JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 3751−3768 **3751**

REVIEWS

Hypoxia Inducible Factor Pathways as Targets for Functional Foods

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The etiology of most chronic angiogenic diseases such as rheumatoid arthritis, atherosclerosis, diabetes complications, and cancer includes the presence of pockets of hypoxic cells growing behind aerobic cells and away from blood vessels. Hypoxic cells are the result of uncontrolled growth and insufficient vascularization and have undergone a shift from aerobic to anaerobic metabolism. Cells respond to hypoxia by stimulating the expression of hypoxia inducible factor (HIF), which is critical for survival under hypoxic conditions and in embryogenesis. HIF is a heterodimer consisting of the O_2-regulated subunit, HIF-1α, and the constitutively expressed aryl hydrocarbon receptor nuclear translocator, HIF-1β. Under hypoxic conditions, HIF-1α is stable, accumulates, and migrates to the nucleus where it binds to HIF-1β to form the complex (HIF-1α + HIF-1β). Transcription is initiated by the binding of the complex (HIF-1α + HIF-1β) to hypoxia responsive elements (HREs). The complex [(HIF-1α + HIF-1β) + HREs] stimulates the expression of genes involved in angiogenesis, anaerobic metabolism, vascular permeability, and inflammation. Experimental and clinical evidence show that these hypoxic cells are the most aggressive and difficult angiogenic disease cells to treat and are a major reason for antiangiogenic and conventional treatment failure. Hypoxia occurs in early stages of disease development (before metastasis), activates angiogenesis, and stimulates vascular remodeling. HIF-1α has also been identified under aerobic conditions in certain types of cancer. This review summarizes the role of hypoxia in some chronic degenerative angiogenic diseases and discusses potential functional foods to target the HIF-1α pathways under hypoxic and normoxic conditions. It is reported that dietary quinones, semiquinones, phenolics, vitamins, amino acids, isoprenoids, and vasoactive compounds can down-regulate the HIF-1 pathways and therefore the expression of several proangiogenic factors. Considering the lack of efficiency or the side effects of synthetic antiangiogenic drugs at clinical trials, down-regulation of hypoxia-induced angiogenesis by use of naturally occurring functional foods may provide an effective means of prevention.

KEYWORDS: Hypoxia; angiogenesis; bioreductive functional foods; vasoactive functional foods; antiangiogenic functional foods; nutraceuticals

1. INTRODUCTION

The "angiogenic switch" is the conversion of quiescent endothelial cells to a proliferative state whereby a tumor acquires the ability to recruit host blood vessels to grow and disseminate throughout the host's body. It has been recognized for its role in the progression and complications of several angiogenic diseases such as cancer, diabetic retinopathy, nephropathy, and angiopathy, atherosclerosis, HIV, AIDS, bowl disease, multiple sclerosis, chronic inflammation, and arthritis (*1*−*4*).

The direct medical costs and lost worker productivity and burdens to families for these chronic diseases, in the United States and around the world, are staggering in the billions of dollars. The Center for Disease Control and Prevention (CDC) and the American Cancer Society have identified cancer, from all sites, as the second leading cause of death among Americans. Cancer is responsible for one of every four deaths in the United States. In 2005, about 570 280 Americans are expected to die of cancer from all sites, up 10 000 more from 2004 combined cancer deaths. An estimated 1.4 million new cases were diagnosed in 2004. Despite advances in medical interventions, it is also predicted that aging and the increasing size of the U.S. population will cause the total number of cancer cases to double by 2050. The financial costs of cancer, for 2003, were estimated at more than $189 billion overall with $64 billion for direct

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10.1021/jf0479719 CCC: $30.25
Published on Web 04/16/2005

medical costs and $125 billion for lost productivity. Arthritis, which comprises over 100 different diseases such as osteoarthritis, rheumatoid arthritis (RA), and gout, affects nearly 70 million Americans with nearly two-thirds of people affected younger than 65 years. From CDC statistics, in 1995, arthritis cost U.S. medical care nearly $22 billion and loss of productivity cost the economy about $60 billion. The number is projected to increase dramatically and so will the cost. The National Institute of Diabetes and Digestive and Kidney Diseases of the NIH estimated that in 2002, 18.2 million Americans or 6.3% of the population had diabetes. The U.S. government statistics show that, in 2002, diabetes cost the country $132 billion. Indirect costs, including disability payments, time lost from work, and premature death, totaled $40 billion; direct medical costs for diabetes care, including hospitalizations, medical care, and treatment supplies, totaled $92 billion.

A variety of proangiogenic factors, which include hypoxia, growth factors, hormones, matrix metalloproteinases, serine proteinases, aspartic proteinases, cysteine proteinases, proteasome, signal transduction enzymes, proteins, cell adhesion molecules, metals, and oncogenes, have been identified and recognized for their ability to stimulate the angiogenesis machinery that leads to disease progression, metastasis, and death. The stimulators of angiogenesis have thus become the subject of intense research efforts, to elucidate their mechanisms of action and allow rational design of effective and selective inhibitors of angiogenesis.

