· PERSPECTIVES ·

Oxidative stress in tumor microenvironment ——Its role in angiogenesis

Armando ROJAS*, Raúl SILVA*, Héctor FIGUEROA*, Miguel A MORALES△

【CLC】R734.2 DOI:10.3779/j.issn.1009-3419.2008.03.019

*School of Medicine, Catholic University of Maule, Chile, and △ICBM, University of Chile, Chile
Corresponding author: Dr. Armando Rojas, E-mail: arojasr@ucm.cl

【Abstract】 The tumor angiogenesis process is believed to be dependent on an "angiogenic switch" formed by a cascade of biologic events as a consequence of the "cross-talk" between tumor cells and several components of local microenvironment including endothelial cells, macrophages, mast cells and stromal components. Oxidative stress represents an important stimulus that widely contributes to this angiogenic switch, which is particularly relevant in lungs, where oxidative stress is originated from different sources including the incomplete reduction of oxygen during respiration, exposure to hypoxia/reoxygenation, stimulated resident or chemoattracted immune cells to lung tissues, as well as by a variety of chemicals compounds. In the present review we highlight the role of oxidative stress in tumor angiogenesis as a key signal linked to other relevant actors in this complex process.

【Key words】 Oxidative stress Tumor angiogenesis NOX enzymes Nitric oxide VEGF Cadherins Advanced glycation

Reactive Oxygen Species (ROS) in vasculature

Vascular cells produce energy by reducing molecular oxygen to water during aerobic respiration. During this process, reactive species such as superoxide anion ($O_2^{-}\cdot$) hydrogen peroxide (H_2O_2), peroxynitrite ($OONO^-$), hypochlorous acid (HOCl) and the hydroxyl radical ($OH\cdot$), among others are generated[1,2]. Under homeostatic conditions, these molecules play regulatory roles in cellular function, and antioxidant defences are critical to modulate their steady state balance, which is now recognized as a key mechanism for the maintenance of vascular health[3].

An important consequence of vascular oxidant stress, with dramatic results on vascular homeostasis, is the impaired nitric oxide (NO) bioavailability produced by its inactivation by superoxide anion. The latter rapidly reacts with NO and eliminates its biological activity[4].

Main ROS sources

In the vascular wall, several enzymatic systems produce O_2^{-} and its derivatives in the vasculature, including NAD(P)H oxidases (NOXs), xanthine oxidase (XO), NO synthases (NOS) and myeloperoxidase (MPO).

NAD(P)H oxidase

Compelling evidence suggests that NAD(P)H oxidases, also known as NOX enzymes, constitute the main enzymatic source of endothelial and vascular $O_2\cdot$. Nox proteins represent the catalytic subunits of these enzymes and vary in terms of their mode of activation and need for cofactor activation[5]. Nox1 protein levels are quite low in vascular cells, but can be induced by stimuli such as platelet-derived growth factor (PDGF) and angiotensin II[5]. Nox2, previously known as gp91phox, is expressed in endothelial and adventitial cells of large vessels and in the vascular smooth muscle cells of smaller vessels[6-8]. Nox4 is constitutively expressed and active in vascular smooth muscle (VSMC) and endothelial cells (EC)[9,10]. All Nox enzymes require p22phox, which serves as a docking protein for other subunits and stabilizes the Nox proteins[10].

Although endothelial and vascular oxidases appear to be constantly active, generating low levels of ROS, they are regulated by humoral factors, as demonstrated for cytokines,

Conflict of interest statement. None of the authors have conflicts of interest.
Corresponding author: Dr. Armando Rojas, School of Medicine, Catholic University of Maule, Ave San Miguel # 3605, Talca, Chile.
E-mail: arojasr@ucm.cl Phn: 56-71-203134 Fax: 56-71-413657.

growth factors, and vasoactive agents as well as by physical factors, including stretch, pulsatile strain and shear stress[11]. Interestingly, hydrogen peroxide and lipid peroxides can stimulate the activity of the NADPH oxidases in vascular smooth muscle cells, leading to a feed-forward increase in ROS production in the vascular wall[11,13].

The protagonic role of this enzymatic system in vascular disease has been evidenced by several reports showing that increased levels of p22phox, p47phox, p67phox and Nox subunits are present in both human atherosclerotic coronary arteries[14] and diabetic vessels[15], in association with increased superoxide production. This suggests that upregulated gene expression and/or post-transcriptional increases in protein levels are important in mediating increased NAD(P)H oxidase activity in human vascular disease. For example, angiotensin II increases NAD(P)H oxidase activity by transcriptional upregulation of subunits expression[16]. However, it is clear that the cytosolic regulatory proteins p47phox, p67phox and the small g protein Rac-1 also play an important part in regulating NAD(P)H oxidase activity in vascular diseases by acute activation of the enzyme complex, i.e. by phosphorylation and translocation of p47phox[17] .

COX

Cyclooxygenase is another source $O_2^{-}\cdot$ of production, particularly in the cerebral circulation[18]. PGH synthase and lipooxygenase are able to co-oxidize substances such as NAD(P)H[19].

X/XO

Another source of vascular ROS is the xanthine oxidoreductase enzyme system. The xanthine dehydrogenase (XDH) activity present in vascular endothelium is readily converted into XO by processes including thiol oxidation and/or proteolysis[20] and the ratio of XO to XDH in the cell is therefore critical to determine the amount of ROS produced by these enzymes. Xanthine oxidase metabolizes hypoxanthine, xanthine, and NADH to form O_2^{-} and H_2O_2, and appears to be an important source of ROS production in ischemia/reperfusion[21] hypercholesterolemia[22]. Thus, xanthine oxidase has the potential to be an important source of ROS production under certain pathophysiological conditions.

