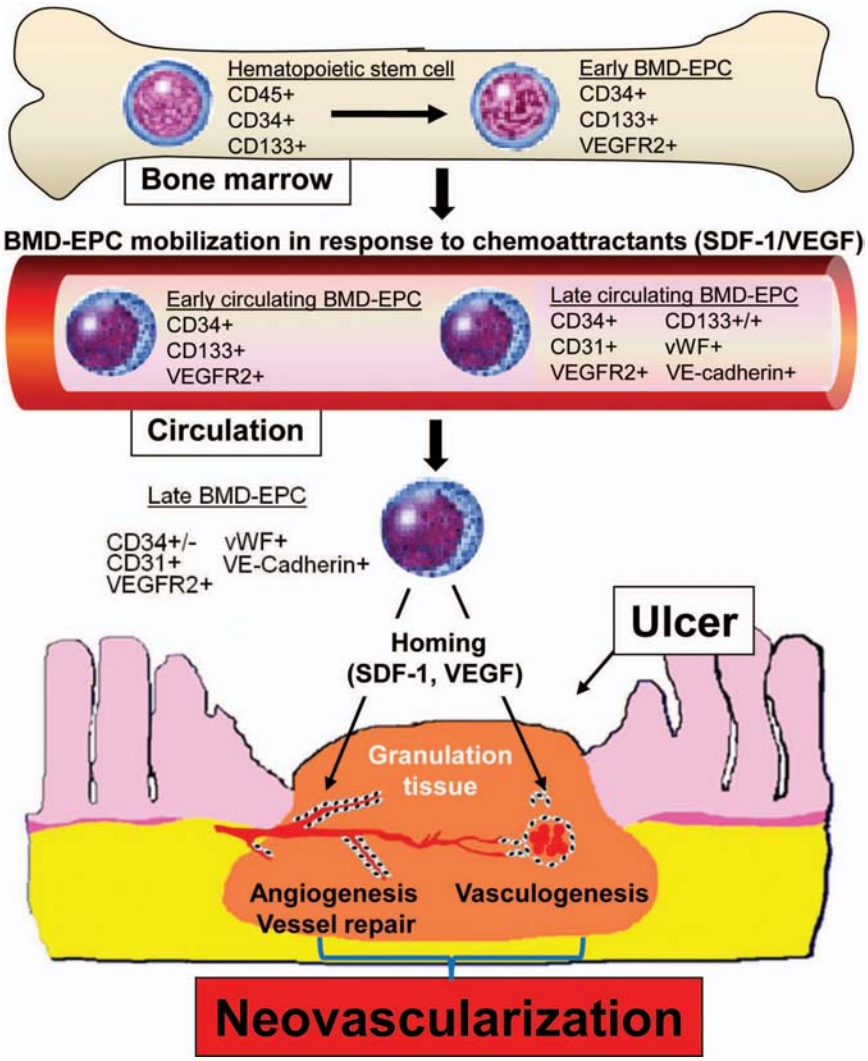

Fig. (1). A diagram of BMD-EPC mobilization and homing to granulation tissue for neovascularization in gastrointestinal ulcer healing.

granulations of wound area. They differentiate into endothelial cells and form capillaries, arterioles and venules *in vivo* [31]. The newly formed blood vessels are stabilized by smooth muscle cells, pericytes and the extracellular matrix. The use of cytokines and growth factors such as SDF-1 and VEGF to stimulate the bone marrow release of progenitor stem cells for purposes of wound healing or therapeutic neovascularization has been considered [32-34]. Normal wound/ulcer healing proceeds through an orderly sequence of steps that require the control of contamination and infection, resolution of inflammation, regeneration of the connective tissue matrix, angiogenesis/vasculogenesis, wound constriction and reepithelialization. Chronic wounds/ulcers are those that have failed to follow this sequence and do not achieve a sustained anatomic and functional regeneration [35]. The hypoxic nature of all wounds/ulcers has been demonstrated, but when hypoxia is pathologically increased wound/ulcer healing is impaired [36-38]. Local oxygen tensions in the vicinity of the wound/ulcer are approximately half the values observed in normal, non-wounded tissue [39]. Angiogenesis and vasculogenesis, fibroblast preliferation, and collagen deposition in granulation tissue are oxygen-sensitive responses essential for wound/ulcer healing [40-42]. Granulation tissue is an important component of wound/ulcer healing process because it supplies blood microvessels and connective tissue cells for the mucosal regeneration [43-45]. For these reasons, a number of investigators have focused on the role of hypoxia and neovascularization in wound/ulcer healing [46-48]. Until now, this area remains an open field for investigation because the healing of chronic wounds/ulcers constitutes a major clinical problem.

MUCOSAL HYPOXIA, ABNORMAL MICROVASCULATURE, AND IMPAIRED HEALING IN UC PATIENTS AND ANIMAL MODELS

Although it is generally assumed that macroscopically "normal" appearing colonic mucosa in patients with UC in remission (or the "healed" lesions in experimental animals) has restored mucosal structures and functions, several studies demonstrate that "healed" colonic mucosa in UC has structural abnormalities [49,50]. The colonic mucosa has prominent vascular network that is separated from the anaerobic and nonsterile lumen of the gut by an epithelial cell layer. As such, intestinal epithelial cells, which line the mucosa, experience a uniquely steep physiologic oxygen gradient. During UC, increased tissue metabolism and vascular injury renders the chronically inflamed mucosa and particularly the epithelium hypoxic, giving rise to the activation of the hypoxia-responsive transcription factor HIF.

Recent studies using confocal laser endomicroscopy (CLE) indicate that normal regular pattern of crypt distribution is distorted and disorganized, and the lamina propria is expanded in chronic UC [51]. CLE also showed increase in number, size and tortuosity of capillary blood microvessels *in vivo* reflecting increased pathologic neovascularization in patients with UC in remission [52,53]. These findings in human UC were also seen by CLE in experimental UC induced by dextran sulfate sodium in rat and mouse [54,55]. It was postulated that the poor healing, refractory inflammation, recurrent ulceration and damage in colonic mucosa of UC could depend on microvascular dysfunction and endothelial barrier defect, resulting in sustained tissue hypoperfusion and ischemia in the colon [56]. One of the consequences of vascular ischemia is the development of tissue hypoxia, which, in turn, may induce intestinal endothelial and epithelial cell injury due to increased levels of oxygen-derived toxic free radicals and the production of inflammatory mediators. Hypoxia is a key regulatory factor in activating neovascularization, glycolysis and cell migration [57].

