Oxidative stress in tumor microenvironment
——Its role in angiogenesis

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【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

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 (O2−) hydrogen peroxide (H2O2), peroxynitrite (ONOO−), hypochlorous acid (HOCl) and the hydroxyl radical (OH·), 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 O2− 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 O2−. 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,
growth factors, and vasoactive agents as well as by physical factors, including stretch, pulsatile strain and shear stress\textsuperscript{[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\textsuperscript{[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\textsuperscript{[14]} and diabetic vessels\textsuperscript{[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\textsuperscript{[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\textsuperscript{[17]}. COX

Cyclooxygenase is another source \(O_2^-\) of production, particularly in the cerebral circulation\textsuperscript{[18]}. PGH synthase and lipooxygenase are able to co–oxidize substances such as NAD(P)H\textsuperscript{[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\textsuperscript{[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\textsuperscript{[21]} hypercholesterolemia\textsuperscript{[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 immunoactivity to XO in endothelial cells or other cardiovascular tissues\textsuperscript{[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\textsuperscript{[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\textsuperscript{[26]}. Recent evidence suggests that increased mitochondrial \(O_2^-\) generation in endothelial cells is particularly prominent in some pathological settings. Hyperglycemia induces mitochondrial \(O_2^-\) production, which is involved in the pathogenesis of diabetic complications\textsuperscript{[27]}. Similarly, the adipokine leptin also induces mitochondrial \(O_2^-\) production by increasing fatty acid oxidation\textsuperscript{[28]}. In hypoxia–reoxygenation and ischemia–reperfusion, mitochondrial–derived \(O_2^-\) radicals are increased, where the enhanced \(O_2^-\) is at least partially responsible for a rise in endothelial permeability\textsuperscript{[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\textsuperscript{[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\textsubscript{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\textsubscript{4} partially restores NOS III–dependent NO production and reduces NOS uncoupling in hypertension\textsuperscript{[31]}, hypercholesterolemia\textsuperscript{[32]}, and smokers\textsuperscript{[33]}. Thus, BH\textsubscript{4} availability is a crucial factor in the balance between NO and \(O_2^-\) 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. For more than 100 years, tumors had been observed to be more vascularized than normal tissues. 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. Thirty-two years later, Judah Folkman 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. Furthermore, Weidner and colleagues in 1999 found that the microvascular density of the primary tumor is 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. 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 and growth factors (GF) released by tumor associated inflammatory cells are now considered important elements in local tumor microenvironment. Mast cells (MCs) are known to accumulate at the sites of angiogenesis and produce many angiogenesis promoters such as histamine, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), heparin and tryptase. Likewise, tumor associated macrophage (TAMs) are thought to induce angiogenesis through secretion of several factors, including tumor necrosis factor-alfa (TNF-α), VEGF, angiogenin and urokinase. 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. A growing body of evidences clearly support a relevant role of platelets by releasing proangiogenic substances in the tumor microenvironment as demonstrated for VEGF, platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), endothelial cell growth factor (ECGF), transforming growth factor (TGF), insulin-like growth factor (IGF), angiopoietin 1, sphingosine-1-phosphate, and matrix metalloproteinases (MMPs). However, platelets also secrete inhibitors of angiogenesis such as thrombospondin, platelet factor 4, plasminogen activator inhibitor and angiostatin. Thus, increased activation of platelets is thought 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.

**Oxidative stress as a key signal in angiogenesis switch**

The tumor angiogenesis 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. 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 chemomitted 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[60, 70]. NAD(P)H oxidases also influence tumor cell proliferation via the redox regulated transcription factor NF–κB 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
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, 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–sensitive mechanism\[116\].

At present, there are compelling evidences that oxidative stress is relevant in neoplastic 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 effects of many classical antioxidants such as, ascorbic acid\[118\], vitamin E\[109\], green tea catechins\[120\], resveratrol\[121\], and beta-carotene\[122\], 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


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