However, the presence of XDH in endothelium is still matter of controversy because other studies based on immunohisto-chemical techniques studies have failed to demonstrate immunoreactivity to XDH in endothelial cells or other cardiovascular tissues[23]. It has been suggested that XO in endothelial cells originates from other organs and that the enzyme is probably taken-up via heparin binding sites[24, 25].

Mitochondria

The contribution of mitochondria to the production of ROS in vascular wall is less understood, although significant contributions have been made in the last five years[26].

Recent evidence suggests that increased mitochondrial $O_2^{-}\cdot$ generation in endothelial cells is particularly prominent in some pathological settings. Hyperglycemia induces mitochondrial $O_2^{-}\cdot$ production, which is involved in the pathogenesis of diabetic complications[27]. Similarly, the adipokine leptin also induces mitochondrial $O_2^{-}\cdot$ production by increasing fatty acid oxidation[28]. In hypoxia-reoxygenation and ischemia-reperfusion, mitochondrial-derived $O_2^{-}\cdot$ radicals are increased, where the enhanced $O_2^{-}\cdot$ is at least partially responsible for a rise in endothelial permeability[29].

Dysfunctional or uncoupled endothelial nitric oxide synthase (NOS III)

NOS III is a complex homodimeric oxidoreductase that shuttle electrons from the reductase domain to the oxidase domain that contains the heme active site. Under some conditions, NOS generates superoxide rather than NO[30], a phenomenon that is known as NOS uncoupling which means that electrons flowing from the NOS III reductase domain to the oxygenase domain are diverted to molecular oxygen rather than to L-arginine. One of NOS cofactors, tetrahydrobiopterin (BH_4) appears to have a key role in regulating NOS function by "coupling" the reduction of molecular O_2 to L-arginine oxidation. Exogenous BH_4 partially restores NOS III-dependent NO production and reduces NOS uncoupling in hypertension[31], hypercholesterolemia[32], and smokers[33]. Thus, BH_4 availability is a crucial factor in the balance between NO and $O_2^{-}\cdot$ generation by NOS III.

Tumor angiogenesis

Angiogenesis, the formation of new blood vessels from the pre-existing vasculature, is one main mechanism of vascularisation during the embryonic development, growth, regeneration, wound healing and some physiological processes such as formation of the corpus luteum and endometrium. On the other hand, angiogenesis is also involved in a number of pathological processes including tumor growth, invasion, metastasis, diabetic retinopathy and arthritis[34,35].

For more than 100 years, tumors had been observed to be more vascularized than normal tissues[36]. Understanding this condition has been a major task in biomedical research. In 1939, it was hypothesized for the first time that tumor hyperemia could be related to new blood vessel growth instead of vasodilatation[37]. Thirty-two years later, Judah Folkman[38] proposed that tumor growth is angiogenesis dependent. In 1974, Gimbrone et al, suggested that tumors are able to release diffusible stimulators, as demonstrated by the sprouting of new capillaries when angiogenic tumors were implanted in avascular region such as the cornea[39]. Furthermore, Weidner and colleagues in 1991[40] found that the microvascular density of the primary tumor was a highly significant prognostic marker for human breast cancer.

Today it is widely accepted that tumor growth is heavily conditioned by the availability of an adequate vasculature. Angiogenesis is thus becoming essential when tumor diameter reaches (1-2) mm in diameter, in order to supply adequate oxygenation and nutrition to tissues and to eliminate toxic molecules[41].

Tumor angiogenesis is a very complex process which is regulated by a delicate balance between several proangiogenic and antiangiogenic molecules released by tumor and host cells, including endothelial cells, macrophages, mast cells and stromal components.

A cascade of biologic events is switched on following the "cross-talk" between tumor cells and several components of local microenvironment. For example, homeostatic modifications under hypoxic oxidative or mechanical stresses may act as potent stimulators of tumor angiogenesis and induce the expression of multiple pro-angiogenic factors. Tumors promote angiogenesis by the secretion of growth factors that stimulate endothelial cell migration and proliferation, proteolytic activity, and capillary morphogenesis. Furthermore, angiogenesis-promoting factors andgrowth factors (GF) released by tumor associated inflammatory cells are now considered important elements in local tumor microenviroment. Mast cells (MCs) are known to accumulate at the sites of angiogenesis and produce many angiogenesis promotors such as histamine, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), heparin and tryptase[42-44]. Likewise, tumor associated macrophage (TAMs) are thought to induce angiogenesis through secretion of several factors, including tumor necrosis factor-alfa (TNF-alfa), VEGF, angiogenin and urokinase[45]. Additionally, eosinophils, are also able to produce some angiogenic factors, which has been correlated with angiogenesis in inflammatory conditions characterized by increased tissue eosinophilia, such as asthmatic airways and nasal polyps[46,47]. A growing body of evidences clearly support a relevant role of plateles by releasing proagngiogenic substances in the tumor microenvironment as demonstrated for VEGF[48], platelet derived growth factor (PDGF) [49], basic fibroblast growth factor (bFGF)[50], endothelial cell growth factor (ECGF)[51], transforming growth factor (TGF)[52], insulin-like growth factor (ILGF)[53], angiopoietin 1[54], sphingosine-1-phosphate[55], and matrix metalloproteinases (MMPs)[56]. However, platelets also secrete inhibitors of angiogenesis such as thrombospondin I[57], platelet factor 4[58], and plasminogen activator inhibitor I[59], and angiostatin[60]. Thus, increased activation of platelets is though to support tumor angiogenesis by releasing of growth factors within the prothrombotic tumor microcirculation. Furthermore, many tumors also secrete factors like GM-CSF, G-CSF, IL-1 and IL-6, which have been discussed to increase platelet counts[61, 62].

