Commentary

Gut Mucosal Healing: Is the Science Relevant?

Marc D. Basson

From the Departments of Surgery, John D. Dingell VA Medical Center and Wayne State University

Historical Perspective

Injuries to the gut mucosa have been of interest to clinicians for centuries. Among the case reports inscribed on the votive columns of the Asclepian temple at Epidaurus is the description of the cure of a patient with a gastric ulcer.¹ Many of the fundamental concepts of gut mucosal biology, including the development of experimental models for study, the identification of restitution and proliferation as separate and complementary processes, and the nature of gastric acid and pepsin secretion were identified before 1900. For instance, Friederich Gunzberg² proposed in 1852 that gastric acidity might cause ulcers. Experimental gut mucosal healing was first studied in 1863 when Pavy³ studied the resolution of a surgically created gastric mucosal defect in a dog, and this seminal work was followed in 1888 by two important papers by Bizzozero⁴ and Griffini and Vassale⁵ pointing out the importance of restitution and mucosal proliferation respectively in healing mucosal wounds.

During the same era, the pioneering work of scientists like Bernard, Heidenhain, Langley, and Prout, along with clinicians like Beaumont and Cooper laid the groundwork for the study of the biochemistry of gastric secretion. During the first half of the twentieth century, Boris Babkin proposed that vagal stimulation released acetylcholine that stimulated histamine secretion that stimulated acid secretion. However, the absence of a pharmacological or surgical mechanism to block acid secretion or promote restitution impeded the application of physiological knowledge to the clinic. Thus, Babkin⁶ felt compelled to apologize for the irrelevance of gastrointestinal (GI) physiology in 1934. "Whenever I speak before a medical audience on the physiology of the gastrointestinal tract, I feel somewhat guilty lest I be communicating things which have at present very little or no practical value. Gastroenterology today is chiefly an empirical science—the theory and the practice in the field of knowledge stand at the present so far apart."

In the mid-twentieth century, interaction between basic and clinical GI science became more pronounced and cross-fertilization accelerated progress. Billroth's original observation that a third of his gastrojejunostomy patients

developed anastomotic ulcers laid the foundation for the Mann Williamson canine ulcer model that permitted the first systematic animal research into the genesis and treatment of peptic ulcer disease. In turn, such physiological observations supported Lester Dragstedt's work⁷ championing surgical vagotomy as the first definitive treatment for peptic ulcer disease and ultimately led to the development of H₂ blockers by James Black in 1972.⁸ GI cell biologists of the current era should also recall that modern work on humoral effects in the gut was presaged by clinical observations on the effect of pregnancy on ulcer disease and by animal studies demonstrating that urogastrone, a factor in urine more accurately known today as epidermal growth factor (EGF), inhibited gastric secretion and promoted ulcer healing in canine models. The history of this work and indeed of GI secretory research in general during this era has been elegantly reviewed by Davenport in his book,⁹ to which the above discussion is indebted.

The initial interest of students of mucosal injury concentrated on the factors that might injure the gut mucosa and how they could be blocked, including acid, pepsin, bile acids, non-steroidal anti-inflammatory drugs, and other noxious agents. However, the gut mucosa is constantly subject to injury during normal function,¹⁰ leading many mucosal biologists to shift focus from how the gut is injured to how and why it heals (or fails to heal). Among the recent advances in this regard have been the description of adaptive cytoprotection by mild exposure to noxious irritants by Robert,¹¹ studies of the matrix-driven and growth factor-modulated signal transduction events that regulate mucosal cell motility and proliferation,^{12–18} and most notably the recent shift in emphasis to the effects of *H. pylori* and non-steroidal anti-inflammatory drugs on mucosal healing. These studies were inspired by clinico-pathological observations and clinical science and have more recently inspired a legion of investigators to begin to characterize the effects of *H. pylori* toxins at the basic science level. Conversely, however, the elucidation of the basic cell biology of growth factor effects on gut mucosal healing has yet to achieve clinical application.

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Address reprint requests to Dr. Marc Basson, Chief, Surgical Service, John D. Dingell VA Medical Center, 4646 John R. Street, Detroit, MI 48201-1932. E-mail: marc.basson@med.va.gov.