Tumor angiogenesis has become the focus of extensive biomedical investigations, and its inhibition is emerging as a rational and potentially valuable new approach to cancer therapy because angiogenesis is required for tumor growth and metastasis (*4*−*6*). Activated endothelial cells are the primary targets of antiangiogenic compounds. There are several advantages to consider antiangiogenic therapy over conventional therapies. In healthy individuals, angiogenesis is normally restricted, with only about 0.01% of adult endothelial cells undergoing the process of angiogenesis at any given time. Therefore, the side effects of inhibitors of angiogenesis on normal tissues should be negligible (*4*, *7*, *8*). The inhibitors of angiogenesis affect tumor growth indirectly by targeting the steps involved in the process leading to the formation of new blood vessels and are mainly cytostatic (*9*). The inhibitors of angiogenesis can be classified in different categories: (i) endothelial growth factors inhibitors, (ii) endothelial cell signaling transduction inhibitors, (iii) inhibitors of the urokinase plasminogen activator system, (iv) inhibitors of matrix metalloproteinases, (v) inhibitors of cell proliferation, (vi) inhibitors of endothelial cell survival, and (vi) inhibitors of endothelial bone marrow precursor cells (*10*). Although acquired resistance has never been demonstrated in preclinical trials of antiangiogenesis therapy, clinical evidence has shown a gradual loss of activity by antiangiogenic drugs such as STI571 or Gleevec when these compounds were administered in monotherapy (*11*, *12*). Potential factors involved in the acquired resistance to antiangiogenic drugs include among others: (i) antiapoptotic functions of activated endothelial cells, (ii) genetic alterations such as *p53*, (iii) redundancy of tumor cell-secreted growth factors, (iv) impact of tumor microenvironment such as hypoxia and alteration of the hypoxia inducible factor-1α (HIF-1α) pathway, (v) advanced stage of the disease and age of patients while preclinical data are often collected using young animals with relatively small tumors (*13*). Vascular endothelial growth factor (VEGF) is one of the most potent stimulators of physiological and pathological angiogenesis. Hypoxia is one of the major stimulators for VEGF. Three strategies were suggested to improve the efficacy of antiangiogenic drugs: (i) inhibition of oncogene-mediated signal transduction, (ii) use of hypoxic cell cytotoxins, and (iii) vascular targeting agents (*14*).

The early stages of the angiogenic diseases are critical to the spread of the diseases and could be used as targets in disease prevention (*15*, *16*). Several antiangiogenic factors, some endogenous to tumor cells such as endostatin, angiostatin, thrombospondin-1 (TSP-1), tissue inhibitors of metalloproteinases (TIMPs), and others from dietary sources or synthesis, have been identified, characterized, and used in cell cultures, animal models, and clinical trials (*16*−*21*). Endostatin is a 20 kDa C-terminal proteoglycan fragment of collagen XVIII produced by hemangioendothelioma (*22*). Endostatin inhibits endothelial cell migration in vitro and experimental tumor growth in vivo (*22*). The ability of endostatin to bind Zn^{2+} is essential for its antiangiogenic activity (*23*). Angiostatin is a 38 kDa internal proteolytic fragment of plasminogen (*4*, *24*). Metalloproteinases such as matrix metalloproteinase (MMP)-3, MMP-7, MMP-9, and MMP-12 can generate angiostatin from plasminogen (*25*). The antiangiogenic mechanism of angiostatin remains an enigma, and it is difficult to predict the ultimate outcome of ongoing clinical trials because the mechanism of action of angiostatin is not well-known (*26*). TSP-1 is a 450 kDa homotrimeric extracellular matrix protein expressed by both normal and tumor cells (*27*). TSP-1 has been shown to inhibit tumorigenesis, angiogenesis, and prevent metastasis in several tumor models including breast, skin, and lung carcinomas, melanoma, and malignant glioma (*28*). Repression of TSP-1 promotes tumor growth (*31*, *32*). TIMPS are a family of closely related 21−32 kDa proteins found in the extra cellular matrix that regulate the activity of MMPs and have substantial influence on the activation process of MMP zymogens. Four TIMPS [TIMP-1 (29 kDa), TIMP-2 (22 kDa), TIMP-3 (24 kDa), and TIMP-4 (23 kDa)] have been identified to date (*33*).

Despite early enthusiasm for many of the endogenous and synthetic inhibitors of angiogenesis, phase III trials have not yet demonstrated significant increases in overall survival, and in some cases, toxicity remains an issue (*34*). This review concentrates on hypoxia and the potential contribution of functional foods against hypoxia inducible transcription factor pathways.