Oxidative stress as a key signal in angiogenesis switch

The tumor angiogesis process is believed to be dependent on an "angiogenic switch" which initiates a series of events starting with the release of tumor-related proangiogenic factors, leading to the activation of endothelial cells, the release of proteolytic enzymes, degradation of the basement membrane, followed by endothelial cell migration, proliferation, and capillary tube formation[63]. The new capillaries formed in tumors lack the same supporting architecture as their parent vessels and are thin-walled and highly permeable. Consequently, the

leakiness of the new tumor vasculature provides access to the circulation for tumor cells to metastasise.

Oxidative stress represents an important stimulus that widely contributes to tumor angiogenesis mediating the angiogenic switch[64, 65].

In lungs, oxidative stress is originated from different sources including the incomplete reduction of oxygen during respiration, exposure to hypoxia/reoxygenation, stimulated resident or chemoattracted immune cells to lung tissues, as well as by a variety of chemicals compounds.

ROS are also produced by cancer cells at higher concentrations and thus contribute to neoplastic transformation and angiogenesis. Contrarily, tumor cell proliferation and endothelial cell differentiation are promoted by lower concentrations[66-68].

The NOX family of NAD(P)H oxidases is a key cellular source of ROS, which are well-represented in lung tissues, which has been associated with the angiogenic switch in non tumor tissue[69,70]. NAD(P)H oxidases also influence tumor cell proliferation via the redox regulated transcription factor NF-kB which in turn regulates numerous genes involved in apoptosis, cell proliferation, metastasis and angiogenesis[71], as well as to induce IL-8 expression in endothelial cells, favouring the expression of an angiogenic phenotype[72].

The effects of ROS on angiogenesis may be in part mediated by the up-regulation of VEGF expression at both the protein and mRNA levels[73,74]. VEGF and its receptors (VEGFR) are among the most intensively studied angiogenic regulators in basic and clinical cancer research[75], which regulates endothelial proliferation, permeability, and survival. VEGF is secreted by many cell types, and its expression is regulated by a myriad of growth factors and cytokines and there is considerable evidence that VEGF is a major tumor angiogenesis factor and its mRNA is up regulated in a large number of tumor types[76,77]. There is an extensive body of data documenting that inhibition of VEGF activity results in suppression of growth of a wide variety of tumor cell lines in murine models[78,79].

It is now known that in several cancer types, including lung cancer, there is an excess production of oxidative stress[80,81]. Furthermore, a significant correlation between oxidative stress and VEGF levels in bronchoalveolar lavage fluid of lung cancer patients after chemotherapy or radiotherapy and chemotherapy has been reported[82], suggesting a possible induction of VEGF production by oxidative stress. Similar correlation has been also reported between serum VEGF levels and oxidative stress in most malignancies, including lung cancer[83-85].

One important aspect of the biology of VEGF is the regulation of VEGF-gene transcription mediated by HIF-1 (hypoxia inducible factor-1). In hypoxic conditions, the heterodimer HIF1α/HIF β may translocate to the cell nucleus and, after binding to specific promoters, may lead to increased transcription of some genes (hypoxia-induced genes) encoding for proteins involved in the angiogenesis process, such as VEGF, PDGF-beta, TGF-alpha, and even erythropoietin[86].

Another redox protein, thioredoxin, activates HIF-dependent pathways by similar mechanisms[87]. Overexpression of thioredoxin is observed in several human tumors, which may contribute to the HIF-induced transcriptional up-regulation of VEGF and tumor angiogenesis[88,89].

Nitric oxide (NO) has been shown to mediate angiogenesis by direct and indirect mechanisms[90]. Contrarily, anti-angiogenic effects of NO have been also reported. This apparent discrepancy might be explained by differences in concentration, cellular compartment as well duration of exposure. Many angiogenic factors are known to increase nitric oxide production by activation endothelial nitric oxide synthase, as demonstrated for VEGF, sphingosine-1-phosphate, angiopoietins, estrogens[91,92].

Additionally, NO induces the synthesis and activation of hypoxia-inducible factor 1α (HIF1α) the transcription factor, which in turn upregulates VEGF[93].

There is increasing evidence that Angiotensin II (Ang II), a major regulator of blood pressure and cardiovascular homeostasis, is also involved in tumor progression, tumor vascularisation and metastasis, mainly through AT1 receptor, as demonstrated with specific receptor blockers[94]. It has been demonstrated that Ang II, a potent stimulus for ROS generation, promotes in vitro angiogenesis by enhancing VEGF expression, through both p38- and p44/42 MAPKs-dependent mechanism[95].

Vascular endothelial (VE) cadherin has emerged as a key molecule in angiogenesis processes. The first evidences of VE-cadherin implication in angiogenesis emerged from in

vitro and *in vivo* angiogenesis models using anti-VE cadherin antibodies[96-99].

VE-cadherin, initially considered as a constitutive protein with unregulated expression, has been demonstrated to be under a very complex expression control[100,101].