Mucosal Healing versus Submucosal Angiogenesis

Although much of the work that has been done on mucosal healing has focused on the renewal of the mucosa, the hypothesis that angiogenesis is required to support mucosal reconstitution is an attractive one. In this issue of *The American Journal of Pathology*, Baatar and colleagues¹⁹ describe the induction of vascular endothelial growth factor (VEGF) expression by a hypoxia-inducible transcription factor (Hif-1 α) in an rodent ischemic esophageal ulcer model and demonstrate that a single local injection of a cDNA for VEGF enhances angiogenesis and accelerates esophageal ulcer healing. The manuscript should be read together with another recent publication by the same group²⁰ in which they describe similar efficacy for VEGF gene transfection in ischemic gastric ulcers. (The authors correctly point out the substantial differences in anatomy between esophagus and stomach, so that successful results in one organ would not necessarily predict results in another.)

These results are potentially exciting for at least three reasons. First, they appear to provide solid evidence for the importance of angiogenesis in the healing of esophageal and gastric ulcers. Second, the present report provides new information about the mechanisms that regulate VEGF synthesis in response to esophageal ulceration. Third, if extrapolated to human pathophysiology, these results appear to predict that a novel approach, a single endoscopic VEGF cDNA injection, might accelerate the healing of mucosal injuries in the esophagus and stomach. Extrapolation to mucosal lesions in other organs (ie, duodenal ulceration) or from other pathologies (ie, inflammatory bowel disease) would be premature but would merit further study. Each of these issues merits comment.

Angiogenesis in Mucosal Healing

Any physician who has watched an open wound fill with granulation tissue has observed angiogenic wound healing at the macroscopic level, but angiogenesis is obviously critical not only for filling in space but also for supporting the healing of the overlying epithelium. It does so by constructing a matrix substrate across which restitution can occur and by providing a mechanism for nutrient and oxygen delivery to the new epithelium. The importance of this process was presaged by Longmire's²¹ demonstration a half century ago that mucosal restitution is more rapid in experimental canine ulcers if the muscularis mucosae is left intact.

At its simplest, angiogenesis is the production of new blood vessels, an expansion of the vascular system from its existing plexus. It occurs in settings as diverse as neoplasm, corneal neovascularization, wound healing, arthritis, and surgical adhesion formation. Although the stimulation of angiogenesis has long been ascribed to "ischemia" and "inflammatory mediators" on clinical and teleologic grounds, the initial delineation of the angiogenic effects of growth factors such as fibroblast growth

factor (FGF) opened up a new paradigm for study. Understanding of the role of angiogenesis in wound healing was then dramatically enhanced by the identification of the VEGF family of peptides.²² Although the mechanisms by which VEGF supports angiogenesis remain an active subject for investigation, at least one mechanism involves the protection of new endothelial cells uncovered by pericytes.²³ VEGF stimulates expression of antiapoptotic proteins such as Bcl-2 and A1 in these cells.²⁴

There is little information about the role of angiogenesis in the healing of esophageal mucosal injuries. Koukourakis and colleagues²⁵ have reported that oral administration of a stabilized preparation of granulocyte macrophage colony-stimulating factor (GM-CSF) ameliorates radiation-induced esophagitis in patients undergoing radiotherapy, and have attributed this effect to the promotion of angiogenesis by this factor. Interestingly, Barrett's esophageal epithelium is characterized by overexpression of VEGF in the Barrett's-specific goblet cells and strong neovascularization even in the absence of visible esophageal mucosal injury.²⁶

However, both angiogenesis and VEGF have been observed in healing gastric mucosal ulcers, and indeed, neutralizing anti-VEGF antibody significantly reduces the angiogenic response to gastric mucosal necrosis induced by luminal ethanol in rats.²⁷ In addition to extending the concept of promoting mucosal healing via angiogenesis to the esophagus, the recent work by Tarnawski and colleagues goes beyond this previous work in demonstrating that specific promotion of angiogenesis by VEGF gene induction can accelerate the healing of a mucosal injury. Thus, VEGF-induced angiogenesis does seem to promote the healing of at least some esophageal and gastric mucosal injuries. Which of the two VEGF receptors is involved and what signals mediate the VEGF effects of gut mucosal angiogenesis remain to be established.

