Available online at www.sciencedirect.com
ScienceDirect
Free Radical Biology & Medicine 44 (2008) 180–192
www.elsevier.com/locate/freeradbiomed

Review Article

Redox regulation of the VEGF signaling path and tissue vascularization: Hydrogen peroxide, the common link between physical exercise and cutaneous wound healing

Sashwati Roy *, Savita Khanna, Chandan K. Sen

Laboratory of Molecular Medicine, Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, OH 43210, USA

Received 24 October 2006; revised 14 December 2006; accepted 5 January 2007
Available online 19 January 2007

Abstract

Vascularization, under physiological or pathophysiological conditions, typically takes place by one or more of the following processes: angiogenesis, vasculogenesis, arteriogenesis, and lymphangiogenesis. Although all of these mechanisms of vascularization have sufficient contrasting features to warrant consideration under separate cover, one common feature shared by all is their sensitivity to the VEGF signaling pathway. Conditions such as wound healing and physical exercise result in increased production of reactive oxygen species such as H_2O_2, and both are associated with increased tissue vascularization. Understanding these two scenarios of adult tissue vascularization in tandem offers the potential to unlock the significance of redox regulation of the VEGF signaling pathway. Does H_2O_2 support tissue vascularization? H_2O_2 induces the expression of the most angiogenic form of VEGF, VEGF-A, by a HIF-independent and Sp1-dependent mechanism. Ligation of VEGF-A to VEGFR2 results in signal transduction leading to tissue vascularization. Such ligation generates H_2O_2 via an NADPH oxidase-dependent mechanism. Disruption of VEGF–VEGFR2 ligation-dependent H_2O_2 production or decomposition of such H_2O_2 stalls VEGFR2 signaling. Numerous antioxidants exhibit antiangiogenic properties. Current evidence lends firm credence to the hypothesis that low-level endogenous H_2O_2 supports vascular growth.

Keyword: Free radicals

Contents

Primary mechanisms of tissue vascularization . . . 181
The VEGF family . . . 181
Adult tissue vascularization in response to wound . . . 183
Adult tissue vascularization in response to exercise . . . 184
Inflammation and adult tissue vascularization . . . 184
Inflammation-derived oxidants support and antioxidants resist adult tissue vascularization . . . 185
Induction of VEGF family members by oxidants . . . 186
Oxidants as central messengers in VEGF signaling . . . 186
Conclusion . . . 187
Acknowledgments . . . 188
References . . . 188

* Corresponding author. Fax: +1 614 247 7818.
E-mail address: Sashwati.Roy@osumc.edu (S. Roy).

0891-5849/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2007.01.025

Wounding and physical exercise are two relatively well characterized triggers of vascularization of the adult tissue. Both cause inflammation and marked shifts in the redox environment of tissues toward oxidation. Understanding these two scenarios of adult tissue vascularization in tandem offers the potential to unlock the significance of redox regulation of the vascular endothelial growth factor (VEGF) signaling pathway. The objective of this review article is to critically appraise that potential in light of current developments. The redox environment of a linked set of redox couples as found in a biological fluid, organelle, cell, or tissue has been defined as the summation of the products of the reduction potential and reducing capacity of the linked redox couples present [1]. In biological tissues, H_2O_2 is ubiquitously generated and represents one major determinant of the redox environment. Given the central regulatory role of H_2O_2 in vascular signaling and function [2–4], this article focuses on the significance of reactive oxygen species in the VEGF signaling pathway.

Primary mechanisms of tissue vascularization

Vascularization, under physiological or pathophysiological conditions, typically takes place by one or more of the following processes: angiogenesis, vasculogenesis, arteriogenesis, and lymphangiogenesis. Angiogenesis refers to the process by which new blood vessels develop from preexisting blood vessels by sprouting or intussusception. De novo formation of new blood vessels from primitive cells during, say, early embryonic development takes place by vasculogenesis. As it is now known that under certain conditions endothelial precursor cells present in the bone marrow may be recruited and contribute to adult angiogenesis, there seems to be an overlap between angiogenesis and vasculogenesis [5–9]. The concept of collateral artery growth had been modified by the introduction of the term arteriogenesis, which describes the growth of arterioles into mature arteries [10]. In other words, arteriogenesis describes the remodeling of preexisting arterio-arteriolar anastomoses to completely developed and functional arteries [11]. Stimulation of collateral artery growth provides a potential alternative option for the treatment of patients suffering from occlusive arterial disease. By definition, arteriogenesis represents one aspect of angiogenesis. Thus, the significance of a separate definition for arteriogenesis has been questioned [12]. The merit in dealing with the two processes separately lies in the observation that the physiological signals that induce angiogenesis or arteriogenesis seem to be distinctly separate [11]. The lymphatic vasculature forms a vessel network that drains interstitial fluid from tissues and returns it to the blood. Lymphatic vessels are an essential part of the body's immune defense. Lymphangiogenesis refers to the formation of lymphatic vessels from preexisting lymphatic vessels, in a method believed to be similar to blood vessel development or angiogenesis. Lymphangiogenesis is of outstanding significance in lymphatic-associated disorders such as wound healing, lymphedema, and tumor metastasis. Lymphatic endothelial cells and extracellular matrix microenvironment represent primary players in lymphangiogenesis [13]. Although all of the above-mentioned mechanisms of vascularization have sufficient contrasting features to warrant consideration under separate cover, one common feature shared by all is their sensitivity to the VEGF signaling pathway.

The VEGF family

Members of the VEGF family are central regulators of vascularization and are classified as cystine knot growth factors. Proteins of the VEGF family specifically bind cellular receptor tyrosine kinases VEGFR1, VEGFR2, and VEGFR3 with high but variable affinity and selectivity. The VEGF family has been recently expanded and currently comprises seven members: VEGF-A, VEGF-B, placenta growth factor (PlGF), VEGF-C, VEGF-D, viral VEGF (also known as VEGF-E), and snake venom VEGF (also known as VEGF-F). Although all members are structurally homologous, there is molecular diversity among the subtypes, and several isoforms, such as VEGF-A, VEGF-B, and PlGF, are generated by alternative exon splicing. These splicing isoforms exhibit differing properties, particularly in binding to coreceptor neuropilins and heparin. Splice variants of VEGF-A include VEGF121, VEGF165, and VEGF189. VEGF121 and VEGF165 promote the proliferation of endothelial cells and angiogenesis. VEGF165 contains the 44 additional amino acids encoded by exon 7 of the VEGF gene. These amino acids confer upon VEGF165 a heparin binding capability, which VEGF121 lacks. VEGF165 binds to all three VEGFR, whereas VEGF121 selectively binds to VEGFR2.

VEGF is a disulfide-bonded dimeric glycoprotein. Initially identified as human vascular permeability factor, VEGF was recognized as a disulfide-linked dimeric 40-kDa protein that promoted dermal blood vessel leakage [14]. It is now known that the two heparin-binding sites of the VEGF165 dimer interact simultaneously with highly sulfated S-domain regions of the heparan sulfate chain that can be linked through a stretch of transition sequence [15]. VEGF family members bear three loops produced via three intramolecular disulfide bonds, and cooperation between loop-1 and loop-3 is necessary for the specific binding and activation of VEGFR2 for angiogenesis.

VEGF-A, the prototype VEGF ligand, binds and activates two tyrosine kinase receptors: VEGFR1 (Flt-1) and VEGFR2 (KDR/Flk-1). VEGFR1, which occurs in transmembrane and soluble forms, negatively regulates vasculogenesis and angiogenesis during early embryogenesis, but it also acts as a positive regulator of angiogenesis and inflammatory responses, playing a role in several human diseases such as rheumatoid arthritis and cancer. The soluble VEGFR1 is overexpressed in the placenta of preeclampsia patients. VEGFR2 has a typical tyrosine kinase receptor structure with seven immunoglobulin-like domains in the extracellular region, as well as a long kinase insert in the tyrosine kinase domain (Fig. 1). It utilizes a unique signaling system for DNA synthesis in vascular endothelial cells, i.e., a phospholipase C γ–protein kinase C–Raf–MAP kinase pathway. Although VEGF-A binds two receptors, VEGFR1 and VEGFR2, a newly isolated ligand VEGF-E (Orf-virus-derived VEGF) binds and activates only VEGFR2. VEGFR2 has critical functions in physiological and pathological angiogenesis