VE-cadherin phosphorylation is a key step to the angiogenic phenotype induced by VEGF which, in turn, triggers cell-cell disruption, possibly through modification of adherens junction composition[102].

Oxidative stress is a key element in VE cadherin regulation because of VE disappearance from adherent junctions as well as VE cadherin phosphorylation induced by angiogenic signals is markedly reverted by antioxidant treatments[101,103].

Type 2 diabetes and even moderately elevated glucose levels have been associated with several cancer types[104-106].

Advanced glycation end-products (AGEs) and its receptor (RAGE) is now emerging as an important element in tumor biology and angiogenesis[107].

The formation of AGEs, by the so called Maillard reaction, is a complex cascade of condensations, rearrangements, fragmentations, and oxidative modifications that leads to poorly characterized heterogeneous products. Glucose possesses a reactive aldehyde moiety that reacts non enzymatically with the amino groups of proteins, forming slowly reversible Amadori products. Rearrangement reactions then occur to produce a chemically related group of moieties, termed AGEs, which remain irreversibly bound to proteins[108]. RAGE ligands, which include the S100/calgranulins and high-mobility group box 1 (HMGB1) ligands, are expressed and secreted by cancer cells and are associated with increased metastasis and poorer outcomes in a wide variety of tumors. These ligands can interact in an autocrine manner to directly activate cancer cells and stimulate proliferation, invasion, and metastasis[107]. On the other hand, RAGE ligands can also influence a variety of important cell types within the tumor microenvironment, including fibroblasts, leukocytes, and vascular cells, leading to increased fibrosis, inflammation, and angiogenesis[107].

RAGE expression is closely associated with gastric[109], colorectal[110], prostate[111] and lung cancer[112]. Blockade of the RAGE signal suppresses tumor growth and metastasis in these cancers[113].

Endogenous secretory RAGE (esRAGE), has been identified in some cancer types including lung cancer[114]. esRAGE is similar to, but more stable than, a previously described form of secretory RAGE (sRAGE). By functioning as a decoy receptor, esRAGE is able to protect vascular cells from injury[115]. This suggests that esRAGE is a mediator that controls RAGE-associated cell responses. Loss of esRAGE expression was associated with an increased risk of mortality after complete surgical resection, and was an independent predictor of the clinical outcome in patients with non-small cell lung carcinoma.

Endothelial cell migration, a key early event during new vessel formation, is also induced by AGEs in human endothelial cells markedly affecting VE-cadherin distribution by a redox-sentivive mechanism[116].

At present, there are compelling evidences that oxidative stress is relevant in neoplasic diseases and particularly in lung cancer[117]. Oxidative stress at tumor microenvironment represents an important element favouring both tumor growth and angiogenesis, as demonstrated by the antiangiogenic affects of many classical antioxidants such as, ascorbic acid[118], vitamin E[119], green tea catechins[120], resveratrol[121], and beta-caroten[122], as well as new compounds derived from plants used in traditional Chinese medicine[123,124], therefore might be considered as a potential target to pharmacological manipulation.

References

1 Bergamini CM, Gambetti S, Dondi A, et al. Oxygen, reactive oxygen species and tissue damage. Curr Pharm Des, 2004, 10(14): 1611-1626.

2 Poli G, Leonarduzzi G, Biasi F, et al. Oxidative stress and cell signalling. Curr Med Chem, 2004, 11(9): 1163-1182.

3 Schulz E, Anter E, Keaney JF Jr. Oxidative stress, antioxidants, and endothelial function. Curr Med Chem, 2004, 11(9): 1093-1104.

4 Gryglewski RJ, Palmer RM, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature, 1986, 320(6061): 454-456.

5 Lassegue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. Am J Physiol Regul Integr Comp Physiol, 2003, 285(2): R277-R297.

6 Griendling KK. Novel NAD(P)H oxidases in the cardiovascular system. Heart, 2004, 90(5): 491-493.

7 Gorlach A, Brandes RP, Nguyen K, et al. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ Res, 2000, 87(3): 26-32.

8 Touyz RM, Chen X, Tabet F, et al. Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. Circ Res, 2002, 90(11): 1205-1213.

9 Ago T, Kitazono T, Ooboshi H, et al. Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. Circulation, 2004, 109(2): 227-233.

10 Hilenski LL, Clempus RE, Quinn MT, et al. Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol, 2004, 24(4): 677-683.

11 Touyz RM, Tabet F, Schiffrin EL. Redox-dependent signalling by angiotensin II and vascular remodelling in hypertension. Clin Exp Pharmacol Physiol, 2003, 30(11): 860-866.

12 Li WG, Stoll LL, Rice JB, et al. Activation of NAD(P)H oxidase by lipid hydroperoxides: mechanism of oxidant-mediated smooth muscle cytotoxicity. Free Radic Biol Med, 2003, 34(7): 937-946.

13 Li WG, Miller FJ Jr, Zhang HJ, et al. H(2)O(2)-induced O(2) production by a non-phagocytic NAD(P)H oxidase causes oxidant injury. J Biol Chem, 2001, 276(31): 29251-29256.

14 Sorescu D, Weiss D, Lassegue B, et al. Superoxide production and expression of nox family proteins in human atherosclerosis. Circulation, 2002, 105(12): 1429-1435.