This finding, while important, certainly does not render irrelevant all of the work that has been done in understanding mucosal re-epithelialization. Dysregulated angiogenesis is likely to be complementary to deficient epithelial restitution and proliferation rather than a competing cause of poor mucosal healing. Indeed, growth factors such as EGF and hepatocyte growth factor (HGF) that stimulate restitution also stimulate VEGF expression in primary gastric epithelial cells in culture,²⁸ further suggesting the possibility of synergy between these processes.

VEGF Synthesis

Hypoxia (as well as some growth factors and activation of some oncogenes) results in increases in the level of hypoxia-inducible factor 1 (HIF-1).²⁹ In view of the known propensity of diabetics to poor wound healing, it is also interesting to note that insulin is one of the peptides which activates HIF1 under normoxic conditions.^{30,31} AP-1 is also activated by hypoxia in some cell lines and may act synergistically with HIF-1.³²

HIF-1 consists of two subunits, HIF-1 α and HIF-1 β , and has variously been reported to be induced by PI3-kinase/

Akt signaling^{33,34} or MAPK signaling^{32,35} in response to diverse stimuli. The two pathways may be synergistic, as they act on different domains of HIF-1 α .³⁶ Sen and colleagues³⁷ have hypothesized that reactive oxygen species released by neutrophils and macrophages recruited to wounds may also induce VEGF expression in epithelial keratinocytes independently of HIF-1 *via* Sp1 activation. HIF-1 β is constitutively expressed but HIF- α is an unstable protein, so that HIF-1 α levels generally regulate HIF-1 activity. HIF1 α undergoes rapid proteasome-dependent degradation in a ubiquitin complex associated with the von Hippel Lindau (VHL) tumor suppressor protein. Heat shock protein 90 may act as a chaperone to promote this interaction and further accelerate HIF-1 α degradation. In addition, HSP90 may inhibit HIF-1 α transcription.³⁸

Increases in active HIF-1 in turn increase the levels of various gene products, including VEGF. At least one report suggests that HIF-1 may also stabilize VEGF mRNA.³⁹ In some systems, HIF-2 α induction has similar effects to those reported for HIF-1 α induction in other systems, and the relative contributions of these two subunits *in vivo* may vary with the cell type and stimulus.⁴⁰ Smad-mediated TGF- β signaling may also be synergistic with hypoxic HIF activation in some settings and HIF-1 α associates with Smad3.⁴¹

The study by Baatar and colleagues¹⁹ in this issue of *The American Journal of Pathology* demonstrates that HIF-1 α is activated in parallel with VEGF mRNA and protein levels in the setting of esophageal ulceration induced by serosally applied acetic acid. The relative contributions of the additional signal elements delineated above to this effect remains to be delineated.

Clinical Relevance

Thus, the work by Baatar and colleagues¹⁹ represents interesting science. Is it relevant? We must first consider whether the model used appears likely to resemble human pathophysiology and second whether it points the way toward potential therapeutic intervention.

It should be pointed out that this study has been performed in an ischemic ulcer model, based on serosal and transmural chemical injury by acetic acid. These results might not necessarily extrapolate to non-ischemic ulcers associated with luminal noxious agents such as acid, pepsin, bile, NSAID's, or *H. pylori*. Indeed, normoxic regulation of VEGF secretion has been reported to differ from that which occurs during hypoxia once hypoxia-inducible factors become involved.⁴²

This does not invalidate the importance of the current result, but it does require caution in extrapolation. Acute ethanolic gastric mucosal injury in rats also stimulates HIF-1 α at the edge of acute gastric ulcers⁴³ while VEGF has been reported to be up-regulated in active gastric ulcers.⁴⁴ Furthermore, there is some evidence that angiogenic peptides can accelerate healing of alcohol-induced or cysteamine-induced ulcers.^{45,46} However, of some concern in this regard are recent observations by Konturek and colleagues⁴⁷ in a similar serosal acetic acid model of gastric ulceration in rats. These investiga-

tors reported that intraperitoneal injection of *E. coli* lipopolysaccharide resulted in further up-regulation of VEGF but inhibition of ulcer healing. Endotoxin injection produces numerous effects other than VEGF induction that could have opposed the promotion of healing by VEGF, and certainly the promotion of ulcer healing in such models by VEGF gene induction is more specific. However, such observations suggest the complexity of the *in vivo* situation and the difficulties of extrapolating from a pure laboratory model to a more complex setting. Interestingly, VEGF secretion by gastric epithelial cell lines may also be increased by aspirin,⁴⁸ a seemingly paradoxical effect, while indomethacin inhibition of ulcer healing in the serosal acetic acid model is not explicable by VEGF effects.⁴⁹ Portal hypertensive gastropathy is also characterized by increased VEGF.⁵⁰