2. HYPOXIA AND ANGIOGENESIS

Hypoxia is a reduction in the normal level of tissue oxygen tension (≤6% O_2) and is a feature of most malignant and benign proliferative angiogenic diseases including cancer (*13*, *35*, *36*). Tissue pO_2 can be measured by oxygen-detecting probes such as a polarographic needle microelectrode (Ependorf, Hamburg, Germany), a luminescence-based fiber optic sensor (OxyLite from Oxford Optronix, Oxford, United Kingdom), or the comet assay (*37*). As a tumor grows, the abnormal vascular system of solid tumors (highly irregular, tortuous, and dilated, with increased vascular permeability and irregular blood flow) results in reduced or even abolished O_2 delivery to the neoplastic and stromal cells. Chemicals such as nitrite, cobaltous chloride (iron antagonist), and desferrioxamine (iron chelator) mimic one aspect of hypoxia, which is the induction of hypoxia-inducible factor-1 (HIF-1) (*38*−*42*). Anemia and the formation of methemoglobin or carboxyhemoglobin can reduce O_2 transport capacity and induce hypoxia (*43*). Anemia has been shown to induce or aggravate hypoxia and ischemic complications and is a poor prognostic factor in lymphoma, leukemia, and many types of cancer (*44*−*46*). There are two types of hypoxia:

transient and chronic hypoxia. Transient hypoxia is a temporary reduction in oxygen availability. The inadequate vascular geometry relative to the volume of oxygen-consuming tumor cells creates diffusion-limited O_2 delivery, which results in chronic hypoxia (*47*).

In response to chronic hypoxic conditions, cells in the hypoxic environment shift from aerobic (TCA cycle) to anaerobic metabolism (glycolysis, also known as Warburg effect) and respond to low O_2 levels by up-regulating the synthesis of HIF. The HIF family comprises the HIF-1α, HIF-1β, HIF-2α, and HIF-3α subunits (*48*). HIF-1α and HIF-2α, which are both regulated by cellular oxygen concentrations in a similar fashion, have been identified as key transcription factors responsible for gene expression in response to hypoxia and up-regulated in many cancers (*49*). The β-subunit of HIF, also known as aryl hydrocarbon receptor nuclear translocator, is a constitutive nuclear protein present in normoxic cells (*50*). Under normoxic conditions, the HIF-1α subunit is undetectable because it undergoes rapid ubuquitination and proteosomal degradation (*51*, *52*). Under hypoxic conditions, HIF-1α is stabilized, accumulates, translocates to the nucleus, and dimerizes with the HIF-1β subunit to form the (HIF-1α + HIF-1β) complex. The complex binds to hypoxia response elements (HREs) to form the [(HIF-1α + HIF-1β) + HREs] complex. The latter complex activates the expression of numerous hypoxia response genes of which products mediate angiogenesis, cell proliferation and survival, migration, and invasion (*51*).

Hypoxia is associated with the induction of HIF-1α activity and expression of HIF-1α genes including VEGF, angiopoietin-2 (Ang2), erythropoietin, glycolytic enzymes, increased telomerase and carbonic anhydrase-9 and -12 activities, and increased MMP and PKC activities (*17*, *50*, *52*−*54*). VEGF is mostly associated with angiogenesis and vascular permeability; erythropoietin is associated with red blood cell production; glycolytic enzymes are associated with glucose metabolism and energy; and carbonic anhydrase is associated with pH and adaptation to oxidative stress (*47*, *55*). These genes participate in the normal adaptive response of the cell to hypoxia. Under hypoxic conditions, a mutation in the tumor suppressor gene *p53* can lead to clonal selection of tumor cells with mutated *p53*, which in turn facilitates a more malignant phenotype and diminished apoptotic potential (*55*, *56*).

Hypoxia is not the only inducer of angiogenesis. Genetic alterations, such as loss of function of tumor suppressor genes such as the von Hippel-Lindau (*VHL*), *p53*, and $p16^{INK4a}$ or the activation of oncogenes including *ras*, *raf*, *HER2*/*erbB2* (*neu*), and *src*, result in increased expression of HIF-1α and HIF-1 inducible genes (*56*, *57*). HIF-1α and HIF-2α are required for normal embryogenesis because they are central to oxygen homeostasis. HIF-1α and HIF-2α knockout mice died early or had syndromes of multiple organ pathology that included retinopathy, cardiac hypertrophy, mitochondrial abnormalities, hypoglycemia, altered Krebs cycle, and several biochemical abnormalities (*17*, *49*, *58*, *59*). However, HIF-1α is overexpressed in a large number of human tumors and its overexpression correlates with poor prognosis, treatment failure, and mortality (*51*, *60*). HIF-1 is therefore an important target for cancer therapy. Tumors can also contain areas of more severe hypoxia (0.1% O_2) or anoxia (<0.1% O_2). Under these conditions, cells undergo *p53*-dependent apoptosis (*61*). The mechanisms for survival under anoxia are HIF-1α independent and, thus, differ from the hypoxic response.