15 Guzik TJ, Mussa S, Gastaldi D. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation, 2002, 105(14): 1656-1662.

16 Mollnau H, Wendt M, Szocs K. Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. Circ Res, 2002, 90(4): E58-E65.

17 Touyz RM, Chen X, Tabet F. Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. Circ Res, 2002, 90(11): 1205-1213.

18 Marshall JJ, Kontos HA. Endothelium and cerebral vascular diseases. In: Rubanyi GM, ed. Cardiovascular Significance of Endothelium-Derived Vasoactive Factors. Mount Kisco, NY: Futura Publishing Co, 1991.125-145.

19 Kukreja RC, Kontos HA, Hess ML, et al. PGH synthetase and lipoxygenase generate superoxide in the presence of NADH or NADPH. Circ Res, 1986, 59(6): 612-619.

20 Granger DN. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am J Physiol, 1988(1), 255: H1269-H1275.

21 Granger DN. Ischemia-reperfusion: mechanisms of microvascular dysfunction and the influence of risk factors for cardiovascular disease. Microcirculation, 1999, 6(3): 167-178.

22 Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest, 1993, 91(6): 2546-2551.

23 Linder N, Rapola J, Raivio KO. Cellular expression of xanthine oxidoreductase protein in normal human tissues. Lab Invest, 1999, 79(8): 967-974.

24 Fukushima T, Adachi T, Hirano K. The heparin-binding site of human xanthine oxidase. Biol Pharm Bull, 1995, 18(1): 156-158.

25 Houston M, Estevez A, Chumley P, et al. Binding of xanthine oxidase to vascular endothelium. Kinetic characterization and oxidative impairment of nitric oxide-dependent signaling. J Biol Chem, 1999, 274(8): 4985-4994.

26 Genova ML, Pich MM, Bernacchia A, et al. The mitochondrial production of reactive oxygen species in relation to aging and pathology. Ann N Y Acad Sci, 2004, 1011(4): 86-100.

27 Du XL, Edelstein D, Rossetti L, et al. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. Proc Natl Acad Sci USA, 2000, 97(22): 12222-12226.

28 Yamagishi SI, Edelstein D, Du XL, et al. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem, 2001, 276(27): 25096-25100.

29 Pearlstein DP, Ali MH, Mungai PT, et al. Role of mitochondrial oxidant generation in endothelial cell responses to hypoxia. Arterioscler Thromb Vasc Biol, 2002, 22(4): 566-573.

30 Vasquez-Vivar J, Kalyanaraman B, Martasek P, et al. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc Natl Acad Sci USA, 1998, 95(16): 9220-9225.

31 Cosentino F, Patton S, d'Uscio LV, et al. BH4 alters superoxide and nitric oxide release in prehypertensive rats. J Clin Invest, 1998, 101(7): 1530-1537.

32 Stroes E, Kastelein J, Cosentino F, et al. BH4 restores endothelial function in hypercholesterolemia. J Clin Invest, 1997, 99(7): 41-46.

33 Heitzer T, Brockhoff C, Mayer B, et al. BH4 improves endothelium-dependent vasodilation in chronic smokers: evidence for a dysfunctional nitric oxide synthase. Circ Res, 2000, 86 (2): E36-E41.

34 Carmeliet P. Angiogenesis in health and disease. Nat Med, 2003, 9(6): 653-660.

35 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med, 1995, 1(1): 27-31.

36 Warran BA. The vascular morphology of tumors. In: Peterson H.I. editor, Tumor blood circulation:Angiogenesis, vascular morphology and blood flow of experimental human tumors. CRC Press, 1979. Miami, FL1-4.

37 Ide AG, Baker NH, Warren SL. Vascularization of the Brown-Peatce rabbit epithelioma transplant as seen in the transparent ear chamber, Am J Roentgenol, 1939, 42: 891-899.

38 Folkman J. Tumor angiogenesis: therapeutic implications, N Engl J Med, 1971, 285(21): 1182-1188.

39 Gimbrone M Jr, Cotran R, Leapman SB, et al. Tumor growth and neovascularization: An experimental model using the rabbit cornea. J Natl Cancer Inst, 1974, 52(2): 413-427.

40 Weidner N, Semple J, Welch W, et al. Tumor angiogenesis and metastasis correlation in invasive breast carcinoma. N Engl J Med, 1991, 324(1): 1–8.

41 Nishida T. Angiogenesis, which is essential for cancer growth, is a diagnostic and therapeutic target. Gastroenterology, 2005, 40(3): 320–321.

42 Qu Z, Liebler JM, Powers MR, et al. Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. Am J Pathol, 1995, 147(3): 564–573.

43 Azizkhan RG, Azizkhan JC, Zetter BR, et al. Mast cell heparin stimulates migration of endothelial cells *in vitro*. J Exp Med, 1980, 152(4): 931–944.

44 Blair RJ, Meng H, Marchase MJ, et al. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. J Clin Invest, 1997, 99(11): 2691–2700.

45 Allavena P, Sica A, Solinas G, et al. The inflammatory micro–environment in tumor progression: The role of tumor–associated macrophages. Crit Rev Oncol Hematol, 2008, 66(1): 1–9.

46 Elovic DT, Weller PF, Matosian K, et al. Expression of transforming growth factors–alpha and beta–1 messenger RNA and product by eosinophils in nasal polyps. J Allergy Clin Immunol, 1994, 93(5): 864–869.

47 Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. J Allergy Clin Immunol, 2001, 107(2): 295–301.

48 Nolte I, Przibylla H, Bostel T, et al. Tumor–platelet interactions: Glioblastoma growth is accompanied by increasing platelet counts. Clin Neurol Neurosurg, 2008, 110(4): 339–342.