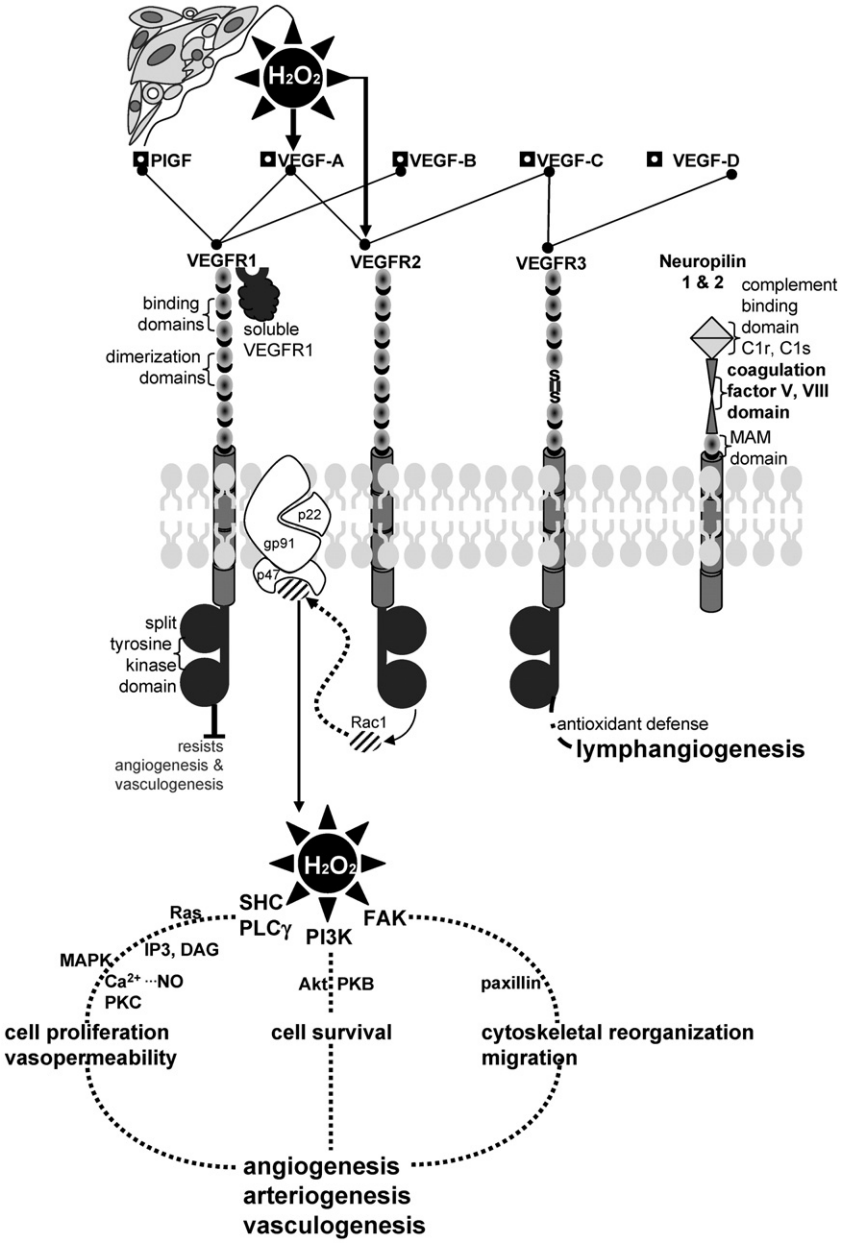

Fig. 1. Hydrogen peroxide: an endogenous vascular growth factor? NADPH oxidases in cells resident in the tissue microenvironment, as well as in recruited inflammatory cells, generate superoxides, which rapidly dismutate to H_2O_2. Extracellular H_2O_2 induces the transcription and release of VEGF-A. In addition, extracellular H_2O_2 induces VEGFR2 expression. Human VEGF family members and their receptors are depicted. Ligation of VEGF-A to VEGFR2 results in activation and translocation of the small GTPase Rac1 into the plasma membrane. Rac1 stimulates gp91phox-containing NAD(P)H oxidase in endothelial cells and thus NADPH oxidase-dependent H_2O_2 generation. Intracellular H_2O_2 serves as a messenger to execute angiogenic VEGF-A signaling. Decomposition of H_2O_2 stalls VEGFR2-dependent angiogenic signaling. Thus, extracellular H_2O_2 induces the angiogenic form of VEGF. Intracellular H_2O_2 is required for the successful propagation of angiogenic signal in response to VEGF–VEGFR2 binding. PlGF, placental growth factor; VEGF, vascular endothelial growth factor; VEGFR1–3, VEGF receptors 1–3, respectively; MAM, membrane proximal meprin-A5-mu domain; SHC, Src homology 2 domain containing; PLC, phospholipase C; PI3K, phosphatidylinositol 3-kinase; FAK, focal adhesion kinase; IP3, inositol 3-phosphate; DAG, diacylglycerol; PKB, protein kinase B; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; gp91, gp91phox; p22, p22phox.

through distinct signal transduction pathways regulating proliferation and migration of endothelial cells. Ligation of VEGF to VEGFR2 enhances SH-PTP1 activity and eNOS expression, which in turn leads to two diverse events. First, SH-PTP1 dephosphorylates VEGFR2 and ERK/MAPK, which weakens VEGF mitogenic activity. Second, eNOS increases nitric oxide production, which in turn lowers SH-PTP1 activity via S-nitrosylation [16]. VEGFR3, a receptor for the lymphatic growth factors VEGF-C and VEGF-D, but not for VEGF-A, regulates vascular and lymphatic endothelial cell function during embryogenesis. VEGFR3 and its ligands VEGF-C and VEGF-D play a role in cell-to-cell signaling in adult blood vessel angiogenesis. The expression of VEGFR3 in VEGF-A-induced iris neovascularization and in preexisting blood vessels

exposed to VEGF-A suggests that this receptor and possibly its ligands are recruited in VEGF-A-driven angiogenesis [17].

The VEGF signaling pathway is of central importance in vascular development including vasculogenesis, arteriogenesis, and lymphangiogenesis as well as both physiological and pathophysiological angiogenesis. Adult vasculogenesis occurs through *in situ* recruitment, proliferation, and tubulization of circulating bone marrow-derived cells [18]. Employing two distinct murine bone marrow transplantation models it has been demonstrated that VEGF supports vasculogenesis by functioning downstream of its effect to enhance either mobilization or survival of circulating endothelial progenitor cells (EPC). VEGFR1 (Flt1) and VEGFR2 (Flk1) are expressed on culture-expanded human EPC and are involved in VEGF-induced EPC migration. In addition, PlGF-VEGFR1 signaling supports tumor vasculogenesis [8]. Independent studies note that VEGF-A165 supports vasculogenesis by recruiting bone marrow-derived $CD34^+$stem cells [19]. The VEGF signaling pathway supports arteriogenesis and therefore the remodeling of ischemic tissue at the capillary and arteriolar levels [20]. VEGFR tyrosine kinase activity is essential for collateral arteriogenesis [21]. eNOS function is a critical prerequisite enabling VEGF-dependent arteriogenesis [22]. VEGF-induced increased expression of the telomerase reverse transcriptase and telomerase activity in skeletal muscles and satellite and endothelial cells are also involved in this process [23]. Lymphangiogenesis plays an important role in several normal and pathological conditions, such as wound healing, pathogen infection, inflammation, and the metastatic formation of endothelial malignancies. VEGFR3 is essential for embryonic cardiovascular development. Thereafter VEGFR3 becomes confined to the lymphatic endothelium in adult tissues. Growth of lymphatic vessels or lymphangiogenesis is induced by secreted proteins VEGF-C and VEGF-D that induce growth of lymphatic vessels via activation of VEGFR3 (Flt-4) localized on the surface of lymphatic endothelial cells [24–27]. Whereas lymphatic endothelial cells migrate and organize unidirectionally, in the direction of interstitial fluid flow, they do not sprout into the region but rather migrate as single cells that later join together into vessels. Furthermore, in a modified "shunted flow" version of the model, infiltrated lymphatic endothelial cells fail to organize into functional vessels, indicating that interstitial fluid flow is necessary for successful functional lymphangiogenesis [28]. The VEGF-C/VEGFR3 (Flt-4) axis, through upregulation of contactin-1, stimulates lymphangiogenesis [29]. Loss-of-function variants of VEGFR3 have been identified in lymphedema. VEGFR3 protects against oxidative damage in endothelial cells. Patients with hereditary lymphedema are susceptible to reactive oxygen species (ROS)-induced cell damage [30]. Formation of tumor lymphatics may be stimulated by tumor-produced VEGF-C, allowing increased spread of tumor metastases through the lymphatics [31]. VEGF-C enhances angiogenesis and lymphangiogenesis in the wound and significantly accelerates wound healing. VEGF-C also recruits inflammatory cells, some of which express VEGFR3. On the other hand, when the function of endogenous VEGF-C/VEGF-D is blocked, wound closure is delayed [32]. VEGF-E, a VEGFR2-selective ligand, stimulates NO release and tube formation in human endothelial cells by a cGMP-independent pathway. Inhibition of phospholipase C γ with U73122 abrogates VEGF-E-induced endothelial cell migration, tube formation, and NO release. In addition, inhibition of eNOS blocks VEGF-E-induced NO release and angiogenesis [33].

Adult tissue vascularization in response to wound

Wound repair can be divided into a series of overlapping phases including formation of fibrin clot, inflammatory response, granulation tissue formation incorporating reepithelialization and angiogenesis, and finally, matrix formation and remodeling. Among many known growth factors, VEGF is believed to be the most prevalent, efficacious, and long-term signal that is known to stimulate angiogenesis in wounds [34]. Although hypoxia is one of the most extensively studied inducers of VEGF production, paradoxically it impairs wound angiogenesis [35,36]. Because the core of most wounds is almost always hypoxic [37,38], it is likely that other factors that positively regulate VEGF expression and release might concomitantly appear at the wound site [39]. Plasmin-catalyzed cleavage of VEGF-A isoform VEGF165 results in the loss of its carboxyl-terminal heparin-binding domain and significant loss in its bioactivity. Plasmin activity is increased at the wound site and compromises angiogenesis *in vivo*. Inactivation of the plasmin cleavage site Arg110/Ala111 has the potential to preserve the biological function of VEGF165 in therapeutic angiogenesis under conditions under which proteases are highly active, such as in wound repair and inflammation [40].

Mild wound ischemia triggers arteriogenesis. In a model of hind-limb ischemia caused by femoral artery dissection, hind-limb perfusion, measured by laser Doppler imaging, was noted to be higher in mdx mice than in wild-type mice. Increased arteriole length density in mdx mice explained the observed difference in perfusion. The enhanced arteriogenesis response was not limited to the ischemic skeletal muscle. In a wound-healing assay, mdx mice showed an accelerated wound closure rate compared with wild-type mice [41]. The evidence in support of the significance of vasculogenesis in adult tissue repair is mounting. However, compared to angiogenesis, the relative contribution of this mechanism to wound vascularization remains limited [42]. Transient lymphangiogenesis occurs in parallel with angiogenesis in healing wounds. VEGFR3 becomes upregulated in blood vessel endothelium in chronic inflammatory wounds. In healing incisional and punch biopsy wounds VEGFR3-positive vessels have been noted in the granulation tissue from the late inflammatory phase (day 5). These vessels are distinct from typical blood vessels and fewer in number. They sprouted from preexisting VEGFR3-positive lymphatic vessels at the wound edge. Unlike the blood vessels, very few VEGFR3-positive lymphatic vessels persist on day 9 and none on day 14 after wounding. In chronic wounds such as ulcers and decubitus wounds of the lower extremity of humans, VEGFR3 is also weakly expressed in the vascular endothelium [43].

Adult tissue vascularization in response to exercise

Physical exercise triggers expansion of the capillary network or angiogenesis [44–47]. This process is known to be VEGF dependent [48–51] and involves NO, perhaps upstream of VEGF [44,52–54]. eNOS is necessary for maintaining a suitable physical capacity. Under conditions of attenuated eNOS function, even moderate exercise poses the risk of worsening energy metabolism specifically in oxidative skeletal muscle [55]. PlGF does not seem to be necessary for exercise-induced angiogenesis [56]. In humans, intense intermittent endurance training induces capillary growth and a transient proliferation of endothelial cells within 4 weeks, with a similar growth occurring around type I versus type II muscle fibers [57]. Exercise training augments the number of circulating endothelial progenitor cells in patients with cardiovascular risk factors and coronary artery disease and is associated with improved vascular function and NO synthesis [58]. Even a single acute bout of exercise can increase endothelial progenitor cells and circulating angiogenic cells [59].

In its role as an endothelial cell proliferation and migration factor, VEGF is thought to affect peripheral circulation and therefore impact maximal oxygen consumption ($\dot{V}O_{2max}$). VEGF gene polymorphisms seem to directly influence aerobic capacity in humans. The effects of the VEGF–2578/ –1154/ –634 promoter region haplotype on VEGF gene expression in human myoblasts have been investigated. AAG and CGC haplotypes resulted in significantly higher inducible VEGF gene expression than the AGG and CGG haplotypes. Consistent with these results, it has been noted that individuals with at least one copy of the AAG or CGC haplotype have higher $\dot{V}O_{2max}$ before and after aerobic exercise training than do subjects with only the AGG and/or CGG haplotype [60]. In humans, skeletal muscle capillarization and the VEGF mRNA response to acute exercise are lower in aged adults compared with young adults. In mice, aging does not impair the potential for nonpathological angiogenesis. Acute exercise induces VEGF mRNA in the soleus, plantaris, and gastrocnemius muscles, which differ considerably in fiber type percentage [61]. Submaximal exercise, with and without reduced leg blood flow, has been studied to test whether ischemia-induced metabolic stress is an important physiological stimulus responsible for upregulating the VEGF-A system in humans. This study, addressing both VEGF-A ligand and receptors, implicated metabolic perturbation as a regulator of human muscle angiogenesis and elegantly demonstrated that VEGF-A splice variants are distinctly regulated. All three receptor genes studied were noted to exhibit different pretranslational regulation in response to exercise in humans [49]. Endurance training induces angiogenesis in a subpopulation of type IIb + IId/x fibers before switching to type IIa fibers [62]. VEGF is an essential survival factor for muscle capillarity. Insufficient VEGF-dependent signaling leads to apoptosis in mouse skeletal muscle [63].