3. CLINICAL SIGNIFICANCE OF HYPOXIA

Clinically, the association of hypoxia to angiogenic diseases aggressiveness has been supported by the finding that hypoxic primary diseased cells are associated with a higher rate of metastasis, genetic instability, and resistant phenotypes (*43*). Chronic and transient hypoxia have been detected in animal tumor models, and both types occur in human cancers as well (*47*). Hypoxia is often associated with low glucose concentration, high lactate levels, and low extracellular pH (*62*). Hypoxia can induce the production of stress proteins, which protect the hypoxic cells against drugs such as methotrexate and doxorubicin (*47*). However, poor outcome of treatment is not necessarily indicative of hypoxia-associated intrinsic resistance (*55*). The following paragraphs briefly illustrate the interplay between hypoxia and some major chronic angiogenic diseases.

3.1. Tumor Hypoxia. HIF-1α overexpression, as a result of either intratumoral hypoxia or genetic alteration, has been demonstrated in human cancers and leads to increased transcription of genes that encode angiogenic stimulators. The products of these genes contribute to basement membrane degradation, metastasis, and patient mortality, which are the defining features of cancer cells (*63*). HIF-1α is stabilized during hypoxia and is a major regulator of cell cycle arrest in primary cells during hypoxia (*64*, *65*). Low oxygen tension in tumors was associated with increased HIF-1α overexpression, metastasis, treatment failure, and/or mortality (*60*, *62*). Hypoxia occurs in the early stages of tumor development (before metastasis), may induce angiogenesis, and is commonly observed in noninvasive tumors such as intraductal breast cancer. Hypoxia within solid tumor cells larger than 1 mm^2 reduces the sensitivity of tumor cells to conventional (both radio- and chemotherapy) modalities, influences growth, and may increase malignant progression within nonirradiated tumors (*13*, *66*). The oxygen diffusion limit prevents diffusion of sufficient concentrations of many chemotherapeutic drugs 100−150 μm from a functional vascular capillary to be toxic to hypoxic tumor cells.

HIF-1α expression can also occur under aerobic conditions in some human cancer cells. Zhong et al. (*67*) demonstrated that human prostatic cancer lines DU 145, PC-3, PPC-1, and TSU, most notably PC-3 cells, express HIF-1α protein and HIF-1 DNA binding activity under aerobic conditions, and expression is further increased in response to hypoxia. Under normoxic conditions, growth factors such as the epidermal growth factor (EGF) and insulin-like growth factor (IGF) induce the expression of HIF-1α (*68*, *69*). HIF-1α is also activated by peptides such as insulin and interleukin-1 (IL-1) in human hepatoma cell cultures (line HepG2) under aerobic conditions (*70*). The growth factors bind to their receptors and activate respective tyrosine kinases, and the latter activate the phosphatidyl-3-inositol kinase/Akt kinase/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway that contributes to the expression of HIF-1α under aerobic conditions (*71*). Park et al. (*72*) showed that under aerobic conditions, HIF-1α is activated in gastric cancer. These authors also showed that infection with *Helicobacter pylori*, which is associated with the initiation and progression of gastric cancer, stimulates reactive oxygen species (ROS) production and stabilizes HIF-1α. The ROS-mediated stabilization of HIF-1α contributes to the transcriptional activity of HIF-1α.

Efforts to develop therapies against the hypoxic regions of tumor cells include among others (i) restoring tumor normal oxygenation; (ii) targeting the biological responses to hypoxia and the pathways leading to hypoxia tolerance through the use of bioreductive compounds, which are selectively active against

hypoxic tumor cells, and hypoxia specific gene delivery systems by exploiting the reduced pH of hypoxic environment; (iii) using inhibitors of glycolysis; and (iv) antiangiogenic compounds and vascular targeting agents (*35*, *64*). Vascular targeting is based on the finding that vessels within a tumor are phenotypically different from those in normal tissue and are therefore selectively recognizable by antibodies or ligands of the target molecules (*73*). Vascular targeted antiangiogenic compounds are based on the dependence of angiogenesis on a functional blood vessel system for survival, proliferation, and metastasis. Vascular-targeting agents aim at compromising the integrity and functionality of the tumor vessel to force a shutdown of the tumor vascular system and consequently cell death. However, at present, the narrow therapeutic window of these agents (vascular-targeting activity is achieved only at doses approaching or exceeding their maximum tolerated dose) has prevented their development (*73*). It has been reported that inhibition of HIF-1 alone will not eradicate tumor growth (*51*).

Cells expressing *src* and *ras* oncogenes up-regulate and stabilize HIF-1 under both hypoxic and aerobic conditions (*74*, *75*). Farnesyl transferase inhibitors block farnesylation and can prevent the translocation of the mature *ras* protein to the cell surface membrane, thereby reducing *ras* signaling that can promote the growth and proliferation of cancer cells (*76*). Bioactive compounds that inhibit the activity of *ras* oncogene and *src* kinase may inhibit HIF-1.