Another concern that should be raised is that these studies, like most animal studies of mucosal injury, are based on an acute injury. However, acute mucosal injuries in humans (and likely in animals) generally heal spontaneously. Pathophysiologically important ulcerations are those which fail to heal and become chronic. Whether VEGF plays a similarly important role in the pathobiology of such chronic mucosal wounds must also await study.

If VEGF is important in chronic esophageal mucosal ulcerations or those elsewhere in the GI tract, would VEGF gene therapy make clinical sense? Again, the data appear promising, but numerous cautions must be raised, some practical, and some theoretical.

The strength of this approach is that only a single dose is required and that gene expression is transient, long enough to accelerate ulcer healing, but hopefully not long enough for side effects. However, would this accelerate healing of a chronic ulcer in the setting of chronic ulcerogenic stimulation, as opposed to a single acute ulcerogenic injury? There are also likely to be important technical differences between transluminal endoscopic injection into an established ulcer bed characterized by inflammation, exposure to luminal contents and acid, and probably a fair amount of DNase activity (as would presumably have to be done for clinical use of such a technique) and transserosal injection before the actual ulcer formation, as was done here. In addition, whether VEGF itself would be the optimal gene to use for these purposes and whether naked gene injection might yield better results than other transfection techniques also awaits further study. For instance, Tarnawski and colleagues²⁰ have previously observed similar effects in the stomach with another growth factor, basic FGF, while others have proposed the use of an adeno-associated viral vector containing the VEGF gene under control of a *cis*-acting hypoxia-responsive element⁵¹ or naked DNA encoding a HIF1 α /VP16 hybrid alone or in combination with naked DNA encoding VEGF⁵² in a different setting.

Even if a single endoscopic VEGF cDNA injection could be shown to accelerate ulcer healing, this might not necessarily be a desirable event. Tarnawski's group^{53,54} has previously pointed out in the setting of duodenal ulcers that one must consider not only the speed at which the mucosa reconstitutes but the function of that mucosa, raising concerns about the "quality of ulcer healing"

based on neomucosal architecture and differentiation in some settings. Would a more rapidly reconstituted esophageal mucosa be as functional as a barrier, for instance? There might also be other safety concerns. For instance, ulcerations in Barrett's esophagus may be a sign of malignancy.⁵⁵ VEGF treatment in this setting might be contraindicated not only because (like proton pump inhibitors in some malignant gastric ulcers) it might actually stimulate mucosal healing over a malignancy and thus mask a neoplasm but also because VEGF overexpression might facilitate tumor growth. Finally, even if proven safe and effective in humans, the role for such therapy would remain to be established within the context of more conventional therapy based on relative cost, efficacy, side effects, and safety.

Conclusion

In sum, there is certainly a great deal of work to be done before anyone seriously advocates injecting VEGF DNA into GI mucosal ulcers to heal them. Indeed the entire approach may turn out to be ineffective, expensive, or even dangerous. However, none of these concerns should detract from the essential accomplishments of the work by Baatar and colleagues.¹⁹ Not only have they elucidated an important step in the healing of esophageal ulcers, but also this work may point the way to a closing of the gap between the basic science of mucosal healing and clinical application. Even if this turns out to be the wrong gene or the wrong method, this work suggests to the cynical clinician that there may be a point to studying all these proteins after all.