Diabetes causes vasculopathy of microvessels. Diabetic skeletal muscle is characterized by compromised ability for angiogenesis. In the skeletal muscle of diabetics, levels of VEGF-A, VEGF-B, neuropilin-1, VEGFR1, and VEGFR2 are reduced and the levels of antiangiogenic thrombospondin-1 and retinoblastoma-like-2 are increased. A favorable effect of exercise training to partially offset such handicap has been recently reported [64]. Exercise training has been also found to be effective in offsetting age-induced compromise in angiogenic function [65]. Preischemic exercise training stimulates angiogenesis and reduces brain injury in stroke [66].

Inflammation and adult tissue vascularization

Both wounds and physical exercise are often associated with inflammation [67–71]. Inflammatory cells, namely monocytes/macrophages, T lymphocytes, and neutrophils, represent an integral component of the angiogenic process. They release cytokines, which in turn regulate endothelial cell proliferation, migration, and activation [72,73]. Mast cells are granulated secretory cells that have long been recognized as a rich source of biologically active mediators such as biogenic amines, prostaglandins, leukotrienes, proteases, cytokines, and chemokines. Most of their biological functions remain elusive. Emergent findings ascribe mast cells with the function of regulating angiogenesis [74]. The inflammation microenvironment regulates many aspects of tissue vascularization by providing diverse mediators implicated in maintaining tissue homeostasis, e.g., soluble growth and survival factors, matrix remodeling enzymes, and other bioactive molecules. Angiogenic mediators form a complex interactive network that regulates the perpetuation of angiogenesis. Often, proangiogenic agents also exhibit proinflammatory properties [75,76]. Leukocytes modulate inflammation-associated angiogenesis. Endothelial cells are involved in leukocyte extravasation underlying inflammation. A number of adhesion molecules play a role in leukocyte –endothelial interactions. Leukocyte extravasation and activation are followed by the expression and release of leukocyte-derived factors that modulate neovascularization. The intricate interplay between the endothelium and the immune cells has been well recognized in the context of immune responses. However, the fact that this interrelation extends well beyond immune regulation has been understood only recently. The observation that TNF-α activates endothelial cells and induces angiogenesis directly links inflammation and angiogenesis [77]. Various diseases that involve chronic inflammation, such as asthma, psoriasis, rheumatoid arthritis, and bowel disease, have been associated with vascular development [78,79]. Arteriogenesis is induced after the occlusion of a major artery, which induces hemodynamic and mechanical effects on the collateral vessel wall, which occur with increasing blood flow velocity due to the low pressure at the reentrant site of the collateral vessel. Inflammatory cytokines act by stimulating endothelial and smooth muscle cell proliferation and migration. Akin to angiogenesis, the recruitment and activation of monocytes is central to arteriogenesis [80]. Products of inflammation support vasculogenesis by the recruitment of EPC and stem cells. VEGF, nitric oxide, PDGF, TGF-β3, and integrins are some examples that facilitate such recruitment [81–84].

The lymphatic system plays an essential physiological role in homeostasis, interstitial fluid composition, and immunity, whereas impaired lymphatic function has been implicated in a number of pathological conditions, including arthritis and delayed wound healing [85]. The lymphatic vascular system is necessary for the return of extravasated interstitial fluid and macromolecules to the blood circulation, for immune defense, and for the uptake of dietary fats [86]. Edema occurs in inflammatory diseases when the rate of plasma leakage from blood vessels exceeds the drainage through lymphatic vessels and other routes [87]. Inflammation and accompanying fluid overload are cardinal factors in wound healing, lymphedema, the pathogenesis of some forms of lymphangiomatosis, and lymphangiogenesis. The panel of proinflammatory and anti-inflammatory molecules that orchestrate the inflammatory response abounds with cytokines and chemokines that foster survival, migration, and proliferation of lymphatic endothelial cells. Increased angiogenesis as well as lymphangiogenesis is noted in inflammatory versus noninflammatory breast cancer [88]. In inflamed lymph nodes, B-cell-driven lymphangiogenesis enhances dendritic cell mobilization [89]. Taken together, it is generally recognized that inflammation supports adult tissue vascularization.

Inflammation-derived oxidants support and antioxidants resist adult tissue vascularization

In addition to being rich in cytokines/growth factors, the inflammation site is very rich in oxidants. Among all biological fluids, the highest level of hydrogen peroxide is found in the wound fluid. Using the Hunt–Schilling cylinder approach to harvest fluid from the site of inflammation, the presence of 0.1–0.3 mM hydrogen peroxide has been reported in mice with highly efficient healing mechanisms [90]. NADPH oxidases, especially those found in phagocytic cells, contribute to the burst of oxidant production at the site of inflammation [68,91]. In the endothelial cell, the major source of reactive oxidants is an NADPH oxidase that consists of Nox1, Nox2 (gp91phox), Nox4, p22phox, p47phox, p67phox, and the small G protein Rac1. The specific mechanisms by which NADPH oxidase subunits may contribute to angiogenesis have been recently reviewed [92].

Over a decade ago, it was proposed that in biological systems oxidants are not necessarily always the triggers for oxidative damage and that oxidants such as hydrogen peroxide could actually serve as signaling messengers and drive several aspects of cellular signaling [93]. Today, that concept is much more developed and mature. Evidence supporting the role of oxidants such as hydrogen peroxide as signaling messenger is compelling [4,68,94–102]. Current findings indicate that the oxidant factor in inflammation plays a central role in supporting tissue vascularization [103]. Decomposition of endogenous hydrogen peroxide at the wound site by adenoviral catalase gene transfer impairs wound tissue vascularization [90]. Consistently, impairment in healing responses is noted in NADPH oxidase-deficient mice and humans [68,90,104]. Direct evidence identifying macrophage-derived oxidants as angiogenic factors has been noted [105–107]. Physical exercise is commonly associated with inflammation and increased oxidant production by the muscles [70,108–110]. Whether oxidants produced during physical exercise contribute to training-induced tissue vascularization remains to be investigated.

Evidence demonstrating the ability of antioxidants to oppose vascularization suggests a proangiogenic role for oxidants. Antioxidants stall physiological angiogenesis *in vivo* [111,112]. GSH has been observed to resist tumor angiogenesis [113]. Among thiol antioxidants, pyrrolidine dithiocarbamate inhibits inducible NF-κB, a mediator of inflammation, and arrests myocardial angiogenesis [114]. NF-κB activation is known to be sensitive to a wide range of inducers, including hydrogen peroxide [93,115]. *N*-Acetyl-L-cysteine, an analogue and precursor of GSH, also inhibits angiogenesis by suppressing inducible VEGF gene expression. The thiol antioxidant thiram-tetramethylthiuram disulfide, a chelator of heavy metals, possesses antiangiogenic properties [116]. An antiangiogenic action of the dietary antioxidant Se, especially methylated Se metabolites, has been recognized as well [117]. Dietary antioxidant flavonoids and polyphenols are known to have potent antiangiogenic functions [118–121]. Inducible VEGF release is prevented by flavonoid and phenolic antioxidants [122]. Edible berries, rich in antioxidants, are potently antiangiogenic both *in vitro* and *in vivo* [123,124]. Quercetin, one of the most abundant flavonoids in edible berries and in the human diet, is a known antioxidant and inhibitor of angiogenesis [105]. The presence of different numbers of phenolic moieties on the B-ring of the flavonols seems to contribute to their antioxidant activity as well as to their potency for resisting angiogenesis [125]. Tea polyphenols are antiangiogenic as well [126,127]. Resveratrol, a polyphenolic compound found in grapes and other fruits, inhibits angiogenesis [128,129]. The inhibition of VEGF-induced angiogenesis by resveratrol was mediated by disruption of ROS-dependent Src kinase activation and the subsequent vascular endothelial cadherin tyrosine phosphorylation [128]. Polyphenol curcumin (diferuloylmethane, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), obtained from the spice turmeric, exhibits antiangiogenic activity [130,131]. The soy flavonoid genistein, 4′,5,7-trihydroxyisoflavone, is potently antiangiogenic [132,133]. Found in fruits, vegetables, and whole grains commonly consumed by humans, phytoestrogens are antioxidants that include isoflavones, coumestans, and lignans. Antiangiogenic functions of phytoestrogens have been reported [134]. Known for its ability to prevent the oxidative modification of LDL, probucol is antiangiogenic [135]. Long-term α-tocopherol supplementation is associated with lower serum vascular endothelial growth factor levels [136]. Vitamin C has also been identified as an angiostatic factor [137]. Inducible VEGF and VEGFR2 expression in apoE$^{-/-}$ vasculature is downregulated by vitamins C and E, at least partially through their antioxidant properties [138]. Taken together, a large number of structurally unrelated antioxidants demonstrate angiostatic function, suggesting a proangiogenic role for endogenous oxidants.

Induction of VEGF family members by oxidants

In biological systems proteins are at risk of oxidative modification and inactivation [139–141]. However, VEGF is protected from oxidative damage by the extracellular chaperone glypican-1 expressed in the vascular system. Glypican-1 can restore the receptor binding ability of VEGF165 that has been damaged by oxidation [142]. Glypican-1 is a member of a family of glycosylphosphatidylinositol-anchored cell surface heparan sulfate proteoglycans implicated in the control of cellular growth and differentiation. Among the glypicans, glypican-1 is the only member that is expressed in the vascular system. Glypican-1 interacts with VEGF165 but not with VEGF121. The binding of glypican-1 to VEGF165 is mediated by the heparan sulfate chains of glypican-1, as heparinase treatment abolishes this interaction. Only an excess of heparin or heparan sulfates, and not other types of glycosaminoglycans, inhibited this interaction. Glypican-1 potentiates the binding of VEGF165 to a soluble extracellular domain of VEGFR2 [142]. It seems that nature has a way of defending VEGF against oxidants because oxidants are required for tissue vascularization [90,92,143]. Recently it has been identified that ROS support electrical field-induced angiogenesis of embryonic stem cells [144]. Today, numerous lines of evidence point toward the conclusion that oxidants support VEGF-dependent tissue function. This section focuses on the induction of members of the VEGF family by oxidants.

At micromolar concentrations, H_2O_2 induces VEGF-A (VEGF165 and VEGF121) expression [145]. Under conditions of coexistence characteristic of any inflammatory site, the effects of TNF-α and H_2O_2 on VEGF induction were additive. Using deletion mutant constructs of a 2.6-kb VEGF promoter fragment (bp −2361 to +298, relative to the transcription start site) ligated to a luciferase reporter gene it has been established that the sequence from bp −194 to −50 of the VEGF promoter is responsible for the H_2O_2 response. The 144-bp VEGF element (bp −194 to −51) that conferred H_2O_2-mediated transcriptional induction is GC rich and contains four closely spaced GC boxes (bp −94 to −51) that have been identified to have Sp1-binding function. Studies with Sp1 luciferase reporter constructs have identified that H_2O_2-induced VEGF expression is Sp1 dependent. These studies have also proven that H_2O_2-induced VEGF expression is HIF-independent [145]. The above-mentioned findings have been verified and extended in subsequent studies. Signaling studies identified a cascade comprising Ras –Raf –MEK1 –ERK1/2 as the main pathway mediating H_2O_2-induced VEGF-A transcription [146]. In skeletal myotubes, oxidants seem to induce VEGF release via a PI3K/Akt-dependent pathway [147]. Angiotensin II stimulation of VEGF mRNA translation requires production of ROS [148]. Furthermore, angiopoietin 1-induced H_2O_2 plays an important role in Ang1-induced angiogenesis by modulating p44/42 MAPK activity [149]. Several lines of evidence consistently support that mild oxidizing conditions favor VEGF release. Partial cellular glutathione deficiency results in increased VEGF-A release [150]. H_2O_2 modulates vascular permeability via upregulation of VEGF expression [151].