ACTIVATION OF HIF-1α IN UC DEVELOPMENT AND HEALING

HIF-1α is a hypoxia-regulated transcriptional factor, which controls the expression of a variety of genes responsible for angiogenesis, glycolysis and the inhibition of apoptosis. A number of studies have demonstrated mucosal hypoxia in UC [57]. Consistent with the presence of hypoxia, the expression of HIF-1α in the colonic mucosa of UC is significantly up-regulated, focally in epithelial cells, stromal fibroblasts, and myocytes. Our recent study demonstrated both increased hypoxia of colonic surface epithelial cells as well as increased HIF-1α prior to epithelial damage in experimental UC [58]. HIF-1α accumulation and its transcriptional activity in response to hypoxia lead to increased mRNA expression of VEGF. Surgical specimens from patients with UC have revealed prominent HIF-1α activation associated with increased vascular density in diseased areas [59]. Other studies in humans demonstrated that a number of microvascular endothelial abnormalities may contribute to diminished blood flow to the intestine in UC, including the loss of endothelial nitric oxide generation and enhanced tissue vasoconstrictor production [60]. Recent studies utilizing conditional intestinal epithelial HIF-1α null mice showed a protective role for epithelial HIF-1α in these murine models of UC. Such protection occurs, at least in part, through HIF-dependent induction of barrier-protective genes in the epithelium. More recently, studies employing pharmacologic activation of HIF-1α via inhibition of HIF prolyl hydroxylases revealed a significantly protective effect of these agents in murine models of UC. HIF-1α is also a master regulator for neovascularization via regulation of the expression of VEGF, and SDF-1 as well as their receptors Flk-1 and CXCR4 [57].

EXPRESSION OF VEGF AND ITS RECEPTOR VEGFR2/FLK-1 IN HUMAN AND EXPERIMENTAL UC

VEGF is a fundamental regulator of neovascularization and an endothelial cell specific mitogen because its receptor 2 (VEGFR2/Flk-1) is primarily restricted to endothelial cells [61]. The loss of a single VEGF allele results in defective vascularization and an early embryonic death [62]. Binding of VEGF to its specific receptors (VEGF-R1/Flt-1 and VEGF-R2/Flk-1) on endothelial cells, initiates phosphorylation of numerous cytosolic proteins involved in the signal transduction that triggers endothelial cell proliferation, migration and microvascular tube formation [63]. VEGF has been implicated in the stimulation of pathologic angiogenesis that underlies proliferative diabetic retinopathy, tumor growth and metastasis [64,65]. Since neovascularization and tissue regeneration are integral components of the inflammatory process, the role of VEGF in IBD has been broadly studied [66-68]. Both serum and tissue levels of VEGF were significantly higher in active UC patients [69]. Griga *et al*. reported increased serum VEGF level in patients with active UC but not in patients with inactive UC [70]. In a subsequent study the same group identified the colonic mucosa as the source of the increased serum level of VEGF [71]. Kanazawa reported increased VEGF level in the serum and colonic tissue of patients with active UC [72]. A recent study by Konno *et al*. showed an elevated VEGF level in UC [73]. Although a number of studies have demonstrated that VEGF is associated with pathologic angiogenesis and inflammation [74,75], however, there is no evidence indicating that VEGF plays a beneficial role in UC, similar to its established role in healing of other ulcer diseases such as upper gastrointestinal ulcers and diabetic skin ulcer [76-78]. Our recent study demonstrated that gene and protein expressions of both VEGF and VEGFR@ (Flk-1) were increased in UC development but decreased in healing stage in iodoacetamide-induced UC in rats (Fig. **2**). This may implicate that VEGF plays a dual role and the low levels of VEGF in healing period may lead to impaired healing of UC.

Recent findings indicate a novel role for VEGF that is analogous to that described for hemotopoietic stem cells-modulating cytokines in the regulation of postnatal neovascularization. Previous reports of endogenous VEGF expression in the bone marrow environment [79] or bone marrow stromal cell lines are consistent with the concept of a physiologic regulatory function for VEGF in BMD-EPC mobilization, similar to the role established for other