Oxidative stress is a common feature of hypoxia (*14*). Oxidative stress causes inactivation of antioxidant enzymes and favors disease progression by a rapid degeneration of endothelial cell function. Malignant cells in general are under intrinsic oxidative stress and, thus, are more vulnerable to damage by ROS generating agents. The intrinsic oxidative stress in primary human leukemia and ovarian cancer cells was associated with the up-regulation of superoxide dismutase (SOD) and catalase protein expression for O_2^- elimination, likely as a mechanism to tolerate increased ROS stress (*77*).

3.2. Hypoxia and Diabetes. Chronic hyperglycemia, in diabetic patients, is a critical factor associated with biochemical changes leading to chronic vascular complications such as retinopathy and nephropathy in concert with alteration in the expression of several growth factors and their receptors (*78*). Growth factors such as VEGF, IGF-I, platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and EGF have been associated with either the early stage or the late stages of diabetes complications and lead to endothelial cell migration and proliferation, basement membrane thickening, pericytes loss, microaneurysms, and retinal capillary nonperfusion (*78*, *79*). Retinal capillary closure causes hypoxia, which in turn leads to retinal neovascularization.

The therapeutic strategy to prevent diabetes-induced renal damage involves (i) oral administration of antidiabetic agents for glycemic control and the use of angiotensin-converting enzyme (ACE) inhibitors as primary prevention in patients with no clinical and biochemical signs of renal damage; (ii) blood pressure, glycemic control, and antihypertensive agents other than ACE inhibitors as secondary prevention to slow or prevent the progresssion from micro- to macroalbuminuria; and (ii) blood pressure control, a slightly hypoproteic diet, and dyslipidemia control as tertiary prevention to reduce the rate of renal failure (*80*). Endothelin blockage, amelioration of hypoxia by correcting reduced hemoglobin levels, an interference with the formation and accumulation of advanced glycosylation end products (AGE), and gene therapy are among the promising new trends in the therapy for diabetes nephropathy (*80*). However, graft hypoxia-induced ROS production in the early stages of islet transplantation has been identified as the major factor associated with the failure of this promising therapy for type I diabetes (*81*).

3.3. Hypoxia and Obesity. Hypoxia, in adipocytes, stimulates the HIF-1α pathway and enhances the expression of proangiogenic factors such as leptin, VEGF, MMP-2, and MMP-9 (*82*, *83*). Using differentiated 3T3-F442A adipocytes exposed to hypoxia mimics, Lolmede et al. (*83*) demonstrated rapid accumulation of HIF-1α protein levels in nuclei and the up-regulation of the expression of leptin, VEGF, and MMP-2 and MMP-9. Wound and tissue hypoxia were common in obese patients in the perioperative period and most pronounced during surgery, and supplemental oxygen tissue did not restore oxygen tension to a level free of infection risk (*84*).

3.4. Hypoxia and Atherosclerosis. Atherosclerosis, a progressive disease of large arteries, is the principal cause of heart attack, stroke, and peripheral vascular disease and is the primary cause of 50% of all mortality cases in the western world. Atherosclerosis can develop as early as the second decade of life and progress into clinical disease over time. The etiology of the disease is complex; however, epidemiological studies have delineated genetic (family history, obesity and diabetes, elevated levels of low-density lipoproteins/very low-density lipoproteins, and others) and environmental (diet, smoking, antioxidant levels, lack of exercise, and infectious agents) risk factors (*87*, *88*). Several factors that stimulate atherosclerotic lesion initiation and progression to fibrous plaques are associated with angiogenesis in vivo and include a wide range of growth factors (VEGF, angiopoietin-1, platelet-activating factor, $\alpha_v\beta_3$-integrin, glucose transporter 1, HGF, MMP-2, MMP-3, MMP-7, MMP-9, and IL-8) family of proteins (*87*−*90*). Atherosclerosis, like many other angiogenic diseases, develops cells that grow away from blood vessels and under low oxygen ($\leq$6% oxygen) conditions, i.e., hypoxic cells.

3.5. Hypoxia and Chronic Inflammation. Hypoxia regulates the expression of certain inflammatory mediators. Hypoxia affects the production of IL-2, IL-4, and interferon-γ. The early steps of RA are characterized by an alteration in blood vessel density and prominent neovascularization. These steps are followed by chronic destruction involving thickening of the synovial membrane lining the joints, inflammation and hyperproliferation of synovial cells, proinflammatory cytokines cascade, leukocyte infiltration, and tissue damage and bone resorption. In RA, angiogenesis may not keep pace with synovial proliferation, which leads to regions of hypoperfusion and hypoxia (*91*). VEGF is elevated in the serum of RA patients (*92*).