Evidence supporting the synergism of oxidative stressors in inducing VEGF expression is also present [152]. Studies aiming at characterizing the chemical nature of oxidants capable of inducing VEGF expression provide evidence that hydrogen peroxide, not hydroxyl radicals, triggers induction of the growth factor. These studies demonstrated that inducible VEGF expression is sensitive to copper and that the angiogenic potential of copper may be harnessed to accelerate dermal wound contraction and closure. Copper shared some of the pathways utilized by hypoxia to regulate VEGF expression [153]. In addition to inducing VEGF transcription, oxidants also enhance VEGF release by increasing VEGF mRNA stability [154]. Whereas oxidant-induced VEGF expression is helpful in the case of dermal wound healing [90], the situation can be different in other disease settings such as H_2O_2 inhalation [151]. Excessive reactive oxygen species pose a threat to angiogenesis. Thus, reactive oxygen species have been noted to have biphasic effects on angiogenesis, which indicate that pharmacologically regulating cellular oxidant levels might serve as an antiangiogenic or angiogenic tool [155].

Recent studies support the previous observation identifying H_2O_2 as a VEGF-inducing signal and show that H_2O_2 also induces expression of VEGFR2 by an NF-κB-dependent pathway. VEGFR1 was not H_2O_2-sensitive [156]. Oxidized low-density lipoprotein, however, downregulates VEGFR1, minimizing VEGF entrapment by this receptor and improving the availability of VEGF to support angiogenesis [157]. In sum, these findings support that H_2O_2 favors a vascularization response. Mitochondria have been identified as a proximal target specific to H_2O_2-induced signaling and VEGFR2 transactivation [158].

Oxidants as central messengers in VEGF signaling

The involvement of oxidants in the VEGF signaling pathway is not limited to induction of VEGF. After VEGF binds to its specific receptors, especially VEGFR2, oxidants seem to be required for the signaling leading to the angiogenic response of VEGF [92,159–161]. ROS are involved in the mitogenic cascade initiated by the tyrosine kinase receptors of several growth factor peptides, including VEGF. Insulin induces VEGF expression through H_2O_2 production [162]. Evidence supporting the involvement of ROS in vanadate-and hyperoxia-induced expression of VEGF is also reported [163,164]. Although it has been known for a long time that cytokines induce superoxide generation by endothelial cells [165], the physiological significance of such oxidant production remains to be appreciated in full [3]. Early evidence indicating that the binding of VEGF to VEGFR in endothelial cells leads to NADPH oxidase-induced oxidant production led to questions about the significance of such oxidants in VEGF signaling [166]. It was soon recognized that VEGF-induced oxidant production was required to activate NF-κB, which in turn was required for vascular smooth muscle cell migration, an integral component of angiogenesis [167]. In porcine aortic endothelial cells stably expressing human VEGFR2, receptor activation by VEGF is followed by a rapid rise in intracellular

hydrogen peroxide. Genetic and pharmacological studies suggest that such oxidant burst requires, as upstream events, the activation of phosphatidylinositol 3-kinase and the small GTPase Rac-1 and is likely initiated by lipoxygenases. Inhibition of VEGFR2-dependent generation of ROS attenuates early signaling events including receptor autophosphorylation and binding to a phospholipase Cγ–glutathione *S*-transferase fusion protein. Moreover, catalase, the lipoxygenase inhibitor nordihydroguaiaretic acid, the synthetic ROS scavenger EUK-134, and phosphatidylinositol 3-kinase inhibitor wortmannin all diminish ERK phosphorylation in response to VEGF. Finally, cell culture and stimulation in a nearly anoxic environment mimics the effects of ROS scavengers on receptor and ERK phosphorylation, reinforcing the idea that oxidants are necessary components of the mitogenic signaling cascade initiated by VEGFR2 [168]. EPR evidence also supports that VEGF stimulates superoxide production, which is inhibited by the NAD(P)H oxidase inhibitor diphenylene iodonium, as well as by overexpression of dominant-negative Rac1 (N17Rac1) and transfection of gp91phox antisense oligonucleotides in human umbilical vein endothelial cells [169]. Antioxidants, including *N*-acetylcysteine, various NAD(P)H oxidase inhibitors, and N17Rac1, significantly attenuate not only VEGF-induced VEGFR2 tyrosine phosphorylation but also proliferation and migration of endothelial cells. Importantly, these effects of VEGF are clearly inhibited in cells transfected with gp91phox antisense oligonucleotides. In contrast, ROS are not involved in mediating these effects of sphingosine 1-phosphate on endothelial cells [169]. Thus, VEGF-induced endothelial cell signaling and angiogenesis are tightly controlled by the redox microenvironment of the VEGF receptor. Also, NAD(P)H oxidase emerged as a potential therapeutic target for angiogenesis-dependent diseases [169]. IQGAP1 is a scaffolding protein that regulates endothelial cell motility and morphogenesis by interacting directly with cytoskeletal, cell adhesion, and small G proteins, including Rac1. IQGAP1 functions as a VEGFR2-associated scaffold protein to organize ROS-dependent VEGF signaling, thereby promoting endothelial cell migration and proliferation, key components of angiogenesis [170]. The family of proteins involved in redox signaling in response to VEGF–VEGR2 ligation is rapidly expanding. ARF6 is a small GTPase protein involved in membrane trafficking and cell motility. Recently it has been demonstrated that ARF6 is involved in the temporal–spatial organization of caveolae/lipid rafts-and ROS-dependent VEGF signaling in endothelial cells as well as in angiogenesis *in vivo* [171]. It is now established that gp91phox-derived ROS play an important role in mediating VEGF-dependent neovascularization *in vivo* [172].

Hypoxia/reoxygenation-induced changes in endothelial permeability result from coordinated actions of the Rho GTPases Rac1 and RhoA. Rac1 and RhoA rapidly respond to changes in oxygen tension, and their activity depends on NADPH oxidase-and PI3 kinase-dependent production of ROS. Rac1 acts upstream of RhoA, and its transient inhibition by acute hypoxia leads to activation of RhoA followed by stress fiber formation, dispersion of adherens junctions, and increased endothelial permeability. Reoxygenation strongly activates Rac1 and restores cortical localization of F-actin and VE-cadherin. This effect is a result of Rac1-mediated inhibition of RhoA and can be prevented by activators of RhoA, L63RhoA, and lysophosphatidic acid. Cdc42 activation follows the RhoA pattern of activation but has no effect on actin remodeling, junctional integrity, or endothelial permeability. Thus, Rho GTPases act as mediators coupling the cellular redox state to endothelial function [173]. Some of the cytoprotective functions of VEGF are dependent on its ability to induce the mitochondrial antioxidant MnSOD. Receptor ligation of VEGF is uniquely coupled to MnSOD expression through growth factor-specific ROS-sensitive positive (protein kinase C–NF-κB) and negative (PI3K–Akt–forkhead) signaling pathways [174]. At low concentrations, intraendothelial H_2O_2 stimulates proliferation and enhances survival. Also, low concentrations of H_2O_2 stimulate endothelial migration as well as tube formation in an *in vitro* model of angiogenesis. Although low concentrations of H_2O_2 have been shown to be involved in numerous signal transduction pathways and to independently stimulate mitogenesis, there has been little information presented on precisely how mammalian cells respond biochemically to these low concentrations of H_2O_2. Functional proteomic approaches have been employed to identify proteins responsive to low concentrations of H_2O_2 in human endothelial cells [3].

PlGF acts in synergism with VEGF and eNOS to induce neovascularization [175]. This conclusion was drawn from a study looking at the phenotype of PlGF $^{-/-}$, eNOS$^{-/-}$, PlGF$^{-/-}$ eNOS$^{-/-}$, and wild-type C57BL/6J mice in response to surgically induced hind-limb ischemia. PlGF$^{-/-}$ eNOS$^{-/-}$ double knockout mice showed the most severe phenotype, including self-amputation and death in up to 47% of the animals studied. In the ischemic legs, capillary density was severely reduced. Macrophage infiltration and oxidative stress were increased compared to the other groups of animals. These changes were associated with an upregulation of both iNOS and VEGF in the ischemic limbs [175]. Whereas it is clear that NADPH oxidase-derived H_2O_2 is a key player in VEGF signaling, little is known about the redox control of PlGF signaling. Early placental development is characterized by rapid cell differentiation and migration, matrix remodeling, and angiogenesis. NADPH oxidase activity has been studied in placental tissues in early pregnancy and at term. In human placentas from normal deliveries at term substantial basal NADPH activity has been detected. The activity was almost threefold higher in early pregnancy [176]. Whether such increase is implicated in tissue vascularization during early placental angiogenesis remains to be determined.

Conclusion

The VEGF pathway is implicated in a wide variety of physiological and pathophysiological processes. Whereas blockade of the VEGF pathway is the goal in treating diseases such as neoplasms [177] and angiogenesis-related ocular dysfunction [178,179], VEGF-dependent angiogenic response is desired during conditions of tissue repair [90,161]. Thus, understanding the specific processes that drive the VEGF-

signaling pathway is of outstanding clinical significance. VEGF was identified as an angiogenic mitogen almost 2 decades ago. A decade later, the mitogenic function of reactive oxygen species was characterized [180]. Work, especially during the past 5 years, has directly connected the two—H_2O_2 and VEGF signaling. That the function of one mitogen, VEGF, largely depends on the signaling driven by another mitogen, H_2O_2, generates a novel paradigm with major therapeutic implications for a variety of angiogenesis-related disorders. The new paradigm provides a theoretical basis for the development and rational use of novel angiogenic and antiangiogenic drugs.

Acknowledgments

This work was supported by NIH RO1 Grants GM069589, GM077185 and HL 073087 to C.K.S. The authors thank Jonathan Holmes, M.D., for proofreading the manuscript.

References

[1] Schafer, F. Q.; Buettner, G. R. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.* **30:**1191–1212; 2001.

[2] Faraci, F. M. Hydrogen peroxide: watery fuel for change in vascular biology. *Arterioscler. Thromb. Vasc. Biol.* **26:**1931–1933; 2006.

[3] Stone, J. R.; Collins, T. The role of hydrogen peroxide in endothelial proliferative responses. *Endothelium* **9:**231–238; 2002.

[4] Stone, J. R.; Yang, S. Hydrogen peroxide: a signaling messenger. *Antioxid. Redox Signaling* **8:**243–270; 2006.

[5] Tateno, K.; Minamino, T.; Miyauchi, H.; Kunieda, T.; Komuro, I. Application of hematopoietic cells to therapeutic angiogenesis. *Curr. Pharm. Des.* **12:**557–563; 2006.