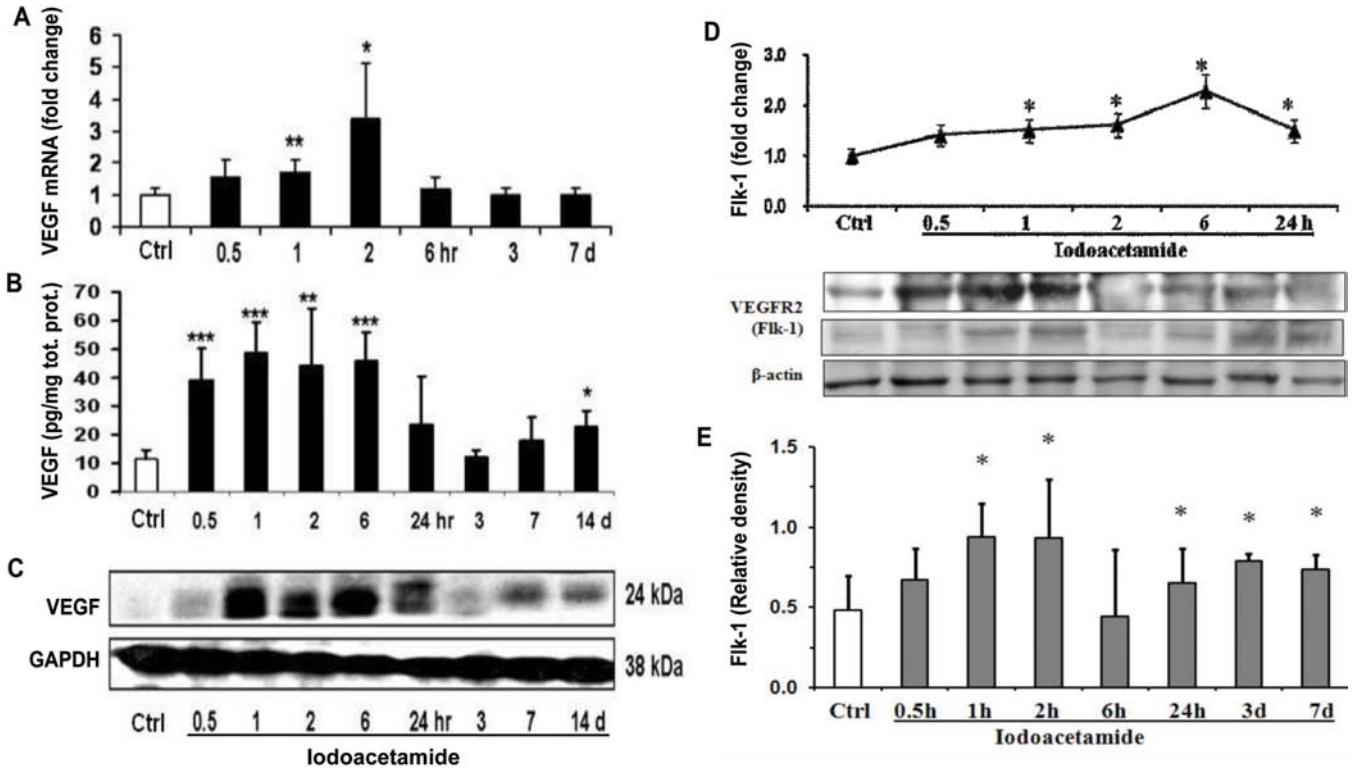

Fig. (2). Expression of VEGF and its receptor 2 (VEGFR2/Flk-1) in colonic mucosa during development (1-48 hr after iodoacetamide) and healing (7-14 days after iodoacetamide) of experimental UC induced by iodoacetamide in rats. **A:** Increased levels of VEGF mRNA in initial developmental stage of UC and decreased levels in healing period measured by Real-time PCR; **B&C:** Increased levels of VEGF protein and decreased VEGF levels during UC measured by ELISA **(B)** and Western blotting **(C); D:** Increased expression of VEGFR2 mRNA measured by Real-time PCR and **E:** Increased levels of VEGFR2 protein measured by Western blotting. **Ctrl:** control. * $p < 0.05$; ** $p < 0.01$.

bone marrow cytokines expressed locally in the bone marrow microenvironment on hemotopoietic stem cell mobilization. Similar to other BMD-EPC homing factor, e.g., SDF-1, VEGF also has a chemoatractive effect on a fraction of bone marrow mononuclear cells and cultured BMD-EPC, as well as on adhesive mononuclear cells [80] and monocytes in peripheral blood [81]. Enhanced BMD-EPC differentiation in culture as early as 4 h following recombinant human VEGF injection reflects the immediate effect of VEGF on BMD-EPC mobilization. In addition to these direct effects, indirect effects of VEGF may help in regulating BMD-EPC mobilization. Permeabilization of the bone marrow vasculature and mobilization of adhesive molecule expression on bone marrow endothelium by VEGF may contribute to recruitment of BMD-EPC into circulation. BMD-EPC mobilized by VEGF from bone marrow enter into peripheral blood and serve as source of BMD-EPC that might facilitate neovascularization, previously attributed exclusively to angiogenesis.

EXPRESSION OF SDF-1 AND ITS RECEPTOR CXCR4 IN HUMAN AND EXPERIMENTAL UC

SDF-1, also known as CXCL12, is firstly characterized as a pre-B cell growth stimulating factor whose specific receptor is CXCR4. The SDF-1/CXCR4 chemokine axis has an important role in the development of cardiovascular, hematopoietic, and central nervous systems, and is also involved in inflammatory diseases such as UC. The SDF-1/CXCR4 axis is a crucial regulator of BMD-EPC function and homing. Recent studies have shown that SDF-1 plays an important role in the regulation of migration, proliferation, and survival of BMD-EPC. SDF-1 regulates the trafficking of CXCR4(+) BMD-EPC, their homing/retention in major BMD-EPC organs and accumulation of CXCR4(+) BMD-EPC in tissues affected by inflammation. Despite the apparently important role of SDF-1/CXCR4 in BMD-EPC migration, proliferation, survival, and neovascularization, it is not known what precise role of SDF-1/CXCR4 plays in mediating mobilization and homing of BMD-EPC to colonic lesions in UC [82,83]. Recent studies demonstrated that SDF-1 and CXCR4 are constitutively expressed on intestinal epithelial cells, lamina propria T cells, and peripheral blood T cells and their expressions are significantly increased during active or developing phase of UC but decreased during healing of UC in patients and experimental animal models [84,85]. Tissue repair and regeneration after injury is thought to involve the selective recruitment of circulating or resident stem cell populations. The importance of SDF-1 in BMD-EPC recruitment has been established by observations that selective expression in injured tissue correlates with adult stem cell recruitment and tissue regeneration [86]. However, the pathophysiologic mechanisms underlying localized expression of SDF-1 and its precise role in UC are still unclear.