References

- Garrison F: An Introduction to the History of Medicine. Philadelphia, W.B. Saunders Company, 1966, pp 84
- Gunzberg F: Zur kritik des magensgeschwurs, insbesondere des perforisierenden. *Arch Physiol Heilk* 1852, 11:516–527
- Pavy FW: On the immunity enjoyed by the stomach from being digested by its own secretion during life. *Philos Trans R Soc Lond* 1863, 153:161–171
- Bizzozero G: Über die regeneration der elemente der schlauchförmigen drüsen und des epithels des magendarmkanals. *Anat Anz* 1888, 3:781–784
- Griffini L, Vassalle G: Ueber die reproduction der magenschleimhaut. *Beitr Pathol Anat* 1888, 3:423–448
- Babkin BP: The "chemical" phase of gastric secretion and its regulation. *Am J Dig Dis* 1934, 1:715–721
- Modlin IM, Basson MD: The Lester Dragstedt centennial. *J Am Coll Surg* 1994, 179:84–102
- Black JW, Duncan WAN, Durant GJ, Ganellin CR, Parsons EM: Definition and antagonism of H₂-receptors. *Nature* 1972, 236:385–390
- Davenport HW: A history of gastric secretion and digestion. New York, Oxford University Press, 1992
- McNeil PL, Ito S: Gastrointestinal cell plasma membrane wounding and resealing in vivo. *Gastroenterology* 1989, 96:1238–1248
- Robert A, Nezamis JE, Lancaster C, Davis JP, Field SO, Hanchar AJ: Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated by prostaglandins. *Am J Physiol* 1983, 245:G113–G121
- Ray RM, Viar MJ, McCormack SA, Johnson LR: Focal adhesion kinase signaling is decreased in polyamine-depleted IEC-6 cells. *Am J Physiol Cell Physiol* 2001, 281:C475–C485
- Yu CF, Sanders MA, Basson MD: Human caco-2 motility redistributes FAK and paxillin and activates p38 MAPK in a matrix-dependent manner. *Am J Physiol Gastrointest Liver Physiol* 2000, 278:G952–G966
- Kinoshita K, Taupin DR, Itoh H, Podolsky DK: Distinct pathways of cell migration and antiapoptotic response to epithelial injury: structure-function analysis of human intestinal trefoil factor. *Mol Cell Biol* 2000, 20:4680–4690
- Ranta-Knuuttila T, Kiviluoto T, Mustonen H, Puolakkainen P, Watanabe S, Sato N, Kivilaakso E: Migration of primary cultured rabbit gastric epithelial cells requires intact protein kinase C and Ca²⁺/calmodulin activity. *Dig Dis Sci* 2002, 47:1008–1014
- Pai R, Szabo IL, Giap AQ, Kawanaka H, Tarnawski AS: Nonsteroidal anti-inflammatory drugs inhibit re-epithelialization of wounded gastric monolayers by interfering with actin, Src FAK, and tensin signaling. *Life Sci* 2001, 69:3055–3071
- Jones MK, Tomikawa M, Mohajer B, Tarnawski AS: Gastrointestinal mucosal regeneration: role of growth factors. *Front Biosci* 1999, 4:D303–D309
- Turner JR, Basson MD: Roles of the extracellular matrix and cytoskeleton in intestinal epithelial restitution. *Gastrointestinal Mucosal Repair and Experimental Therapeutics. Frontiers in Gastrointestinal Research*. Edited by Cho C-H, Wang J-Y. Basel, Switzerland, Karger, 2002, vol 25:1–13