[6] Bao, S.; Wu, Q.; Sathornsumetee, S.; Hao, Y.; Li, Z.; Hjelmeland, A. B.; Shi, Q.; McLendon, R. E.; Bigner, D. D.; Rich, J. N. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res.* **66:**7843–7848; 2006.

[7] Sata, M. Role of circulating vascular progenitors in angiogenesis, vascular healing, and pulmonary hypertension: lessons from animal models. *Arterioscler Thromb. Vasc. Biol.* **26:**1008–1014; 2006.

[8] Li, B.; Sharpe, E. E.; Maupin, A. B.; Teleron, A. A.; Pyle, A. L.; Carmeliet, P.; Young, P. P. VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. *FASEB J.* **20:**1495–1497; 2006.

[9] Rafii, S.; Meeus, S.; Dias, S.; Hattori, K.; Heissig, B.; Shmelkov, S.; Rafii, D.; Lyden, D. Contribution of marrow-derived progenitors to vascular and cardiac regeneration. *Semin. Cell Dev. Biol.* **13:**61–67; 2002.

[10] Zhuang, Z. W.; Gao, L.; Murakami, M.; Pearlman, J. D.; Sackett, T. J.; Simons, M.; de Muinck, E. D. Arteriogenesis: noninvasive quantification with multi-detector row CT angiography and three-dimensional volume rendering in rodents. *Radiology* **240:**698–707; 2006.

[11] Heil, M.; Eitenmuller, I.; Schmitz-Rixen, T.; Schaper, W. Arteriogenesis versus angiogenesis: similarities and differences. *J. Cell. Mol. Med.* **10:** 45–55; 2006.

[12] Chilian, W. M. Editorial comment on Arteriogenesis—Is this terminology necessary? *Basic Res. Cardiol.* **98:**6–7; 2003.

[13] Ji, R. C. Lymphatic endothelial cells, lymphangiogenesis, and extracellular matrix. *Lymphat. Res. Biol.* **4:**83–100; 2006.

[14] Connolly, D. T.; Olander, J. V.; Heuvelman, D.; Nelson, R.; Monsell, R.; Siegel, N.; Haymore, B. L.; Leimgruber, R.; Feder, J. Human vascular permeability factor: isolation from U937 cells. *J. Biol. Chem.* **264:** 20017–20024; 1989.

[15] Robinson, C. J.; Mulloy, B.; Gallagher, J. T.; Stringer, S. E. VEGF165-binding sites within heparan sulfate encompass two highly sulfated domains and can be liberated by K5 lyase. *J. Biol. Chem.* **281:** 1731–1740; 2006.

[16] Cai, J.; Jiang, W. G.; Ahmed, A.; Boulton, M. Vascular endothelial growth factor-induced endothelial cell proliferation is regulated by interaction between VEGFR-2, SH-PTP1 and eNOS. *Microvasc. Res.* **71:**20–31; 2006.

[17] Witmer, A. N.; van Blijswijk, B. C.; Dai, J.; Hofman, P.; Partanen, T. A.; Vrensen, G. F.; Schlingemann, R. O. VEGFR-3 in adult angiogenesis. *J. Pathol.* **195:**490–497; 2001.

[18] Tepper, O. M.; Capla, J. M.; Galiano, R. D.; Ceradini, D. J.; Callaghan, M. J.; Kleinman, M. E.; Gurtner, G. C. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. *Blood* **105:**1068–1077; 2005.

[19] Lee, T. H.; Bolontrade, M. F.; Worth, L. L.; Guan, H.; Ellis, L. M.; Kleinerman, E. S. Production of VEGF165 by Ewing's sarcoma cells induces vasculogenesis and the incorporation of $CD34^+$stem cells into the expanding tumor vasculature. *Int. J. Cancer* **119:**839–846; 2006.

[20] Crottogini, A.; Meckert, P. C.; Vera Janavel, G.; Lascano, E.; Negroni, J.; Del Valle, H.; Dulbecco, E.; Werba, P.; Cuniberti, L.; Martinez, V.; De Lorenzi, A.; Telayna, J.; Mele, A.; Fernandez, J. L.; Marangunich, L.; Criscuolo, M.; Capogrossi, M. C.; Laguens, R. Arteriogenesis induced by intramyocardial vascular endothelial growth factor 165 gene transfer in chronically ischemic pigs. *Hum. Gene Ther.* **14:**1307–1318; 2003.

[21] Lloyd, P. G.; Prior, B. M.; Li, H.; Yang, H. T.; Terjung, R. L. VEGF receptor antagonism blocks arteriogenesis, but only partially inhibits angiogenesis, in skeletal muscle of exercise-trained rats. *Am. J. Physiol. Heart Circ. Physiol.* **288:**H759–H768; 2005.

[22] Yu, J.; deMuinck, E. D.; Zhuang, Z.; Drinane, M.; Kauser, K.; Rubanyi, G. M.; Qian, H. S.; Murata, T.; Escalante, B.; Sessa, W. C. Endothelial nitric oxide synthase is critical for ischemic remodeling, mural cell recruitment, and blood flow reserve. *Proc. Natl. Acad. Sci. U. S. A.* **102:**10999–11004; 2005.

[23] Zaccagnini, G.; Gaetano, C.; Della Pietra, L.; Nanni, S.; Grasselli, A.; Mangoni, A.; Benvenuto, R.; Fabrizi, M.; Truffa, S.; Germani, A.; Moretti, F.; Pontecorvi, A.; Sacchi, A.; Bacchetti, S.; Capogrossi, M. C.; Farsetti, A. Telomerase mediates vascular endothelial growth factor-dependent responsiveness in a rat model of hind limb ischemia. *J. Biol. Chem.* **280:**14790–14798; 2005.

[24] Achen, M. G.; Mann, G. B.; Stacker, S. A. Targeting lymphangiogenesis to prevent tumour metastasis. *Br. J. Cancer* **94:**1355–1360; 2006.

[25] Bando, H.; Weich, H. A.; Horiguchi, S.; Funata, N.; Ogawa, T.; Toi, M. The association between vascular endothelial growth factor-C, its corresponding receptor, VEGFR-3, and prognosis in primary breast cancer: a study with 193 cases. *Oncol. Rep.* **15:**653–659; 2006.

[26] Ding, M. X.; Lin, X. Q.; Fu, X. Y.; Zhang, N.; Li, J. C. Expression of vascular endothelial growth factor-C and angiogenesis in esophageal squamous cell carcinoma. *World J. Gastroenterol.* **12:**4582–4585; 2006.

[27] Yang, J.; Wu, H. F.; Qian, L. X.; Zhang, W.; Hua, L. X.; Yu, M. L.; Wang, Z.; Xu, Y. G.; Sui, X. R. Increased expressions of vascular endothelial growth factor (VEGF), VEGF-C and VEGF receptor-3 in prostate cancer tissue are associated with tumor progression. *Asian J. Androl.* **8:**169–175; 2006.

[28] Rutkowski, J. M.; Boardman, K. C.; Swartz, M. A. Characterization of lymphangiogenesis in a model of adult skin regeneration. *Am. J. Physiol. Heart Circ. Physiol.* **291:**H1402–H1410; 2006.

[29] Su, J. L.; Yang, P. C.; Shih, J. Y.; Yang, C. Y.; Wei, L. H.; Hsieh, C. Y.; Chou, C. H.; Jeng, Y. M.; Wang, M. Y.; Chang, K. J.; Hung, M. C.; Kuo, M. L. The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells. *Cancer Cell* **9:**209–223; 2006.

[30] Wang, J. F.; Zhang, X.; Groopman, J. E. Activation of vascular endothelial growth factor receptor-3 and its downstream signaling promote cell survival under oxidative stress. *J. Biol. Chem.* **279:** 27088–27097; 2004.

[31] Shibuya, M.; Claesson-Welsh, L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp. Cell Res.* **312:**549–560; 2006.

[32] Saaristo, A.; Tammela, T.; Farkkila, A.; Karkkainen, M.; Suominen, E.; Yla-Herttuala, S.; Alitalo, K. Vascular endothelial growth factor-C