REDUCED NUMBER OF CIRCULATING BMD-EPC IN UC

There is strong evidence that BMD-EPC play a significant role in neovascularization and subsequent tissue repair in some disease conditions such as acute myocardial infarction, acute lung injury, and chronic renal disease [87]. However, to date, little is known on the role of BMD-EPC in healing of UC patients and experimental UC. Recently, Masuda *et al.* [88] and Garolla *et al*. [89] reported a reduction in the number and function of circulating BMD-EPC in patients with UC. In contrast, patients with infectious colitis were found to show higher numbers of circulating BMD-EPC, suggesting that prolonged response to chronic inflammation is necessary for UC. Although no direct correlation between the circulating number of BMD-EPC and the degree of clinical disease activity has been

observed in UC, it should be noted that the reduced number of circulating BMC-EPC could induce decreased homing of BMD-EPC to lesions in colon, resulting in defective neovascularization and delayed healing of UC. There are several possible explanations for the reduction in the number of circulating BMD-EPC in patients with UC. Taking into consideration the contention that hematopoietic cells (HPCs) are one putative precursor of BMD-EPC, the ability of HPCs to differentiate into BMD-EPC may be impaired in patients with UC. If this is true, the factor responsible for this reduced ability of the HPCs to differentiate this event may play a crucial role in the pathophysiology of UC [90].

An alternative explanation for the reduction in the number of BMD-EPC in the peripheral blood might be due to the consumption of circulating BMD-EPC at the site of disease. Garolla *et al*. [89] reported that an increased apoptotic BMD-EPC population was found in blood circulation in UC patients and that the isolated BMD-EPC from peripheral blood of UC patients lost the ability to proliferate *in vitro*. The reduction in the number of circulating BMD-EPC was consistently observed in UC regardless of steroid and/or 5-aminosalicylate treatment [91]. However, to date, there are no reports showing whether and to what degree BMD-EPC are involved in the neovascularization and regeneration of colonic mucosa or what molecules are involved in regulating BMD-EPC mobilization and homing to colonic tissue for repair in UC. Thus, further studies are needed to define the mechanisms that underlie the reduction in the circulating number of EPCs and to better understand the pathophysiologic consequences of this event in cases of UC.

ABNORMAL AND/OR IMPAIRED NEOVASCULARIZATION IN UC

Recent evidence shows that pathologic angiogenesis plays a crucial role in pathogenesis of experimental UC [92]. Examination of the relationship between angiogenesis and inflammation in experimental UC shows that initiating factors for these responses simultaneously increase as disease progresses. Recent data provide evidence that differential regulation of the angiogenic mediators involved in UC-associated chronic inflammation is the basis of this pathologic angiogenesis. Many factors are involved in this phenomenon, including growth factors/cytokines, chemokines, adhesion molecules, integrins, matrix-associated molecules, and signaling targets. These factors are produced by various vascular, inflammatory, and immune cell types that are involved in UC pathology. Until recently, the microvascular changes that occur during UC have not been closely investigated, and we are only beginning to understand their involvement in the disease process. Our recent studies demonstrated that differential expression of pro- vs. anti-angiogenic factors regulates pathologic angiogenesis by creating imbalances between these regulatory factors (i.e., up-regulation of pro- over anti-angiogenic factors or relative down-regulation of anti-angiogenic factors) [93,94]. Evidence indicates that some of the up-regulated angiogenic factors in UC may prevent the maturation of vessels, contributing to the pathologic nature of this angiogenesis. Recent studies have shown that VEGF links pathologic angiogenesis and refractory inflammation in UC, and that it is involved in prevention of vessel maturation and ulcer healing [67]. For example, HIF-1α-induced VEGF production has been shown to inhibit vessel maturation and pericyte stabilization, resulting in endothelial cell hyperplasia and abnormal neovascularization [95,96]. These processes could contribute to a pathologic phenotype of neovascularization during UC. The assumption that wound healing related angiogenesis can occur in the form of ulcer repair during remission of UC suggests a role for physiologic angiogenesis in UC and may also represent a return to normal regulation of angiogenic mediators during disease remission and may be the basis for ulceration recurrence. However, the majority of colonic tissue alterations that occur during UC are not fully normalized during remission. Thus, the role of the BMD-EPC in neovascularization may be critical to the process of UC healing, and understanding the regulation of SDF-1 and its receptor CCXCR4 in UC will provide new insight into healing of UC.

THERAPEUTIC POTENTIAL OF BMD-EPC IN HEALING OF UC

UC is the most prevalent form of inflammatory bowel disease with a major clinical and economic impact. The incidence of UC in the U.S. is 205/100,000 persons, which is the second highest in the world and steadily increasing. The current anti-inflammatory therapy does not cure the disease and results in long-term remission only in fewer than 34% of patients [97]. This means that more than 60% of patients are always symptomatic or have frequent disease recurrence, resulting in decreased quality of life and economic loss due to inability to work. Recent studies in animal models of UC indicate that abnormal "pathologic" angiogenesis in colonic mucosa seem to play a critical role in the development and recurrence of UC [74, 98,99]. It was demonstrated that the poorly healing, refractory inflammatory ulceration and damage in colonic mucosa could depend on the microvascular dysfunction and defect, resulting in sustained tissue hypoperfusion and ischemia in the colon with UC [100]. Other studies have demonstrated that stimulation of BMD-EPC release from bone marrow and their homing to injured tissue significantly enhances ventricular function after myocardial infarction by inducing neovasculogenesis [101,102]. However, there is no study showing whether increased mobilization of BMD-EPC improves UC healing.

We recently tested the roles of SDF-1 and its receptor CXCR4 on homing of BMD-EPC to colonic lesions in UC, as well as the therapeutic potential of BMD-SC for improving neovascularization in the healing of UC [103]. Since it has been demonstrated that inhibition of CXCR4 by its antagonist AMD3100 increases BMD-EPC mobilization from bone marrow to blood circulation [104], we tested a hypothesis that increased mobilization of BMD-EPC by CXCR4 antagonist AMD3100 may accelerate healing of experimental UC induced by iodoacetamide. The results showed that colonic lesions were significantly reduced in the rats treated with a single dose of 0.5 mg AMD3100 at 6 hr after iodoacetamide administration compared to saline-treated rats (Fig. **3**). These findings demonstrated that SDF-1/CXCR4 axis plays an important role on healing of UC by regulating mobilization and homing of BMD-EPC to colonic lesions in rats with UC. Although there is no report indicating BMD-EPC application in healing of UC in human and animal models, BMD-SC transplantation has been reported to induce long-term remission in some patients with inflammatory bowel disease in clinical trials [105]. We also demonstrated that intracolonic administration of BMD-SC significantly reduced colonic lesions, while intracolonic administration of IEC-6 cells (normal rat intestinal epithelial cells) had slightly increased colonic lesions when compared to the control (Table **1**). We concluded that this beneficial effect is specific to the stem cells, since IEC-6 had no effect. Thus, BMD-SC therapy seems to achieve a rapid healing of experimental UC, possibly by promoting neovascularization and mucosal regeneration. Taken together, these studies provide new insight into the therapeutic strategies for UC.