4. THERAPEUTIC STRATEGIES FOR HYPOXIA-INDUCED ANGIOGENIC DISEASES

Angiogenesis inhibitors or chemotherapeutic agents cannot efficiently kill hypoxic tumor/diseased cells because such cells proliferate slowly, are usually distant from profused blood vessels, and may have resistant phenotypes (*12*, *17*, *93*). Efforts to develop therapies that exploit the hypoxic environment of tumor cells include (i) targeting the biological responses to hypoxia and the pathways leading to hypoxia tolerance with bioreductive alkylating agents that are toxic to hypoxic cells and hypoxia specific gene delivery systems and exploit the reduced pH of the hypoxic environment; (ii) inhibitors of glycolysis, since hypoxic cells solely rely on this metabolic pathway for ATP production; and (iii) antiangiogenic compounds and vascular targeting agents (*24*, *94*, *95*).

4.1. Bioreductive Alkylating Drugs. The nuclear translocation of HIF-1α and the dimerization process may be desirable targets for the inhibition of HIF-1 activity (*71*). Bioreductive alkylating drugs are compounds that are reduced by enzymes such as flavoproteins, NADPH:cytochrome P450 reductase, NAD(P)H:quinone oxidoreductase (NQO1, DT-diaphorase, EC 1.6.99.2), or nitric oxide synthase (NOS). Under hypoxic conditions, these enzymes reduce bioreductive alkylating agents to cytotoxic, active metabolites, which can then damage and kill hypoxic malignant cells. Bioreductive compounds may be catalyzed by one- or two-electron reductase. Those catalyzed by one-electron reductase are oxygen sensitive and become effective only under hypoxic conditions. In the presence of oxygen, these compounds can be converted back to the inactive parent molecule in a process termed "futile cycling". Bioreductive compounds catalyzed by two-electron reductase utilize DT-diaphorase or NQO1, and in this case, the bioreductive compound activation may be independent of oxygen level in the tumor cells. So, the two-electron reductase is enzyme directed and generally not susceptible to futile cycling, whereas the one-electron reductase is hypoxia selective. Under hypoxic conditions, NQO1 catalyzes the two-electron reduction of quinone to produce radical species that intercalate within DNA and block topoisomerase II and restore the bioreductive compound through redox cycling (*96*−*98*).

Therapeutic initiatives to use bioreductive compounds against hypoxia have been based on some naturally occurring quinones, mitomycin C, and synthetic compounds such as tirapazamine, AQN4, or others co-administered with other drugs (*66*, *94*, *99*, *100*). However, synthetic bioreductive drugs are very toxic to normal cells and mitomycin remains the only clinically approved bioreductive compound (*13*, *101*).

NQO1 protects cells against the toxic effects of quinones (*94*, *102*). NQO1 is elevated in tumor cells, but this might be less relevant if hypoxic one-electron reduction of quinones prevails (*103*, *104*). It has been reported that the aerobic cytotoxicity of quinones and other prooxidant compounds such as the nitroaromatic compounds increases with an increase in their potential of single-electron reduction at pH 7.0 (*103*, *105*). The reactivity of quinones in NQO1-catalyzed reactions is strongly influenced by their steric parameters and does not depend on their reduction potential (*105*). Under aerobic conditions, oxygen can decrease the reduction-dependent DNA damage produced by some quinones such as mitomycin C in intact cells (*106*). Antioxidants such as ascorbate and SOD may counteract the reactivities of quinones under aerobic conditions (*107*). A vitamin C and K_3 (1.5%:0.015%) mixture was orally administered 2 weeks before mouse liver tumor (TLT cells at 10^6) implantation in C3H mice (*108*). Mice were sacrificed 42 days after tumor implantation. Histological examination revealed 40% metastasis in control animals and 27% metastasis in vitamin-treated mice. Co-administration of these vitamins (in a ratio of 100:1, for C and K_3, respectively) produced selective ovarian cancer cell death, and it was suggested that vitamins C and K_3 could be introduced into human clinics as a new, nontoxic adjuvant cancer therapy (*109*).