accelerates diabetic wound healing. *Am. J. Pathol.* **169:**1080–1087; 2006.
[33] Cudmore, M.; Ahmad, S.; Al-Ani, B.; Hewett, P.; Ahmed, S.; Ahmed, A. VEGF-E activates endothelial nitric oxide synthase to induce angiogenesis via cGMP and PKG-independent pathways. *Biochem. Biophys. Res. Commun.* **345:**1275–1282; 2006.
[34] Nissen, N. N.; Polverini, P. J.; Koch, A. E.; Volin, M. V.; Gamelli, R. L.; DiPietro, L. A. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am. J. Pathol.* **152:**1445–1452; 1998.
[35] Allen, D. B.; Maguire, J. J.; Mahdavian, M.; Wicke, C.; Marcocci, L.; Scheuenstuhl, H.; Chang, M.; Le, A. X.; Hopf, H. W.; Hunt, T. K. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. *Arch. Surg.* **132:**991–996; 1997.
[36] Gordillo, G. M.; Sen, C. K. Revisiting the essential role of oxygen in wound healing. *Am. J. Surg.* **186:**259–263; 2003.
[37] Chang, N.; Goodson, F.; Gottrup, W. H. d.; Hunt, T. K. Direct measurement of wound and tissue oxygen tension in postoperative patients. *Ann. Surg.* **197:**470–478; 1983.
[38] Pai, M. P.; Hunt, T. K. Effect of varying oxygen tensions on healing of open wounds. *Surg. Gynecol. Obstet.* **135:**756–758; 1972.
[39] Howdieshell, T. R.; Riegner, C.; Gupta, V.; Callaway, D.; Grembowicz, K.; Sathyanarayana; McNeil, P. L. Normoxic wound fluid contains high levels of vascular endothelial growth factor. *Ann. Surg.* **228:**707–715; 1998.
[40] Roth, D.; Piekarek, M.; Paulsson, M.; Christ, H.; Krieg, T.; Bloch, W.; Davidson, J. M.; Eming, S. A. Plasmin modulates vascular endothelial growth factor-A-mediated angiogenesis during wound repair. *Am. J. Pathol.* **168:**670–684; 2006.
[41] Straino, S.; Germani, A.; Di Carlo, A.; Porcelli, D.; De Mori, R.; Mangoni, A.; Napolitano, M.; Martelli, F.; Biglioli, P.; Capogrossi, M. C. Enhanced arteriogenesis and wound repair in dystrophin-deficient mdx mice. *Circulation* **110:**3341–3348; 2004.
[42] Zwaginga, J. J.; Doevendans, P. Stem cell-derived angiogenic/vasculogenic cells: possible therapies for tissue repair and tissue engineering. *Clin. Exp. Pharmacol. Physiol.* **30:**900–908; 2003.
[43] Paavonen, K.; Puolakkainen, P.; Jussila, L.; Jahkola, T.; Alitalo, K. Vascular endothelial growth factor receptor-3 in lymphangiogenesis in wound healing. *Am. J. Pathol.* **156:**1499–1504; 2000.
[44] Bloor, C. M. Angiogenesis during exercise and training. *Angiogenesis* **8:**263–271; 2005.
[45] Kojda, G.; Hambrecht, R. Molecular mechanisms of vascular adaptations to exercise: physical activity as an effective antioxidant therapy? *Cardiovasc. Res.* **67:**187–197; 2005.
[46] Prior, B. M.; Yang, H. T.; Terjung, R. L. What makes vessels grow with exercise training? *J. Appl. Physiol.* **97:**1119–1128; 2004.
[47] Prior, B. M.; Lloyd, P. G.; Yang, H. T.; Terjung, R. L. Exercise-induced vascular remodeling. *Exerc. Sport Sci. Rev.* **31:**26–33; 2003.
[48] Gu, J. W.; Shparago, M.; Tan, W.; Bailey, A. P. Tissue endostatin correlates inversely with capillary network in rat heart and skeletal muscles. *Angiogenesis* **9:**93–99; 2006.
[49] Gustafsson, T.; Ameln, H.; Fischer, H.; Sundberg, C. J.; Timmons, J. A.; Jansson, E. VEGF-A splice variants and related receptor expression in human skeletal muscle following submaximal exercise. *J. Appl. Physiol.* **98:**2137–2146; 2005.
[50] Yao, Z.; Lafage-Proust, M. H.; Plouet, J.; Bloomfield, S.; Alexandre, C.; Vico, L. Increase of both angiogenesis and bone mass in response to exercise depends on VEGF. *J. Bone Miner. Res.* **19:**1471–1480; 2004.
[51] Brown, M. D.; Hudlicka, O. Modulation of physiological angiogenesis in skeletal muscle by mechanical forces: involvement of VEGF and metalloproteinases. *Angiogenesis* **6:**1–14; 2003.
[52] Frisbee, J. C.; Balch Samora, J.; Peterson, J.; Bryner, R. Exercise training blunts microvascular rarefaction in the metabolic syndrome. *Am. J. Physiol. Heart Circ. Physiol.* **291:**H2483–H2492; 2006.
[53] Suzuki, J. L-Arginine supplementation causes additional effects on exercise-induced angiogenesis and VEGF expression in the heart and hind-leg muscles of middle-aged rats. *J. Physiol. Sci.* **56:**39–44; 2006.
[54] Suzuki, J. Microvascular angioadaptation after endurance training with L-arginine supplementation in rat heart and hindleg muscles. *Exp. Physiol.* **90:**763–771; 2005.
[55] Momken, I.; Lechene, P.; Ventura-Clapier, R.; Veksler, V. Voluntary physical activity alterations in endothelial nitric oxide synthase knockout mice. *Am. J. Physiol. Heart Circ. Physiol.* **287:**H914–H920; 2004.
[56] Gigante, B.; Tarsitano, M.; Cimini, V.; De Falco, S.; Persico, M. G. Placenta growth factor is not required for exercise-induced angiogenesis. *Angiogenesis* **7:**277–284; 2004.
[57] Jensen, L.; Bangsbo, J.; Hellsten, Y. Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle. *J. Physiol.* **557:**571–582; 2004.
[58] Steiner, S.; Niessner, A.; Ziegler, S.; Richter, B.; Seidinger, D.; Pleiner, J.; Penka, M.; Wolzt, M.; Huber, K.; Wojta, J.; Minar, E.; Kopp, C. W. Endurance training increases the number of endothelial progenitor cells in patients with cardiovascular risk and coronary artery disease. *Atherosclerosis* **181:**305–310; 2005.
[59] Rehman, J.; Li, J.; Parvathaneni, L.; Karlsson, G.; Panchal, V. R.; Temm, C. J.; Mahenthiran, J.; March, K. L. Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. *J. Am. Coll. Cardiol.* **43:**2314–2318; 2004.
[60] Prior, S. J.; Hagberg, J. M.; Paton, C. M.; Douglass, L. W.; Brown, M. D.; McLenithan, J. C.; Roth, S. M. DNA sequence variation in the promoter region of the VEGF gene impacts VEGF gene expression and maximal oxygen consumption. *Am. J. Physiol. Heart Circ. Physiol.* **290:** H1848–H1855; 2006.
[61] Gavin, T. P.; Westerkamp, L. M.; Zwetsloot, K. A. Soleus, plantaris and gastrocnemius VEGF mRNA responses to hypoxia and exercise are preserved in aged compared with young female C57BL/6 mice. *Acta Physiol. (Oxford)* **188:**113–121; 2006.
[62] Waters, R. E.; Rotevatn, S.; Li, P.; Annex, B. H.; Yan, Z. Voluntary running induces fiber type-specific angiogenesis in mouse skeletal muscle. *Am. J. Physiol. Cell Physiol.* **287:**C1342–C1348; 2004.
[63] Tang, K.; Breen, E. C.; Gerber, H. P.; Ferrara, N. M.; Wagner, P. D. Capillary regression in vascular endothelial growth factor-deficient skeletal muscle. *Physiol. Genomics* **18:**63–69; 2004.
[64] Kivela, R.; Silvennoinen, M.; Touvra, A. M.; Lehti, T. M.; Kainulainen, H.; Vihko, V. Effects of experimental type 1 diabetes and exercise training on angiogenic gene expression and capillarization in skeletal muscle. *FASEB J.* **20:**1570–1572; 2006.
[65] Iemitsu, M.; Maeda, S.; Jesmin, S.; Otsuki, T.; Miyauchi, T. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *Am. J. Physiol. Heart Circ. Physiol.* **291:** H1290–H1298; 2006.
[66] Ding, Y. H.; Luan, X. D.; Li, J.; Rafols, J. A.; Guthinkonda, M.; Diaz, F. G.; Ding, Y. Exercise-induced overexpression of angiogenic factors and reduction of ischemia/reperfusion injury in stroke. *Curr. Neurovasc. Res.* **1:**411–420; 2004.
[67] Xue, M.; Le, N. T.; Jackson, C. J. Targeting matrix metalloproteases to improve cutaneous wound healing. *Expert Opin. Ther. Targets* **10:**143–155; 2006.
[68] Sen, C. K. The general case for redox control of wound repair. *Wound Repair Regen.* **11:**431–438; 2003.
[69] Harrison, D. G.; Widder, J.; Grumbach, I.; Chen, W.; Weber, M.; Searles, C. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J. Intern. Med.* **259:**351–363; 2006.
[70] Sen, C. K.; Hanninen, O.; Packer, L., eds. *Handbook of oxidants and antioxidants in exercise*. Amsterdam: Elsevier; 2000:1–1191.
[71] Majno, G. Chronic inflammation: links with angiogenesis and wound healing. *Am. J. Pathol.* **153:**1035–1039; 1998.
[72] Naldini, A.; Carraro, F. Role of inflammatory mediators in angiogenesis. *Curr. Drug Targets Inflammation Allergy* **4:**3–8; 2005.
[73] Moldovan, L.; Moldovan, N. I. Role of monocytes and macrophages in angiogenesis. *EXS*127–146; 2005.
[74] Crivellato, E.; Ribatti, D. Involvement of mast cells in angiogenesis and chronic inflammation. *Curr. Drug Targets Inflammation Allergy* **4:**9–11; 2005.
[75] Zheng, Y.; Murakami, M.; Takahashi, H.; Yamauchi, M.; Kiba, A.; Yamaguchi, S.; Yabana, N.; Alitalo, K.; Shibuya, M. Chimeric VEGF-E

(NZ7)/PlGF promotes angiogenesis via VEGFR-2 without significant enhancement of vascular permeability and inflammation. *Arterioscler. Thromb. Vasc. Biol.* **26:**2019–2026; 2006.

[76] Yamaji-Kegan, K.; Su, Q.; Angelini, D. J.; Champion, H. C.; Johns, R. A. Hypoxia-induced mitogenic factor has pro-angiogenic and pro-inflammatory effects in the lung via VEGF and VEGF receptor-2. *Am. J. Physiol. Lung Cell Mol. Physiol.* **291:**L1159–L1168; 2006.

[77] Rajashekhar, G.; Willuweit, A.; Patterson, C. E.; Sun, P.; Hilbig, A.; Breier, G.; Helisch, A.; Clauss, M. Continuous endothelial cell activation increases angiogenesis: evidence for the direct role of endothelium linking angiogenesis and inflammation. *J. Vasc. Res.* **43:**193–204; 2006.

[78] Pousa, I. D.; Gisbert, J. P.; Mate, J. Vascular development in inflammatory bowel disease. *Gastroenterol. Hepatol.* **29:**414–422; 2006.

[79] Koutroubakis, I. E.; Tsiolakidou, G.; Karmiris, K.; Kouroumalis, E. A. Role of angiogenesis in inflammatory bowel disease. *Inflammatory Bowel Dis.* **12:**515–523; 2006.

[80] Buschmann, I.; Heil, M.; Jost, M.; Schaper, W. Influence of inflammatory cytokines on arteriogenesis. *Microcirculation* **10:**371–379; 2003.

[81] Duda, D. G.; Fukumura, D.; Jain, R. K. Role of eNOS in neovascularization: NO for endothelial progenitor cells. *Trends Mol. Med.* **10:**143–145; 2004.

[82] Murphy, M. O.; Ghosh, J.; Fulford, P.; Khwaja, N.; Halka, A. T.; Carter, A.; Turner, N. J.; Walker, M. G. Expression of growth factors and growth factor receptor in non-healing and healing ischaemic ulceration. *Eur. J. Vasc. Endovasc. Surg.* **31:**516–522; 2006.

[83] Dvorak, H. F. VPF/VEGF and the angiogenic response. *Semin. Perinatol.* **24:**75–78; 2000.

[84] Luscinskas, F. W.; Lawler, J. Integrins as dynamic regulators of vascular function. *FASEB J.* **8:**929–938; 1994.

[85] Alitalo, K.; Tammela, T.; Petrova, T. V. Lymphangiogenesis in development and human disease. *Nature* **438:**946–953; 2005.

[86] Karpanen, T.; Makinen, T. Regulation of lymphangiogenesis and mdash; from cell fate determination to vessel remodeling. *Exp. Cell Res.* **312:**575–583; 2006.

[87] Baluk, P.; Tammela, T.; Ator, E.; Lyubynska, N.; Achen, M. G.; Hicklin, D. J.; Jeltsch, M.; Petrova, T. V.; Pytowski, B.; Stacker, S. A.; Yla-Herttuala, S.; Jackson, D. G.; Alitalo, K.; McDonald, D. M. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. *J. Clin. Invest.* **115:**247–257; 2005.

[88] Van der Auwera, I.; Van Laere, S. J.; Van den Eynden, G. G.; Benoy, I.; van Dam, P.; Colpaert, C. G.; Fox, S. B.; Turley, H.; Harris, A. L.; Van Marck, E. A.; Vermeulen, P. B.; Dirix, L. Y. Increased angiogenesis and lymphangiogenesis in inflammatory versus noninflammatory breast cancer by real-time reverse transcriptase-PCR gene expression quantification. *Clin. Cancer Res.* **10:**7965–7971; 2004.

[89] Angeli, V.; Ginhoux, F.; Llodra, J.; Quemeneur, L.; Frenette, P. S.; Skobe, M.; Jessberger, R.; Merad, M.; Randolph, G. J. B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. *Immunity* **24:**203–215; 2006.

[90] Roy, S.; Khanna, S.; Nallu, K.; Hunt, T. K.; Sen, C. K. Dermal wound healing is subject to redox control. *Mol. Ther.* **13:**211–220; 2006.