In summary, UC is characterized by recurrent inflammation, ulcerations, and impaired healing and regeneration of the colonic mucosa. Therapeutic agents currently used are only able to induce temporary remission but do not cure the disease. Although the pathogenesis of UC is not fully understood, the poor healing, refractory inflammatory lesions in colon of UC could be due to abnormal "pathologic" neovascularization, resulting in sustained tissue hypoperfusion and ischemia of colonic mucosa. Previously, regeneration of injured endothelium was assumed to rely solely on the migration and proliferation of endothelial cells from existing blood vessels at the mucosal bordering necrosis. However, accumulating evidence shows that additional mechanisms may exist, and may be mediated by the circulating pool of BMD-EPC, resulting in

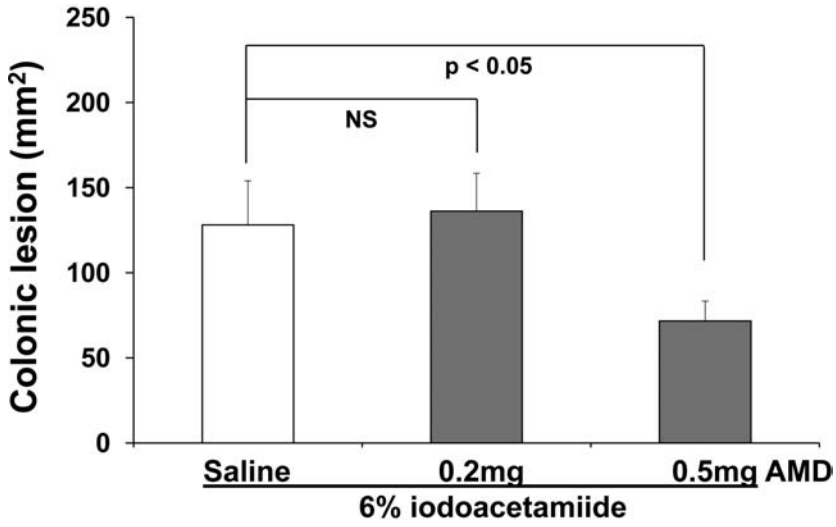

Fig. (3). Effects of CXCR4 on healing of experimental UC induced by iodoacetamide in rats. Transitory inhibition of CXCR4 by its antagonist AMD 3100 (0.5mg/rat) significantly reduced lesions in rats with iodoacetamide-induced UC.

Table 1. Effect of Intracolonic Treatment with BMD-SC on Healing of UC Induced by Iodoacetamide

Group	Colonic lesions (mm^2)	Colon thickness (Scale: 0-3)	Adhesion (Scale: 0-3)	Colon wet weight (g/100g BW)
IA +saline	152.9 47.7	2.1 0.2	2.1 0.3	2.1 0.3
IA + IEC-6	224.8 38.5	2.5 0.2	2.6 0.3	2.3 0.2
IA + BMD-SC	87.7 40.1*	0.8 0.2*	0.9 0.3*	1.1 0.3*

IA: iodoacetamide; IEC-6: normal intestinal epithelial cells;
BMD-SC: bone marrow-derived stem cells. * $p < 0.05$ compared to IA + saline;

vasculogenesis. Recent studies demonstrated abnormal neovascularization in the colonic mucosa of patients with UC and experimental UC. Reduced BMD-EPC number was found in UC patients. The decreased expression of SDF-1 may be associated with reduced BMD-EPC homing to UC lesions, reduced contribution of BMD-EPC to neovascularization in granulation tissue of UC, leading to abnormal microvasculature in mucosa, and impaired mucosal regeneration and reduction in the quality of healing of UC. Thus, it is likely BMD-EPC may play an important role in UC healing and stimulation of BMD-EPC may be a new therapeutic target for improving the neovascularization and quality of mucosal healing in UC.

REFERENCES

[1] Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961; 14: 213-22.

[2] Siminovitch L, McCulloch EA, Till J E. The distribution of colony-forming cells among spleen colonies. J Cell Physiol 1963; 62: 327-36.

[3] Messner HA. Stem cells: the challenge and opportunities. Bone Marrow Transplantation 2008; 42: S57-9.

[4] Pontikoglou C, Deschaseaux F, Sensebé L, Papadaki HA. Bone Marrow Mesenchymal Stem Cells: Biological Properties and Their Role in Hematopoiesis and Hematopoietic Stem Cell Transplantation. Stem Cell Rev 2011; Epub ahead of print.

[5] Jeong JH. Adipose stem cells and skin repair. Curr Stem Cell Res Ther 2010; 5: 137-40.

[6] Witkowska-Zimny M, Walenko K. Stem cells from adipose tissue. Cell Mol Biol Lett 2011; 16: 236-57.

[7] Christ B, Dollinger MM. The generation of hepatocytes from mesenchymal stem cells and engraftment into the liver. Curr Opin Organ Transplant. 2010; Epub ahead of print.

[8] Asahara T, Murohara T, Sullivan A, *et al*. Isolation of putative progenitor endothelial cells for angiogenesis. 1997; Science 275:964-7.

[9] Shi Q, Rafii S, Wu MH, Wijelath ES, *et al*. Evidence for circulating bone marrow-derived endothelial cells. Blood 1998; 92: 362-7.

[10] Crosby JR, Kaminski WE, Schatteman G, *et al*. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. Circ Res 2000; 87: 728-30.

[11] Zhang ZG, Zhang L, Jiang Q, Chopp M. Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. Circ Res 2002; 90: 284-8.