49 Heldin CH, Westermark B, Wasteson A. Platelet–derived growth factor. Isolation by a large–scale procedure and analysis of subunit composition. Biochem J, 1981, 193(3): 907–913.

50 Kaplan DR, Chao FC, Stiles CD, et al. Platelet alpha granules contain a growth factor for fibroblasts. Blood, 1979, 53(6): 1043–1052.

51 Suzuki T, Takahashi K, Nakamura R, et al. Thrombocytosis in patients with tumors producing colony–stimulating factor. Blood, 1992, 80(8): 2052–2059.

52 Yamamoto S, Tsuda H, Takano M, et al. Expression of platelet–derived growth factors and their receptors in ovarian clear–cell carcinoma and its putative precursors. Mod Pathol, 2008, 21(2): 115–124.

53 Karey KP, Sirbasku DA. Human platelet–derived mitogens. II. Subcellular localization of insulinlike growth factor I to the alpha–granule and release in response to thrombin. Blood, 1989, 74(3): 1093–1100.

54 White RR, Shan S, Rusconi CP, et al. Inhibition of rat corneal angiogenesis by a nuclease–resistant RNA aptamer specific for angiopoietin–2. Proc Natl Acad Sci USA, 2003, 100(9): 5028–5033.

55 English D, lch Z, Kovala T, et al. Sphingosine 1–phosphate released from platelets during clotting accounts for the potent endothelial cell chemotactic activity of blood serum and provides a novel link between hemostasis and angiogenesis. FASEB J, 2000, 14(14): 2255–2265.

56 Galt SW, Lindemann S, Allen L, et al. Outside–in signals delivered by matrix metalloproteinase–1 regulate platelet function. Circ Res, 2002, 90(10): 1093–1099.

57 Iruela–Arispe ML, Bornstein P, Sage H. Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells in vitro. Proc Natl Acad Sci USA, 1991, 88(11): 5026–5030.

58 Maione TE, Gray GS, Petro J, et al. Inhibition of angiogenesis by recombinant human platelet factor–4 and related peptides. Science, 1990, 247(4938): 77–79.

59 Booth NA, Simpson AJ, Croll A, et al. Plasminogen activator inhibitor (PAI–1) in plasma and platelets. Br J Haematol, 1988, 70 (3): 327–333.

60 O'Reilly MS, Holmgren L, Shing Y, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell, 1994, 79(2): 315–328.

61 Wislez M, Fleury–Feith J, Rabbe N, et al. Tumor–derived granulocyte–macrophage colony–stimulating factor and granulocyte colony–stimulating factor prolong the survival of neutrophils infiltrating bronchoalveolar subtype pulmonary adenocarcinoma. Am J Pathol, 2001, 159(4): 1423–1433.

62 Hsiao YW, Liao KW, Hung SW, et al. Tumor–infiltrating lymphocyte secretion of IL–6 antagonizes tumor–derived TGF–beta 1 and restores the lymphokine–activated killing activity. J Immunol, 2004, 172(3): 1508–1514.

63 Noonan DM, Benelli R, Albini A. Angiogenesis and cancer prevention: a vision. Recent Results Cancer Res, 2007, 174: 219–224.

64 North S, Moenner M, BIfkalvi A. Recent developments in the regulation of the angiogenic switch by cellular stress factors in tumors. Cancer Lett, 2005, 218(1): 1–14.

65 Rojas A, Figueroa H, Morales MA, et al. Facing up the ROS labyrinth––Where to go? Curr Vasc Pharmacol, 2006, 4(3): 277–289.

66 Yasuda M, Ohzeki Y, Shimizu S, et al. Stimulation of *in vitro* angiogenesis by hydrogen peroxide and the relation with ETS–1 in endothelial cells. Life Sci, 1999, 64(4): 249–258.

67 Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res, 1991, 51(3): 794–798.

68 Eyries M, Collins T, Khachigian LM. Modulation of growth factor gene expression in vascular cells by oxidative stress. Endothelium, 2004, 11(2): 133–139.

69 Arbiser JL, Petros J, Klafter R, et al. Reactive oxygen generated by Nox1 triggers the angiogenic switch. Proc Natl Acad Sci USA, 2002, 99(2): 715–720.

70 Gorlach A, Diebold I, Schini–Kerth VB, et al. Thrombin activates the hypoxia–inducible factor–1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)–containing NADPH oxidase. Circ Res, 2001, 89(1): 47–54.

71 Brar SS, Kennedy TP, Quinn M, et al. Redox signaling of NF–kappaB by membrane NAD(P)H oxidases in normal and malignant cells. Protoplasma, 2003, 221(1–2): 117–127.

72 Lakshminarayanan V, Drab-Weiss EA, Roebuck KA. H_2O_2 and tumor necrosis factor-α induce differential binding of the redox-responsive transcription factors AP-1 and NF-κB to the interleukin-8 promoter in endothelial and epithelial cells. J Biol Chem, 1998, 273(49): 32670-32678.

73 Kuroki M, Voest EE, Amano S, et al. Reactive oxygen intermediates increase vascular endothelial growth factor expression *in vitro* and *in vivo*. J Clin Invest, 1996, 98(7): 1667-1675.

74 Schafer G, Cramer T, Suske G, et al. 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, 2003, 278(10): 8190-8198.

75 Khosravi Shahi P, Fern á ndez Pineda I. Tumoral angiogenesis: review of the literature. Cancer Invest, 2008, 26(1): 104-108.