[91] Cave, A. C.; Brewer, A. C.; Narayanapanicker, A.; Ray, R.; Grieve, D. J.; Walker, S.; Shah, A. M. NADPH oxidases in cardiovascular health and disease. *Antioxid. Redox Signaling* **8:**691–728; 2006.

[92] Ushio-Fukai, M. Redox signaling in angiogenesis: role of NADPH oxidase. *Cardiovasc. Res.* **71:**226–235; 2006.

[93] Sen, C. K.; Packer, L. Antioxidant and redox regulation of gene transcription. *FASEB J.* **10:**709–720; 1996.

[94] Cheng, Y.; Song, C. Hydrogen peroxide homeostasis and signaling in plant cells. *Sci. China C Life Sci.* **49:**1–11; 2006.

[95] Gechev, T. S.; Hille, J. Hydrogen peroxide as a signal controlling plant programmed cell death. *J. Cell Biol.* **168:**17–20; 2005.

[96] Reth, M. Hydrogen peroxide as second messenger in lymphocyte activation. *Nat. Immunol.* **3:**1129–1134; 2002.

[97] Neill, S.; Desikan, R.; Hancock, J. Hydrogen peroxide signalling. *Curr. Opin. Plant Biol.* **5:**388–395; 2002.

[98] Rhee, S. G.; Bae, Y. S.; Lee, S. R.; Kwon, J. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. *Sci. STKE 2000* PE1; 2000.

[99] Jones, R. D.; Morice, A. H. Hydrogen peroxide and mdash;an intracellular signal in the pulmonary circulation: involvement in hypoxic pulmonary vasoconstriction. *Pharmacol. Ther.* **88:**153–161; 2000.

[100] Rhee, S. G. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp. Mol. Med.* **31:**53–59; 1999.

[101] Sen, C. K. Antioxidant and redox regulation of cellular signaling: introduction. *Med. Sci. Sports Exerc.* **33:**368–370; 2001.

[102] Sen, C. K. Cellular thiols and redox-regulated signal transduction. *Curr. Top. Cell Regul.* **36:**1–30; 2000.

[103] Arbiser, J. L.; Petros, J.; Klafter, R.; Govindajaran, B.; McLaughlin, E. R.; Brown, L. F.; Cohen, C.; Moses, M.; Kilroy, S.; Arnold, R. S.; Lambeth, J. D. Reactive oxygen generated by Nox1 triggers the angiogenic switch. *Proc. Natl. Acad. Sci. U. S. A.* **99:**715–720; 2002.

[104] Sen, C. K.; Khanna, S.; Gordillo, G.; Bagchi, D.; Bagchi, M.; Roy, S. Oxygen, oxidants, and antioxidants in wound healing: an emerging paradigm. *Ann. N.Y. Acad. Sci.* **957:**239–249; 2002.

[105] Jackson, S. J.; Venema, R. C. Quercetin inhibits eNOS, microtubule polymerization, and mitotic progression in bovine aortic endothelial cells. *J. Nutr.* **136:**1178–1184; 2006.

[106] Cho, M.; Hunt, T. K.; Hussain, M. Z. Hydrogen peroxide stimulates macrophage vascular endothelial growth factor release. *Am. J. Physiol. Heart Circ. Physiol.* **280:**H2357–H2363; 2001.

[107] Jackson, I. L.; Batinic-Haberle, I.; Sonveaux, P.; Dewhirst, M. W.; Vujaskovic, Z. ROS production and angiogenic regulation by macrophages in response to heat therapy. *Int. J. Hyperthermia* **22:**263–273; 2006.

[108] Atalay, M.; Lappalainen, J.; Sen, C. K. Dietary antioxidants for the athlete. *Curr. Sports Med. Rep.* **5:**182–186; 2006.

[109] Sen, C. K.; Packer, L. Thiol homeostasis and supplements in physical exercise. *Am. J. Clin. Nutr.* **72:**653S–669S; 2000.

[110] Sen, C. K. Oxidants and antioxidants in exercise. *J. Appl. Physiol.* **79:**675–686; 1995.

[111] Polytarchou, C.; Papadimitriou, E. Antioxidants inhibit angiogenesis in vivo through down-regulation of nitric oxide synthase expression and activity. *Free Radic. Res.* **38:**501–508; 2004.

[112] Monte, M.; Davel, L. E.; de Lustig, E. S. Inhibition of lymphocyte-induced angiogenesis by free radical scavengers. *Free Radic. Biol. Med.* **17:**259–266; 1994.

[113] Schwartz, J. L.; Shklar, G. Glutathione inhibits experimental oral carcinogenesis, p53 expression, and angiogenesis. *Nutr. Cancer* **26:** 229–236; 1996.

[114] Sasaki, H.; Zhu, L.; Fukuda, S.; Maulik, N. Inhibition of NF kappa B activation by pyrrolidine dithiocarbamate prevents in vivo hypoxia/reoxygenation-mediated myocardial angiogenesis. *Int. J. Tissue React.* **22:**93–100; 2000.

[115] Sen, C. K.; Roy, S.; Packer, L. Involvement of intracellular Ca^{2+}in oxidant-induced NF-kappa B activation. *FEBS Lett.* **385:**58–62; 1996.

[116] Marikovsky, M. Thiram inhibits angiogenesis and slows the development of experimental tumours in mice. *Br. J. Cancer* **86:**779–787; 2002.

[117] Lu, J.; Jiang, C. Antiangiogenic activity of selenium in cancer chemoprevention: metabolite-specific effects. *Nutr. Cancer* **40:**64–73; 2001.

[118] McCarty, M. F. Polyphenol-mediated inhibition of AP-1 transactivating activity may slow cancer growth by impeding angiogenesis and tumor invasiveness. *Med. Hypotheses* **50:**511–514; 1998.

[119] Shklar, G. Mechanisms of cancer inhibition by anti-oxidant nutrients. *Oral Oncol.* **34:**24–29; 1998.

[120] Stoclet, J. C.; Chataigneau, T.; Ndiaye, M.; Oak, M. H.; El Bedoui, J.; Chataigneau, M.; Schini-Kerth, V. B. Vascular protection by dietary polyphenols. *Eur. J. Pharmacol.* **500:**299–313; 2004.

[121] Kanadaswami, C.; Lee, L. T.; Lee, P. P.; Hwang, J. J.; Ke, F. C.; Huang, Y. T.; Lee, M. T. The antitumor activities of flavonoids. *In Vivo* **19:**895–909; 2005.

[122] Schindler, R.; Mentlein, R. Flavonoids and vitamin E reduce the release of the angiogenic peptide vascular endothelial growth factor from human tumor cells. *J. Nutr.* **136:**1477–1482; 2006.

[123] Atalay, M.; Gordillo, G.; Roy, S.; Rovin, B.; Bagchi, D.; Bagchi, M.; Sen, C. K. Anti-angiogenic property of edible berry in a model of hemangioma. *FEBS Lett.* **544:**252–257; 2003.

[124] Roy, S.; Khanna, S.; Alessio, H. M.; Vider, J.; Bagchi, D.; Bagchi, M.; Sen, C. K. Anti-angiogenic property of edible berries. *Free Radic. Res.* **36:**1023–1031; 2002.

[125] Kim, J. D.; Liu, L.; Guo, W.; Meydani, M. Chemical structure of flavonols in relation to modulation of angiogenesis and immune-endothelial cell adhesion. *J. Nutr. Biochem.* **17:**165–176; 2006.

[126] Yang, C. S.; Maliakal, P.; Meng, X. Inhibition of carcinogenesis by tea. *Annu. Rev. Pharmacol. Toxicol.* **42:**25–54; 2002.

[127] Camouse, M. M.; Hanneman, K. K.; Conrad, E. P.; Baron, E. D. Protective effects of tea polyphenols and caffeine. *Expert Rev. Anticancer Ther.* **5:**1061–1068; 2005.

[128] Lin, M. T.; Yen, M. L.; Lin, C. Y.; Kuo, M. L. Inhibition of vascular endothelial growth factor-induced angiogenesis by resveratrol through interruption of Src-dependent vascular endothelial cadherin tyrosine phosphorylation. *Mol. Pharmacol.* **64:**1029–1036; 2003.

[129] Pezzuto, J. M. Resveratrol: a whiff that induces a biologically specific tsunami. *Cancer Biol. Ther.* **3:**889–890; 2004.

[130] Weber, W. M.; Hunsaker, L. A.; Abcouwer, S. F.; Deck, L. M.; Vander Jagt, D. L. Anti-oxidant activities of curcumin and related enones. *Bioorg. Med. Chem.* **13:**3811–3820; 2005.

[131] Maheshwari, R. K.; Singh, A. K.; Gaddipati, J.; Srimal, R. C. Multiple biological activities of curcumin: a short review. *Life Sci.* **78:**2081–2087; 2006.

[132] Kapiotis, S.; Hermann, M.; Held, I.; Seelos, C.; Ehringer, H.; Gmeiner, B. M. Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. *Arterioscler. Thromb. Vasc. Biol.* **17:**2868–2874; 1997.

[133] Lamartiniere, C.A. Protection against breast cancer with genistein: a component of soy. *Am. J. Clin. Nutr.* **71:**1705S–1707S; discussion 1708S–1709S; 2000.

[134] Kurzer, M. S.; Xu, X. Dietary phytoestrogens. *Annu. Rev. Nutr.* **17:** 353–381; 1997.

[135] Nishimura, G.; Yanoma, S.; Mizuno, H.; Kawakami, K.; Tsukuda, M. An antioxidant, probucol, induces anti-angiogenesis and apoptosis in athymic nude mouse xenografted human head and neck squamous carcinoma cells. *Jpn. J. Cancer Res.* **90:**1224–1230; 1999.

[136] Woodson, K.; Triantos, S.; Hartman, T.; Taylor, P. R.; Virtamo, J.; Albanes, D. Long-term alpha-tocopherol supplementation is associated with lower serum vascular endothelial growth factor levels. *Anticancer Res.* **22:**375–378; 2002.

[137] Ashino, H.; Shimamura, M.; Nakajima, H.; Dombou, M.; Kawanaka, S.; Oikawa, T.; Iwaguchi, T.; Kawashima, S. Novel function of ascorbic acid as an angiostatic factor. *Angiogenesis* **6:**259–269; 2003.

[138] Nespereira, B.; Perez-Ilzarbe, M.; Fernandez, P.; Fuentes, A. M.; Paramo, J. A.; Rodriguez, J. A. Vitamins C and E downregulate vascular VEGF and VEGFR-2 expression in apolipoprotein-E-deficient mice. *Atherosclerosis* **171:**67–73; 2003.

[139] Cecarini, V.; Gee, J.; Fioretti, E.; Amici, M.; Angeletti, M.; Eleuteri, A. M.; Keller, J. N. Protein oxidation and cellular homeostasis: emphasis on metabolism. *Biochim. Biophys. Acta* **1773:**93–104; 2007.

[140] Poppek, D.; Grune, T. Proteasomal defense of oxidative protein modifications. *Antioxid. Redox Signaling* **8:**173–184; 2006.

[141] Gitay-Goren, H.; Cohen, T.; Tessler, S.; Soker, S.; Gengrinovitch, S.; Rockwell, P.; Klagsbrun, M.; Levi, B. Z.; Neufeld, G. Selective binding of VEGF121 to one of the three vascular endothelial growth factor receptors of vascular endothelial cells. *J. Biol. Chem.* **271:**5519–5523; 1996.