[12] Hess DC, Hill WD, Martin-Studdard A, Carroll J, Brailer J, Carothers J. Bone marrow as a source of endothelial cells and NeuN-expressing cells after stroke. Stroke 2002; 33: 1362-8.

[13] Urbich C, Heeschen C, Aicher A, *et al*. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. Circulation 2003; 108: 2511-6.

[14] Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. Br J Haematol 2001; 115: 186-94.

[15] Wang QR, Wang BH, Huang YH, Dai G, Li WM, Yan Q. Purification and growth of endothelial progenitor cells from murine bone marrow mononuclear cells. J Cell Biochem 2008; 103: 21-9.

[16] Jujo K, Hamada H, Iwakura A, Thorne T, *et al.* CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. Proc Natl Acad Sci USA 2010; 107: 11008-13.

[17] Burt RK, Craig RM, Milanetti F, Quigley K, *et al.* Autologous nonmyeloablative hematopoietic stem cell transplantation in patients with severe anti-TNF refractory Crohn disease:long-term follow-up. Blood 2010; 116: 6123-32.

[18] Zaruba MM, Franz WM. Role of the SDF-1-CXCR4 axis in stem cell-based therapies for ischemic cardiomyopathy. Expert Opin Biol Ther 2010; 10: 321-35.

[19] Zhang G, Nakamura Y, Wang X, Hu Q, Suggs LJ, Zhang J. Controlled release of stromal cell-derived factor-1alpha in situ increases c-kit+ cell homing to the infarcted heart. Tissue Eng 2007; 13: 2063-71.

[20] Askari AT, Unzek S, Popovic ZB, *et al.* Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003; 362: 697-703.

[21] Frederick JR, Fitzpatrick JR 3rd, McCormick RC, *et al.* Stromal cell-derived factor-1alpha activation of tissue-engineered endothelial progenitor cell matrix enhances ventricular function after myocardial infarction by inducing neovasculogenesis. Circulation 2010; 122: S107-17.

[22] Ceradini DJ, Gurtner GC. Homing to hypoxia: HIF-1 as a mediator of progenitor cell recruitment to injured tissue. Trends Cardiovasc Med 2005; 15: 57-63.

[23] Lakkisto P, Kyto V, Forsten H, *et al*. Heme oxygenase-1 and carbon monoxide promote neovascularization after myocardial infarction by modulating the expression of HIF-1α, SDF-1α and VEGF-B. Eur J Pharmacol 2010; 635: 156-64.

[24] De Falco E, Avitabile D, Totta P, Straino S, *et al.* Altered SDF-1-mediated differentiation of bone marrow-derived endothelial progenitor cells in diabetes mellitus. J Cell Mol Med 2009; 13: 3405-14.

[25] Gallagher KA, Liu ZJ, Xiao M, *et al.* Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1α. J Clin Invest 2007; 117: 1249-59.

[26] Westenbrink BD, Lipsic E, van der Meer P, *et al.* Erythropoietin improves cardiac function through endothelial progenitor cell and vascular endothelial growth factor mediated neovascularization. Eur Heart J 2007; 28: 2018-27.

[27] Salcedo R, Wasserman K, Young HA, *et al.* Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: *In vivo* neovascularization induced by stromal-derived factor-1α. Am J Pathol 1999; 154: 1125-35.

[28] Bruno S, Bussolati B, Scacciatella P, *et al.* Combined administration of G-CSF and GM-CSF stimulates monocyte-derived pro-angiogenic cells in patients with acute myocardial infarction. Cytokine 2006; 34: 56-65.

[29] Carmeliet, P. Mechanisms of angiogenesis and arteriogenesis. Nat Med 2000; 6: 389-95.

[30] Djonov V, Baum O, Burri PH. Vascular remodeling by intussusceptive angiogenesis. Cell Tissue Res 2003; 314: 107-17.

[31] Tepper OM, Capla JM, Galiano RD, *et al.* Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. Blood 2005; 105: 1068-77.

[32] Di Rocco G, Gentile A, Antonini A, *et al.* Enhanced healing of diabetic wounds by topical administration of adipose tissue-derived stromal cells overexpressing stromal-derived factor-1: biodistribution and engraftment analysis by bioluminescent imaging. Stem Cells Int 2010; 2011: 304562.

[33] Lau TT, Wang DA. Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine. Expert Opin Biol Ther 2011; 11: 189-97.

[34] Tang JM, Wang JN, Zhang L, *et al.* VEGF/SDF-1 promotes cardiac stem cell mobilization and myocardial repair in the infarcted heart. Cardiovasc Res 2011; Epub ahead of print.

[35] Lazarus GS, Cooper DM, Knighton DR, *et al.* Definitions and guidelines for assessment of wounds and evaluation of healing. Arch Dermatol 1994; 130: 489-93.

[36] Ceradini DJ, Kulkarni AR, Callaghan MJ, *et al*. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004; 10: 858-64.

[37] Tamama K, Kawasaki H, Kerpedjieva SS, Guan J, Ganju RK, Sen CK. Differential roles of hypoxia inducible factor subunits in multipotential stromal cells under hypoxic condition. J Cell Biochem 2011; 112: 804-17.

[38] Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, Babilas P. Oxygen in acute and chronic wound healing. Br J Dermatol 2010; 163: 257-68.

[39] Sheffield PJ. Measuring tissue oxygen tension: a review. Undersea Hyperb Med 1998; 25: 179-88.

[40] LaVan FB, Hunt TK. Oxygen and wound healing. Clin Plast Surg 1990; 17: 463-72.

[41] Knighton DR, Silver IA, Hunt TK. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. Surgery 1981; 90: 262-70.

[42] Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt T. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch Surg 2000; 135: 1293-7.

[43] Alonso-Echanove J, Richards C Jr, Horan TC. Supplemental perioperative oxygen to reduce surgicalwound infections. N Engl J Med 2000; 342: 1613.

[44] Lindholm C, Bjellerup M, Christensen OB, Zederfeldt B. A demographic survey of leg and foot ulcer patients in a defined population. Acta Derm Venereol 1992; 72: 227-30.