76 Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev, 1997, 18(1): 4-25.

77 Ferrara N, Davis-Smyth T. VEGF as a therapeutic target in cancer. Oncology, 2005, 69(3): 11-16.

78 Ferrara N, Alitalo K. Clinical applications of angiogenic growth factors and their inhibitors. Nat Med, 1999, 5(12): 1359-1364.

79 Ruegg C, Mutter N. Anti-angiogenic therapies in cancer: achievements and open questions. Bull Cancer, 2007, 94(9): 753-762.

80 Fruehauf JP, Meyskens FL Jr. Reactive oxygen species: a breath of life or death? Clin Cancer Res, 2007, 13(3): 789-794.

81 Azad N, Rojanasakul Y, Vallyathan V. Inflammation and lung cancer: roles of reactive oxygen/nitrogen species. J Toxicol Environ Health B Crit Rev, 2008, 11(1): 1-15.

82 Beinert T, Binder D, Oehm C, et al. Further evidence for oxidant-induced vascular endothelial growth factor up-regulation in the bronchoalveolar lavage fluid of lung cancer patients undergoing radio-chemotherapy. J Cancer Res Clin Oncol, 2000, 126(6): 352-356.

83 Brattstrom D, Bergqvist M, Hesselius P, et al. Elevated preoperative serum levels of angiogenic cytokines correlate to larger primary tumours and poorer survival in non-small cell lung cancer patients. Lung Cancer, 2002, 37(1): 57-63.

84 Tamura M, Ohta Y, Nakamura H, et al. Diagnostic value of plasma vascular endothelial growth factor as a tumor marker in patients with non-small cell lung cancer. Int J Biol Markers, 2002, 17(4): 275-279.

85 Yoshikawa T, Tsuburaya A, Kobayashi O, et al. Plasma concentrations of VEGF and bFGF in patients with gastric carcinoma. Cancer Lett, 2000, 153(1): 7-12.

86 Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med, 2003, 9(6): 677-684.

87 Biaglow JE, Miller RA. The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy. Cancer Biol Ther, 2005, 4(1): 6-13.

88 Denko NC, Fontana LA, Hudson KM, et al. Investigating hypoxic tumor physiology through gene expression patterns, Oncogene, 2003, 22(37): 5907-5914.

89 Welsh SJ, Bellamy WT, Briehl M, et al. The redox protein thioredoxin-1 (Trx-1) increases hypoxia-inducible factor 1alpha protein expression: Trx-1 overexpression results in increased vascular endothelial growth factor production and enhanced tumor angiogenesis, Cancer Res, 2002, 62(17): 5089-5095

90 Ziche M, Morbidelli L. Nitric oxide and angiogenesis. J Neurooncol, 2000, 50(1-2): 139-148.

91 Tanimoto T, Jin ZG, Berk BC. Transactivation of vascular endothelial growth factor (VEGF) receptor Flk-1/KDR is involved in sphingosine 1-phosphate-stimulated phosphorylation of Akt and endothelial nitric-oxide synthase (eNOS). J Biol Chem, 2002, 277(45): 42997-43001.

92 Fulton D, Ruan L, Sood SG, et al. Agonist-stimulated endothelial nitric oxide synthase activation and vascular relaxation. Role of eNOS phosphorylation at Tyr83. Circ Res, 2008, 102(4): 497-504.

93 Kimura H, Esumi H. Reciprocal regulation between nitric oxide and vascular endothelial growth factor in angiogenesis. Acta Biochim Pol, 2003, 50(1): 49-59.

94 Deshayes F, Nahmias C. Angiotensin receptors: a new role in cancer? Trends Endocrinol Metab, 2005, 16(7): 293-299.

95 Feliers D, Gorin Y, Gosh-Choudhury G, et al. Angiotensin II stimulation of VEGF mRNA translation requires production of reactive oxygen species. Am J Physiol Renal Physiol, 2006, 290(4): 927-936.

96 Matsumura T, Wolff K, Petzelbauer P. Endothelial cell tube formation depends on cadherin 5 and CD31 interactions with filamentous actin. J Immunol, 1997, 158(7): 3408-3416.

97 Bach TL, Barsigian C, Chalupowicz DG, et al. VE-Cadherin mediates endothelial cell capillary tube formation in fibrin and collagen gels. Exp Cell Res, 1998, 238(2): 324-334.

98 Orive G, Hern á ndez RM, Gasc ó n AR, et al. Microencapsulation of an anti-VE-cadherin antibody secreting 1B5 hybridoma cells. Biotechnol Bioeng, 2001, 76(4): 285-294.

99 Corada M, Zanetta L, Orsenigo F, et al. A monoclonal antibody to vascular endothelial-cadherin inhibits tumor angiogenesis without side effects on endothelial permeability. Blood, 2002, 100(3): 905-911.

100 Parker BS, Argani P, Cook BP, et al. Alterations in vascular gene expression in invasive breast carcinoma. Cancer Res, 2004, 64(21): 7857-7866.

101 Gonz á lez D, Herrera B, Beltr á n A, et al. Nitric oxide disrupts VE-cadherin complex in murine microvascular endothelial cells. Biochem Biophys Res Commun, 2003, 304(1): 113-118.

102 Weis S, Cui J, Barnes L, et al. Endothelial barrier disruption by VEGFmediated Src activity potentiates tumor cell extravasation and metastasis. J Cell Biol, 2004, 167(2): 223-229.