- Baatar D, Jones MK, Tsugawa K, Pai R, Moon WS, Koh GY, Kim I, Kitano S, Tarnawski AS: Esophageal ulceration triggers expression of hypoxia-inducible factor 1 α and activates vascular endothelial growth factor gene: implications for angiogenesis and ulcer healing. *Am J Pathol* 2002, 161:1449–1457
- Jones MK, Kawanaka H, Baatar D, Szabo IL, Tsugawa K, Pai R, Koh GY, Kim I, Sarfeh IJ, Tarnawski AS: Gene therapy for gastric ulcers with single local injection of naked DNA encoding VEGF and angiopoietin-1. *Gastroenterology* 2001, 121:1040–1047
- Longmire WPJ, Beal JM, Lippman HN, Bishop RC: Studies on the regeneration of gastric mucosa in the experimental animal. *Surgery* 1952, 32:384–394
- Ferrara N: Role of vascular endothelial growth factor in regulation of physiological angiogenesis. *Am J Physiol Cell Physiol* 2001, 280: C1358–C1366
- Benjamin LE, Hemo I, Keshet E: A plasticity window for blood vessel remodeling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. *Development* 1998, 125:1591–1598
- Gerber HP, Dixit V, Ferrara N: Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J Biol Chem* 1998, 273:13313–13316
- Koukourakis MI, Flordellis CS, Giatromanolaki A, Koukouraki S, Kapsoritakis A, Potamianos S, Retalis G, Sivridis E, Salsaa B, Harris AL, Maragoudakis MI: Oral administration of recombinant human granulocyte macrophage colony-stimulating factor in the management of radiotherapy-induced esophagitis. *Clin Cancer Res* 1999, 5:3970–3976
- Auvinen MI, Sihvo EI, Ruotula T, Salminen JT, Koivistoinen A, Siivola P, Ronnholm R, Ramo JO, Bergman M, Salo JA: Incipient angiogenesis in Barrett's epithelium and lymphangiogenesis in Barrett's adenocarcinoma. *J Clin Oncol* 2002, 20:2971–2979
- Jones MK, Itani RM, Wang H, Tomikawa M, Sarfeh IJ, Szabo S, Tarnawski AS: Activation of VEGF and Ras genes in gastric mucosa during angiogenic response to ethanol injury. *Am J Physiol* 1999, 276:G1345–G1355
- Takahashi M, Ogura K, Maeda S, Mori K, Mafune K, Mikami Y, Terano A, Omata M: Promoters of epithelialization induce expression of vascular endothelial growth factor in human gastric epithelial cells in primary culture. *FEBS Lett* 1997, 418:115–118
- Maxwell PH, Ratcliffe PJ: Oxygen sensors and angiogenesis. *Semin Cell Dev Biol* 2002, 13:29–37
- Stiehl DP, Jelkmann W, Wenger RH, Hellwig-Burgel T: Normoxic induction of the hypoxia-inducible factor 1 α by insulin and interleukin-1 β involves the phosphatidylinositol 3-kinase pathway. *FEBS Lett* 2002, 512:157–162
- Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E: Insulin stimulates HIF-1 through a PI-3-kinase/TOR-dependent pathway. *J Biol Chem* 2002, 277:27975–27981
- Michiels C, Minet E, Michel G, Mottet D, Piret JP, Raes M: HIF-1 and