[45] Phillips TJ. Chronic cutaneous ulcers: etiology and epidemiology. J Invest Dermatol 1994; 102: 38S-41S.

[46] Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, Buerk DG. Stem cell mobilization by hyperbaric oxygen. Am J Physiol Heart Circ Physiol 2006; 290: H1378-86.

[47] Goldstein LJ, Gallagher KA, Bauer SM, *et al.* Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. Stem Cells 2006; 24: 2309-18.

[48] Nagano M, Kimura K, Yamashita T, *et al.* Hypoxia responsive mesenchymal stem cells derived from human umbilical cord blood are effective for bone repair. Stem Cells Dev 2010; 19: 1195-210.

[49] Pineton de Chambrun G, Peyrin-Biroulet L, Lémann M, Colombel JF. Clinical implications of mucosal healing for the management of IBD. Nat Rev Gastroenterol Hepatol 2010; 7: 15-29.

[50] Vatn M. Mucosal healing: impact on the natural course or therapeutic strategies. Dig Dis 2009; 27: 470-5.

[51] McLaren WJ, Anikijenko P, Thomas SG, Delaney PM, King RG. *In vivo* detection of morphological and microvascular changes of the colon in association with colitis using fiberoptic confocal imaging (FOCI). Dig Dis Sci 2002; 47: 2424-33.

[52] Tarnawski A, Coron E, Mosnier JF, *et al.* In-vivo detection by confocal endomicroscopy of two distinct structural abnormalities in angioarchitecture and increased VP in colonic mucosa of patients with IBD in remission: mechanistic implications. Gastroenterology 2009; 136: A112.

[53] Cheon JH, Kim WH. Recent advances of endoscopy in inflammatory bowel diseases. Gut Liver 2007; 1: 118-25.

[54] Aychek T, Vandoorne K, Brenner O, Jung S, Neeman M. Quantitative analysis of intravenously administered contrast media reveals changes in vascular barrier functions in a murine colitis model. Magn Reson Med 2011; Epub ahead of print.

[55] McLaren W, Anikijenko P, Barkla D, Delaney TP, King R. *In vivo* detection of experimental ulcerative colitis in rats using fiberoptic confocal imaging (FOCI). Dig Dis Sci 2001; 46: 2263-76.

[56] McGuckin MA, Eri R, Simms LA, Florin TH, Radford-Smith G. Intestinal barrier dysfunction in inflammatory bowel diseases. Inflamm Bowel Dis 2009; 15: 100-13.