[142] Gengrinovitch, S.; Berman, B.; David, G.; Witte, L.; Neufeld, G.; Ron, D. Glypican-1 is a VEGF165 binding proteoglycan that acts as an extracellular chaperone for VEGF165. *J. Biol. Chem.* **274:** 10816–10822; 1999.

[143] Maulik, N. Redox regulation of vascular angiogenesis. *Antioxid. Redox Signaling* **4:**783–784; 2002.

[144] Sauer, H.; Bekhite, M. M.; Hescheler, J.; Wartenberg, M. Redox control of angiogenic factors and CD31-positive vessel-like structures in mouse embryonic stem cells after direct current electrical field stimulation. *Exp. Cell Res.* **304:**380–390; 2005.

[145] Sen, C. K.; Khanna, S.; Babior, B. M.; Hunt, T. K.; Ellison, E. C.; Roy, S. Oxidant-induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing. *J. Biol. Chem.* **277:**33284–33290; 2002.

[146] Schafer, G.; Cramer, T.; Suske, G.; Kemmner, W.; Wiedenmann, B.; Hocker, M. Oxidative stress regulates vascular endothelial growth factor-A gene transcription through Sp1-and Sp3-dependent activation of two proximal GC-rich promoter elements. *J. Biol. Chem.* **278:**8190–8198; 2003.

[147] Kosmidou, I.; Xagorari, A.; Roussos, C.; Papapetropoulos, A. Reactive oxygen species stimulate VEGF production from C(2)C(12) skeletal myotubes through a PI3K/Akt pathway. *Am. J. Physiol. Lung Cell Mol. Physiol.* **280:**L585–L592; 2001.

[148] Feliers, D.; Gorin, Y.; Ghosh-Choudhury, G.; Abboud, H. E.; Kasinath, B. S. Angiotensin II stimulation of VEGF mRNA translation requires production of reactive oxygen species. *Am. J. Physiol. Renal Physiol.* **290:**F927–F936; 2006.

[149] Kim, Y. M.; Kim, K. E.; Koh, G. Y.; Ho, Y. S.; Lee, K. J. Hydrogen peroxide produced by angiopoietin-1 mediates angiogenesis. *Cancer Res.* **66:**6167–6174; 2006.

[150] Sreekumar, P. G.; Kannan, R.; de Silva, A. T.; Burton, R.; Ryan, S. J.; Hinton, D. R. Thiol regulation of vascular endothelial growth factor-A and its receptors in human retinal pigment epithelial cells. *Biochem. Biophys. Res. Commun.* **346:**1200–1206; 2006.

[151] Lee, K. S.; Kim, S. R.; Park, S. J.; Park, H. S.; Min, K. H.; Lee, M. H.; Jin, S. M.; Jin, G. Y.; Yoo, W. H.; Lee, Y. C. Hydrogen peroxide induces vascular permeability via regulation of vascular endothelial growth factor. *Am. J. Respir. Cell Mol. Biol.* **35:**190–197; 2006.

[152] Khanna, S.; Roy, S.; Bagchi, D.; Bagchi, M.; Sen, C. K. Upregulation of oxidant-induced VEGF expression in cultured keratinocytes by a grape seed proanthocyanidin extract. *Free Radic. Biol. Med.* **31:**38–42; 2001.

[153] Sen, C. K.; Khanna, S.; Venojarvi, M.; Trikha, P.; Ellison, E. C.; Hunt, T. K.; Roy, S. Copper-induced vascular endothelial growth factor expression and wound healing. *Am. J. Physiol. Heart Circ. Physiol.* **282:**H1821–H1827; 2002.

[154] Kuroki, M.; Voest, E. E.; Amano, S.; Beerepoot, L. V.; Takashima, S.; Tolentino, M.; Kim, R. Y.; Rohan, R. M.; Colby, K. A.; Yeo, K. T.; Adamis, A. P. Reactive oxygen intermediates increase vascular endothelial growth factor expression in vitro and in vivo. *J. Clin. Invest.* **98:**1667–1675; 1996.

[155] Huang, S. S.; Zheng, R. L. Biphasic regulation of angiogenesis by reactive oxygen species. *Pharmazie* **61:**223–229; 2006.

[156] Gonzalez-Pacheco, F. R.; Deudero, J. J.; Castellanos, M. C.; Castilla, M. A.; Alvarez-Arroyo, M. V.; Yague, S.; Caramelo, C. Mechanisms of endothelial response to oxidative aggression: protective role of autologous VEGF and induction of VEGFR2 by H_2O_2. *Am. J. Physiol. Heart Circ. Physiol.* **291:**H1395–H1401; 2006.

[157] Salomonsson, L.; Svensson, L.; Pettersson, S.; Wiklund, O.; Ohlsson, B. G. Oxidised LDL decreases VEGFR-1 expression in human monocyte-derived macrophages. *Atherosclerosis* **169:**259–267; 2003.

[158] Chen, K.; Thomas, S. R.; Albano, A.; Murphy, M. P.; Keaney Jr., J. F. Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signaling. *J. Biol. Chem.* **279:**35079–35086; 2004.

[159] Maulik, N. Redox signaling of angiogenesis. *Antioxid. Redox Signaling* **4:**805–815; 2002.

[160] Aslan, M.; Ozben, T. Oxidants in receptor tyrosine kinase signal transduction pathways. *Antioxid. Redox Signaling* **5:**781–788; 2003.

[161] Maulik, N. Reactive oxygen species drives myocardial angiogenesis? *Antioxid. Redox Signaling* **8:**2161–2168; 2006.

[162] Zhou, Q.; Liu, L. Z.; Fu, B.; Hu, X.; Shi, X.; Fang, J.; Jiang, B. H. Reactive oxygen species regulate insulin-induced VEGF and HIF-1 {alpha} expression through the activation of p70S6K1 in human prostate cancer cells. *Carcinogenesis* **28:**28–37; 2007.

[163] Gao, N.; Ding, M.; Zheng, J. Z.; Zhang, Z.; Leonard, S. S.; Liu, K. J.; Shi, X.; Jiang, B. H. Vanadate-induced expression of hypoxia-inducible factor

1 alpha and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. *J. Biol. Chem.* **277:**31963–31971; 2002.

[164] Patel, V.; Chivukula, I. V.; Roy, S.; Khanna, S.; He, G.; Ojha, N.; Mehrotra, A.; Dias, L. M.; Hunt, T. K.; Sen, C. K. Oxygen: from the benefits of inducing VEGF expression to managing the risk of hyperbaric stress. *Antioxid. Redox Signaling* **7:**1377–1387; 2005.

[165] Matsubara, T.; Ziff, M. Increased superoxide anion release from human endothelial cells in response to cytokines. *J. Immunol.* **137:**3295–3298; 1986.

[166] Abid, M. R.; Tsai, J. C.; Spokes, K. C.; Deshpande, S. S.; Irani, K.; Aird, W. C. Vascular endothelial growth factor induces manganese-superoxide dismutase expression in endothelial cells by a Rac1-regulated NADPH oxidase-dependent mechanism. *FASEB J.* **15:**2548–2550; 2001.

[167] Wang, Z.; Castresana, M. R.; Newman, W. H. Reactive oxygen and NF-kappaB in VEGF-induced migration of human vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* **285:**669–674; 2001.

[168] Colavitti, R.; Pani, G.; Bedogni, B.; Anzevino, R.; Borrello, S.; Waltenberger, J.; Galeotti, T. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. *J. Biol. Chem.* **277:**3101–3108; 2002.

[169] Ushio-Fukai, M.; Tang, Y.; Fukai, T.; Dikalov, S. I.; Ma, Y.; Fujimoto, M.; Quinn, M. T.; Pagano, P. J.; Johnson, C.; Alexander, R. W. Novel role of gp91(phox)-containing NAD(P)H oxidase in vascular endothelial growth factor-induced signaling and angiogenesis. *Circ. Res.* **91:**1160–1167; 2002.

[170] Yamaoka-Tojo, M.; Ushio-Fukai, M.; Hilenski, L.; Dikalov, S. I.; Chen, Y. E.; Tojo, T.; Fukai, T.; Fujimoto, M.; Patrushev, N. A.; Wang, N.; Kontos, C. D.; Bloom, G. S.; Alexander, R. W. IQGAP1, a novel vascular endothelial growth factor receptor binding protein, is involved in reactive oxygen species-dependent endothelial migration and proliferation. *Circ. Res.* **95:**276–283; 2004.

[171] Ikeda, S.; Ushio-Fukai, M.; Zuo, L.; Tojo, T.; Dikalov, S.; Patrushev, N. A.; Alexander, R. W. Novel role of ARF6 in vascular endothelial growth factor-induced signaling and angiogenesis. *Circ. Res.* **96:**467–475; 2005.

[172] Tojo, T.; Ushio-Fukai, M.; Yamaoka-Tojo, M.; Ikeda, S.; Patrushev, N.; Alexander, R. W. Role of gp91phox (Nox2)-containing NAD(P)H oxidase in angiogenesis in response to hindlimb ischemia. *Circulation* **111:**2347–2355; 2005.

[173] Wojciak-Stothard, B.; Tsang, L. Y.; Haworth, S. G. Rac and Rho play opposing roles in the regulation of hypoxia/reoxygenation-induced permeability changes in pulmonary artery endothelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **288:**L749–L760; 2005.

[174] Abid, M. R.; Schoots, I. G.; Spokes, K. C.; Wu, S. Q.; Mawhinney, C.; Aird, W. C. Vascular endothelial growth factor-mediated induction of manganese superoxide dismutase occurs through redox-dependent regulation of forkhead and IkappaB/NF-kappaB. *J. Biol. Chem.* **279:**44030–44038; 2004.

[175] Gigante, B.; Morlino, G.; Gentile, M. T.; Persico, M. G.; De Falco, S. $Plgf^{-/-}$ $eNos^{-/-}$ mice show defective angiogenesis associated with increased oxidative stress in response to tissue ischemia. *FASEB J.* **20:**970–972; 2006.

[176] Raijmakers, M. T.; Burton, G. J.; Jauniaux, E.; Seed, P. T.; Peters, W. H.; Steegers, E. A.; Poston, L. Placental NAD(P)H oxidase mediated superoxide generation in early pregnancy. *Placenta* **27:** 158–163; 2006.

[177] Morabito, A.; De Maio, E.; Di Maio, M.; Normanno, N.; Perrone, F. Tyrosine kinase inhibitors of vascular endothelial growth factor receptors in clinical trials: current status and future directions. *Oncologist* **11:**753–764; 2006.

[178] Bhisitkul, R. B. Vascular endothelial growth factor biology: clinical implications for ocular treatments. *Br. J. Ophthalmol.* **90:**1542–1547; 2006.

[179] Shams, N.; Ianchulev, T. Role of vascular endothelial growth factor in ocular angiogenesis. *Ophthalmol. Clin. North Am.* **19:**335–344; 2006.

[180] Irani, K.; Xia, Y.; Zweier, J. L.; Sollott, S. J.; Der, C. J.; Fearon, E. R.; Sundaresan, M.; Finkel, T.; Goldschmidt-Clermont, P. J. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. *Science* **275:**1649–1652; 1997.