4.2. Inhibitors of Glycolysis. Hypoxic cells are extraordinarily dependent on anaerobic glycolysis for survival. Hypoxic tumor cells are very sensitive to inhibitors of glycolysis because these cells rely on glycolysis to produce ATP. 2-Deoxy-D-glucose (2-DG) competitively inhibits glycolysis and glycogenolysis by competing directly with glucose for glucose receptors found in the plasma membrane. Oxamate, an analogue of pyruvate, which blocks the step of glycolysis that converts pyruvate to lactic acid, also inhibits glycolysis. Cells under hypoxic conditions succumb to 2-DG or oxamate treatment because these inhibitors shut off ATP synthesis (*110*). Sodium fluoride (NaF), a component of toothpaste, at a concentration as low as 1.0 mM, completely inhibits glucose uptake (*111*). Glycolytic inhibitors could be used to specifically target the slow-growing cells of a tumor and thereby increase the efficacy of current chemotherapeutic and irradiation protocols designed to kill rapidly dividing cells (*112*). Moreover, glycolytic inhibitors could be particularly useful in combination with antiangiogenic agents, which, a priori, should make tumors more anaerobic by reducing or eliminating blood supplies (*112*). This in turn should lead to more anaerobically metabolizing tumor cells, which are naturally hypersensitive to glycolytic inhibitors. Glycolytic inhibitors such as NaF, sodium citrate, and 2-DG also minimize pH decline in muscle tissue. Most tumor cells have a lower pH value than normal cells. Treatment of prerigor muscle with sodium citrate or sodium fluoride maintained the pH of the muscle at 6.18 and 5.93, respectively (*113*). Lactic acid formation was prevented, and glycogen breakdown was delayed or inhibited. Meat tenderness increased. Treated muscle had more NADH than controls. A high NADH concentration has been associated with improved muscle color stability. Fermented wheat germ, known under the trade name of Avemar, was shown to be a strong inhibitor of G6PDH and transketolase activities (*114*). Both enzymes are involved in glucose conversion into the five-carbon nucleotide precursor ribose pool. Avemar is also a strong inhibitor of de novo nucleic acid synthesis. Although inhibition of glycolysis is an alternative to kill hypoxic cells, glycolysis is the main route that provides energy to brain functioning. Inhibition of glycolysis may be associated with the diminution of glucose metabolism observed in the brain of phenylketonuric patients and neurological dysfunction found in these patients (*115*). Therefore, functional food inhibitors of glycolysis may not be good candidates for hypoxia-induced angiogenesis inhibition unless specific tissues or organ sites can be targeted.

4.3. Antiangiogenic Compounds and Vascular Targeting Agents. The abnormal architecture of solid tumor cells offers an ideal target for antiangiogenic and vascular targeting agents (*35*). Synthetic inhibitors (such as thalidomide, Iressa, SU5416, and STI571) and antibodies to angiogenic factors or their receptors (such as avastin, herceptin, or cetuximab) have also been designed. These inhibitors have all been characterized and used in cell cultures, animal models, and/or clinical trials (*4*−*6*, *16*, *17*, *19*, *116*−*121*). The success of avastin (also known as bevacizumab), a humanized monoclonal antibody against VEGF in prolonging the lives of patients with metastatic colorectal cancer, has established the validity of the antiangiogenic approach (*122*−*124*). The success of thalidomide in certain types of cancers needs also to be mentioned (*125*, *126*). Unlike the old generation of antiangiogenic compounds that were cytotoxic in some cases, the newly investigated compounds are cytostatic, have relatively mild side effects, and need to be administered in low dose and continually (metronomic dosing) in order to sustain the inhibition of vascular recruitment by angiogenesis stimulators (*127*). Despite the broad enthusiasm regarding some success with bevacizumab, cetuximab, rituximab, trastuzumab, gefinitib, and imatinib mesylate, progress with several other antiangiogenic compounds has been slow in light of negative results observed in several large-scale phase II and III clinical trials in terms of prolonging the lives of patients with metastatic disease (*126*−*130*). Cetuximab is used against metastatic colorectal and head and neck cancers. Rituxan