103 Usatyuk PV, Parinandi NL, Natarajan V. Redox regulation of 4-hydroxy-2-nonenal-mediated endothelial barrier dysfunction by focal adhesion, adherens, and tight junction proteins. J Biol Chem, 2006, 281(46): 35554-66.

104 Strickler HD, Wylie-Rosett J, Rohan T, et al. The relation of type 2 diabetes and cancer. Diabetes Technol Ther, 2001, 3(2): 263-274.

105 Czyzyk A, Szczepanik Z. Diabetes mellitus and cancer. Eur J Intern Med, 2000, 11(5): 245–252.
106 Stattin P, Björ O, Ferrari P, et al. Prospective study of hyperglycemia and cancer risk. Diabetes Care, 2007, 30(3): 561–567.
107 Logsdon CD, Fuentes MK, Huang EH, et al. RAGE and RAGE ligands in cancer. Curr Mol Med, 2007, 7(8): 777–789.
108 Rojas A, Morales MA. Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. Life Sci, 2004, 76(7): 715–730.
109 Kuniyasu H, Oue N, Wakikawa A, et al. Expression of receptors for advanced glycation end–products (RAGE) is closely associated with the invasive and metastatic activity of gastric cancer. J Pathol, 2002, 196(2): 163–170.
110 Sasahira T, Akama Y, Fujii K, et al. Expression of receptor for advanced glycation end products and HMGB1/amphoterin in colorectal adenomas. Virchows Arch, 2005, 446(4): 411–415.
111 Ishiguro H, Nakaigawa N, Miyoshi Y, et al. Receptor for advanced glycation end products (RAGE) and its ligand, amphoterin are overexpressed and associated with prostate cancer development. Prostate, 2005, 64(1): 92–100.
112 Morbini P, Villa C, Campo I, et al. The receptor for advanced glycation end products and its ligands: a new inflammatory pathway in lung disease? Mod Pathol, 2006, 11: 1437–1445.
113 Huttunen HJ, Fages C, Kuja–Panula J, et al. Receptor for advanced glycation end products–binding COOH–terminal motif of amphoterin inhibits invasive migration and metastasis. Cancer Res, 2002, 62(16): 4805–4811.
114 Kobayashi S, Kubo H, Suzuki T, et al. Endogenous secretory receptor for advanced glycation end products in non–small cell lung carcinoma. Am J Respir Crit Care Med, 2007, 175(2): 184–189.
115 Yan SF, D'Agati V, Schmidt AM, et al. Receptor for Advanced Glycation Endproducts (RAGE): a formidable force in the pathogenesis of the cardiovascular complications of diabetes & aging. Curr Mol Med, 2007, 8(7): 699–710.
116 Otero K, Mart í nez F, Beltr á n A, et al. Albumin–derived advanced glycation end–products trigger the disruption of the vascular endothelial cadherin complex in cultured human and murine endothelial cells. Biochem J, 2001, 359(3): 567–574.
117 Esme H, Cemek M, Sezer M, et al. High levels of oxidative stress in patients with advanced lung cancer. Respirology, 2008, 13(1): 112–116.
118 Ashino H, Shimamura M, Nakajima H, et al. Novel function of ascorbic acid as an angiostatic factor, Angiogenesis, 2003, 6(4): 259–269.
119 Tang FY, Meydani M. Green tea catechins and vitamin E inhibit angiogenesis of human microvascular endothelial cells through suppression of IL–8 production. Nutr Cancer, 2001, 41(1–2): 119–125.
120 Tang FY, Nguyen NM. Meydani M. Green tea catechins inhibit VEGF–induced angiogenesis in vitro through suppression of VE–cadherin phosphorylation and inactivation of akt molecule. Int J Cancer, 2003, 106(3): 871–878.
121 Lin T, Yen ML, Lin CY, et al. Inhibition of vascular endothelial growth factor–induced angiogenesis by resveratrol through interruption of Src–dependent vascular endothelial cadherin tyrosine phosphorylation. Mol Pharmacol, 2003, 64(5): 1029–1036.
122 Guruvayoorappan C, Kuttan G. Beta–carotene inhibits tumor–specific angiogenesis by altering the cytokine profile and inhibits the nuclear translocation of transcription factors in B16F–10 melanoma cells. Integr Cancer Ther, 2007, 6(3): 258–270.
123 Mu P, Gao X, Jia ZJ, et al. Natural antioxidant pedicularioside G inhibits angiogenesis and tumourigenesis *in vitro* and *in vivo*. Basic Clin Pharmacol Toxicol, 2008, 102(1): 30–34.
124 Huang SS, Zheng RL. Rosmarinic acid inhibits angiogenesis and its mechanism of action *in vitro*. Cancer Lett, 2006, 239(2): 271–280.

(Received: 2008–04–03 Revised: 2008–05–13)
(Edited by LIU Qian)

· 启 事 ·

致作者

为了确保图片制版的效果，请作者向本刊投稿时，请遵照以下要求。电泳图需由原始凝胶电泳图拍摄而成，并标注各条带的名称或编码，以及Marker上各迁移带的片段大小，**请勿剪切图片**。X光片及CT片应要求部位明确。组织学照片及荧光照片应注明内部标尺比例或放大倍数及染色方法等。大体标本照片在图中应有尺度标记。所有图片必须平整光滑，具有良好的清晰度和对比度，不能有折痕或缺损。

本刊编辑部