- AP-1 cooperate to increase gene expression in hypoxia: role of MAP kinases. *IUBMB Life* 2001, 52:49–53
33. Gao N, Ding M, Zheng JZ, Zhang Z, Leonard SS, Liu KJ, Shi X, Jiang BH: Vanadate Induced expression of hypoxia-inducible factor 1a and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. *J Biol Chem* 2002, in press
34. Takahashi A, Kureishi Y, Yang J, Luo Z, Guo K, Mukhopadhyay D, Ivashchenko Y, Branellec D, Walsh K: Myogenic Akt signaling regulates blood vessel recruitment during myofiber growth. *Mol Cell Biol* 2002, 22:4803–4814
35. Shemirani B, Crowe DL: Hypoxic induction of HIF-1 α and VEGF expression in head and neck squamous cell carcinoma lines is mediated by stress activated protein kinases. *Oral Oncol* 2002, 38:251–257
36. Sodhi A, Montaner S, Miyazaki H, Gutkind JS: MAPK and Akt act cooperatively but independently on hypoxia inducible factor-1 α in rasV12 up-regulation of VEGF. *Biochem Biophys Res Commun* 2001, 287:292–300
37. Sen CK, Khanna S, Babior BM, Hunt TK, Ellison EC, Roy S: Oxidant induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing. *J Biol Chem* 2002, in press
38. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM: Hsp90 regulates a VHL-independent HIF-1a degradative pathway. *J Biol Chem* 2002, 277:29936–29944
39. Liu LX, Lu H, Luo Y, Date T, Belanger AJ, Vincent KA, Akita GY, Goldberg M, Cheng SH, Gregory RJ, Jiang C: Stabilization of vascular endothelial growth factor mRNA by hypoxia-inducible factor 1. *Biochem Biophys Res Commun* 2002, 291:908–914
40. Akeno N, Robins J, Zhang M, Czyzyk-Krzeska MF, Clemens TL: Induction of vascular endothelial growth factor by IGF-I in osteoblast-like cells is mediated by the PI3K signaling pathway through the hypoxia-inducible factor-2 α . *Endocrinology* 2002, 143:420–425
41. Sanchez-Elsner T, Botella LM, Velasco B, Corbi A, Attisano L, Bernabeu C: Synergistic cooperation between hypoxia and transforming growth factor- β pathways on human vascular endothelial growth factor gene expression. *J Biol Chem* 2001, 276:38527–38535
42. Berra E, Pages G, Pouyssegur J: MAP kinases and hypoxia in the control of VEGF expression. *Cancer Metastasis Rev* 2000, 19:139–145
43. Szabo IL, Kawanaka H, Jones MK, Pai R, Soreghan BA, Baatar D, Husain SS, Tarnawski AS: Activation of hypoxia inducible factor-1 α in gastric mucosa in response to ethanol injury: a trigger for angiogenesis? *Life Sci* 2001, 69:3035–3044
44. Akimoto M, Hashimoto H, Shigemoto M, Yamashita K, Yokoyama I: Changes of nitric oxide and growth factors during gastric ulcer healing. *J Cardiovasc Pharmacol* 2000, 36:S282–S285
45. Szabo S, Vincze A, Sandor Z, Jadus M, Gombos Z, Pedram A, Levin E, Hagar J, Iaquinto G: Vascular approach to gastroduodenal ulceration: new studies with endothelins and VEGF. *Dig Dis Sci* 1998, 43:40S–45S
46. Szabo S, Folkman J, Vattay P, Morales RE, Pinkus GS, Kato K: Accelerated healing of duodenal ulcers by oral administration of a mutein of basic fibroblast growth factor in rats. *Gastroenterology* 1994, 106:1106–1111
47. Konturek PC, Brzozowski T, Konturek SJ, Kwiecien S, Dembinski A, Hahn EG: Influence of bacterial lipopolysaccharide on healing of chronic experimental ulcer in rat. *Scand J Gastroenterol* 2001, 36:1239–1247
48. Pohle T, Becker JC, Markmann A, Luger N, Pauels HG, Konturek JW, Domschke W: Aspirin effects on gastric epithelial cell proliferation and cytokine expression. *Microsc Res Tech* 2001, 53:354–359
49. Suzuki N, Takahashi S, Okabe S: Relationship between vascular endothelial growth factor and angiogenesis in spontaneous and indomethacin-delayed healing of acetic acid-induced gastric ulcers in rats. *J Physiol Pharmacol* 1998, 49:515–527
50. Tsugawa K, Hashizume M, Tomikawa M, Migou S, Kawanaka H, Shiraishi S, Sueishi K, Sugimachi K: Immunohistochemical localization of vascular endothelial growth factor in the rat portal hypertensive gastropathy. *J Gastroenterol Hepatol* 2001, 16:429–437
51. Su H, Arakawa-Hoyt J, Kan YW: Adeno-associated viral vector-mediated hypoxia response element-regulated gene expression in mouse ischemic heart model. *Proc Natl Acad Sci USA* 2002, 99:9480–9485
52. Shyu KG, Wang MT, Wang BW, Chang CC, Leu JG, Kuan P, Chang H: Intramyocardial injection of naked DNA encoding HIF-1 α /VP16 hybrid to enhance angiogenesis in an acute myocardial infarction model in the rat. *Cardiovasc Res* 2002, 54:576–583
53. Tarnawski A, Douglass TG, Stachura J, Krause WJ: Quality of gastric ulcer healing: histological and ultrastructural assessment. *Aliment Pharmacol Ther* 1991, 5(Suppl 1):79–90
54. Tarnawski A, Tanoue K, Santos AM, Sarfeh IJ: Cellular and molecular mechanisms of gastric ulcer healing. Is the quality of mucosal scar affected by treatment? *Scand J Gastroenterol Suppl* 1995, 210:9–14
55. Montgomery E, Bronner MP, Greenson JK, Haber MM, Hart J, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Washington K, Goldblum JR: Are ulcers a marker for invasive carcinoma in Barrett's esophagus? Data from a diagnostic variability study with clinical follow-up *Am J Gastroenterol* 2002, 97:27–31