[57] Taylor CT, Colgan SP. Hypoxia and gastrointestinal disease. J Mol Med 2007; 85: 1295-300.
[58] Tolstanova G, Deng XM, French SW, *et al.* Early endothelial damage and increased colonic vascular permeability in the development of experimental ulcerative colitis in rats and mice. Lab Invest 2011; accepted.
[59] Harris NR, Carter PR, Yadav AS, *et al.* Relationship between inflammation and tissue hypoxia in a mouse model of chronic colitis. Inflamm Bowel Dis 2011; 17: 742-6.
[60] Deban L, Correale C, Vetrano S, Malesci A, Danese S. Multiple pathogenic roles of microvasculature in inflammatory bowel disease: a Jack of all trades. Am J Pathol 2008; 172: 1457-66.
[61] Ferrante M, Pierik M, Henckaerts L, *et al.* The role of vascular endothelial growth factor (VEGF) in inflammatory bowel disease. Inflamm Bowel Dis 2006; 12: 870-8.
[62] Klagsbrun M. Regulators of angiogenesis: stimulators, inhibitors, and extracellular matrix. J Cell Biochem 1991; 47: 199-200.
[63] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 200; 39: 669-76.
[64] Oluleye TS. Diabetic retinopathy: current developments in pathogenesis and management. Afr J Med Med Sci 2010; 39: 199-206.
[65] Wang Q, Diao X, Sun J, Chen Z. Stromal cell-derived factor-1 and vascular endothelial growth factor as biomarkers for lymph node metastasis and poor cancer-specific survival in prostate cancer patients after radical prostatectomy. Urol Oncol 2011; Epub ahead of print.
[66] Kapsoritakis A, Sfiridaki A, Maltezos E, *et al*. Vascular endothelial growth factor in inflammatory bowel disease. Int J Colorectal Dis 2003; 18: 418-22.
[67] Scaldaferri F, Vetrano S, Sans M, *et al*. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology 2009; 136: 585-95.
[68] Ferrante M, Pierik M, Henckaerts L, *et al.* The role of vascular endothelial growth factor (VEGF) in inflammatory bowel disease. Inflamm Bowel Dis 2006; 12: 870-8.
[69] Sandor Zs, Szabo S, Zagoni T, Tulassay Zs. Differential changes of bFGF and PDGF in human serum and biopsy samples in UC and Crohn's disease. Gastroenterology 1998; 114: A1075.
[70] Griga T, Tromm A, Spanger J and May B. Increased serum level of vascular endothelial growth factor in patients with inflammatory bowel disease. Scand J Gastroenterol 1998: 33: 504-8.
[71] Griga T, Voigt E, Gretzer B, *et al.* Increased production of vascular endothelial growth factor by intestinal mucosa of patients with inflammatory bowel disease. Hepato-Gastroenterology 1999; 46: 920-3.
[72] Kanazawa S, Tsunoda T, Onuma E, *et al.* VEGF, basic-FGF, and TGF-beta in Crohn's disease and UC: a novel mechanism of chronic intestinal inflammation. Am J Gastroenterol 2001; 96: 822-8.
[73] Konno S, Iizuka M, Yukawa M, *et al*. Altered expression of angiogenic factors in the VEGF-Ets-1 cascades in inflammatory bowel disease. J Gastroenterol 2004; 39: 931-9.
[74] Chidlow JH Jr, Shukla D, Grisham MB, Kevil CG. Pathogenic angiogenesis in IBD and experimental colitis: new ideas and therapeutic avenues. Am J Physiol Gastrointest Liver Physiol 2007; 293: G5-G18.
[75] Koutroubakis IE, Tsiolakidou G, Karmiris K, Kouroumalis EA. Role of angiogenesis in inflammatory bowel disease. Inflamm Bowel Dis 2006; 12: 515-23.
[76] Deng X, Szabo S, Khomenko T, Jadus MR, Yoshida M. Gene therapy with adenoviral plasmids or naked DNA of vascular endothelial growth factor and platelet-derived growth factor accelerates healing of duodenal ulcer in rats. J Pharmacol Exp Ther 2004; 311: 982-8.
[77] Jones MK, Kawanaka H, Baatar D, *et al.* Gene therapy for gastric ulcers with single local injection of naked DNA encoding VEGF and angiopoietin-1. Gastroenterology 2001; 121: 1040-7.
[78] Kusumanto YH, van Weel V, Mulder NH, *et al.* Treatment with intramuscular vascular endothelial growth factor gene compared with placebo for patients with diabetes mellitus and critical limb ischemia: a double-blind randomized trial. Hum Gene Ther 2006; 17: 683-91.
[79] Katoh O, Tauchi H, Kawaishi K, Kimura A, Satow Y. Expression of the vascular endothelial growth factor (VEGF) receptor gene, KDR, in hematopoietic cells and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. Cancer Res 1995; 55: 5687-92.
[80] Clauss M, Gerlach M, Gerlach H, *et al*. Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. J Exp Med 1990; 172: 1535-45.
[81] Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marmé D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood 1996; 87: 3336-43.
[82] Kucia M, Jankowski K, Reca R, *et al.* CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. J Mol Histol 2004; 35: 233-45.
[83] Zheng H, Fu G, Dai T, Huang H. Migration of endothelial progenitor cells mediated by stromal cell-derived factor-1alpha/CXCR4 via PI3K/Akt/eNOS signal transduction pathway. J Cardiovasc Pharmacol 2007; 50: 274-80.
[84] Katsuta T, Lim C, Shimoda K, *et al.* Interleukin-8 and SDF1-alpha mRNA expression in colonic biopsies from patients with inflammatory bowel disease. Am J Gastroenterol 2000; 95: 3157-64.
[85] Xia XM, Wang FY, Xu WA, *et al.* CXCR4 antagonist AMD3100 attenuates colonic damage in mice with experimental colitis. World J Gastroenterol 2010; 16: 2873-80.
[86] Ceradini DJ, Kulkarni AR, Callaghan MJ, *et al*. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004; 10: 858-64.
[87] Capobianco S, Chennamaneni V, Mittal M, Zhang N, Zhang C. Endothelial progenitor cells as factors in neovascularization and endothelial repair. World J Cardiol 2010; 2: 41120.
[88] Masuda J, Mitsuyama K, Yamasaki H, *et al*. Depletion of endothelial progenitor cells in the peripheral blood of patients with ulcerative colitis. Int J Mol Med 2007; 19: 221-8.
[89] Garolla A, D'Incà R, Checchin D, *et al*. Reduced endothelial progenitor cell number and function in inflammatory bowel disease: a possible link to the pathogenesis. Am J Gastroenterol 2009; 104: 2500-7.
[90] Brittan M, Hunt T, Jeffery R, *et al.* Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. Gut 2002; 50: 752-7.
[91] Okamoto R, Yajima T, Yamazaki M, *et al*. Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. Nat Med 2002; 9: 1011-7.
[92] Danese S, Sans M, de la Motte C, *et al*. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. Gastroenterology 2006; 130: 2060-73.
[93] Sandor Zs, Deng XM, Khomenko T, Tarnawski AS, Szabo S. Altered angiogenic balance in ulcerative colitis: A key to impaired healing. Biochem Biophys Res Commun 2006; 350: 147-50.
[94] Tolstanova G, Deng X, Khomenko T, *et al.* Role of anti-angiogenic factor endostatin in the pathogenesis of experimental ulcerative colitis. Life Sci 2010; 88: 74-81.
[95] Hoenig MR, Bianchi C, Rosenzweig A, Sellke FW. Decreased vascular repair and neovascularization with ageing: mechanisms and clinical relevance with an emphasis on hypoxia-inducible factor-1. Curr Mol Med 2008; 8: 754-67.
[96] Hoenig MR, Bianchi C, Sellke FW. Hypoxia inducible factor-1 alpha, endothelial progenitor cells, monocytes, cardiovascular risk, wound healing, cobalt and hydralazine: a unifying hypothesis. Curr Drug Targets 2008; 9: 422-35.
[97] Herrinton L J, Liu L, Lewis JD, Griffin PM, and Allison J. Incidence and prevalence of inflammatory bowel disease in Northern California managed care organization, 1996-2002. Am J Gastroenterol 2008; 103: 1998-2006.
[98] Chidlow JH Jr, Langston W, Greer JJ, *et al*. Differential angiogenic regulation of experimental colitis. Am J Pathol 2006; 169: 2014-30.
[99] Danese S, *et al*. Angiogenesis blockade as a new therapeutic approach to experimental colitis. Gut 2007; 56: 855-62.
[100] Danese S. Inflammation and the mucosal microcirculation in inflammatory bowel disease: the ebb and flow. Curr Opin Gastroenterol 2007; 23: 384-9.

[101] Shi M, Liu ZW, Wang FS. Immunomodulatory properties and therapeutic application of mesenchymal stem cells. Clin Exp Immunol 2011; 164: 1-8.
[102] To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. Blood 1997; 89: 2233-58.
[103] Chen L, Deng XM, Xiong XM, Szabo S, Sandor Zs. Intracolonical application of bone marrow-derived stem cells accelerates ulcerative colitis healing in rats: A new alternative to bone marrow transplantation. Gastroenterology 2010; 138: S568-9.
[104] Liekens S, Schols D, Hatse S. CXCL12-CXCR4 axis in angiogenesis, metastasis and stem cell mobilization. Curr Pharm Des 2010; 16: 3903-20.
[105] Ditschkowski M, Einsele H, Schwerdtfeger R, *et al*. Improvement of inflammatory bowel disease after allogeneic stem-cell transplantation. Transplantation 2003; 75: 1745-7.

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