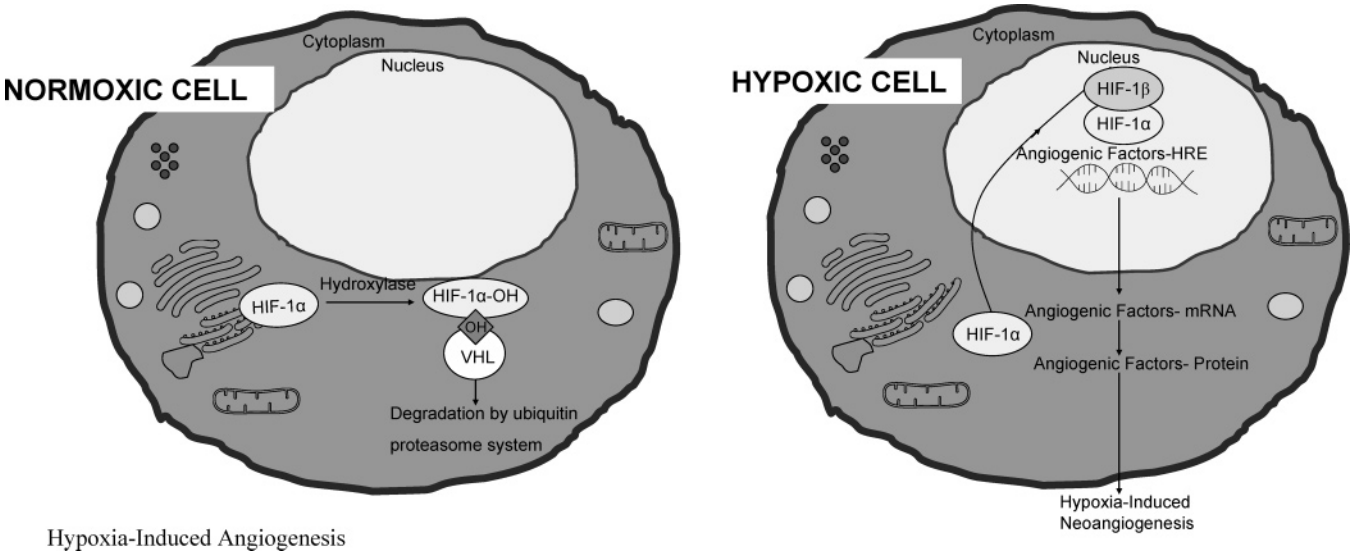

Figure 1. HIF-1α degradation in normoxic cells (**left**) and HIF-1α migration into the nucleus and stimulation of proangiogenic factors in hypoxic cells (**right**).

is a mAb used in non-Hodgkin's lymphoma. Trastuzumab or herceptin is a mAb used in breast cancer. Gefinitib (ZD1839 or Irresa) is a small molecule used in non-small cell lung cancer. Imatinib mesylate (or gleevec) is a small molecule used against chronic myelogenous leukemia. Each one of these antiangiogenic compounds mentioned above has to be used in combination with a chemotherapeutic drug for effectiveness. The first series of MMP inhibitors, such as marimastat, prinomastat, and BAY 12-9566, failed to show clear benefit in patients with advanced metastatic diseases (*12*, *131*, *132*). The failure to recognize the role of MMPs in early stages of the disease as well as the inadequacy of either the employed inhibitors or the proteases to be targeted may explain the lack of success with these series of antiangiogenic compounds. Suggestions have been made that the early steps of hypoxia or angiogenesis are good targets to develop more specific and effective types of therapy (*35*). Selective inhibition of a limited set of MMPs at early stages of tumor evolution may be much more effective than using wide spectrum inhibitors active against most MMP family members and administered to patients at late stages of the disease (*129*, *133*−*135*). Since the onset and/or progression of the angiogenic process may take months or years, sustained ingestion of antiangiogenic compounds, referred to as metronomic ingestion, would be an effective way of chronically inhibiting vascular recruitment by proangiogenic factors (*5*, *15*, *127*). The introduction of novel concepts, such as tumor degradome, and global approaches to protease analysis may facilitate the identification of the relevant MMPs that must be targeted in each individual cancer patient (*130*). The use of functional foods for targeting the early stages of chronic angiogenic diseases in order to prevent pathological angiogenesis and hypoxia-induced angiogenesis may be a valuable concept and a further link between diet and health relationship.

5. BIOREDUCTIVE FUNCTIONAL FOODS

5.1. Introduction. The characteristics of useful bioreductive functional foods should include (i) the ability to penetrate forming and poorly vascularized hypoxic regions, (ii) the ability to be enzymatically converted under hypoxic conditions to cytotoxin whereas the presence of oxygen could not favor such a conversion, and (iii) the ability to be minimally active or completely nontoxic to normoxic cells. Antihypoxic dietary compounds could prove valuable in different ways. First, epidemiological observations show that populations, such as the Japanese and Indians, who consume large amounts of bioactive compounds such as epigallocatechin gallate (EGCG) in green tea, genistein, and the Bowman−Birk inhibitor in soybean, vitamin K_2 in natto, and curcumin in turmeric regularly in their main diets, have lower incidence rates of certain chronic disease-associated disabilities such as age-related macular degeneration, coronary artery disease, colon cancer, diabetes, and hip fracture than those in the Western world, who consume only small amounts of the above-mentioned bioactive compounds (*19*, *20*, *136*−*139*). Second, the relative efficiency and safety of dietary antiangiogenic compounds would allow the classification of these compounds based on their cytotoxicity for hypoxic and normoxic tumor cell biomarkers and for physiologically normal cells. Third, suggestions could be made for dietary patterns that may help reduce the ongoing epidemic of angiogenic diseases such as juvenile obesity and diabetes. Food-derived factors with potential inhibitory activities against hypoxic disease cells are discussed in the following sections with the structures of the bioactive compounds shown in **Figure 2**.

5.2. Quinones. Quinones are a general term for naturally occurring compounds found in plants, fungi, and bacteria, and represent important components of the electron transport chains involved in cellular respiration and photosynthesis. Quinones may also be generated through metabolism of hydroquinones and/or catechols (*140*). Quinones are Michael reaction acceptors, and some quinones are potent redox active compounds. As acceptors, the cytotoxicity of quinones is related to the generation of ROS after redox cycling and/or their reaction with cellular nucleophiles such as glutathione (GSH) or cysteine residues on proteins leading to the depletion of cellular GSH levels and/or protein alkylation and/or oxidative stress (*104*). Other quinones, such as *p*-benzoquinones, have the propensity of reacting with nucleophilic amino groups on proteins or DNA (*141*). The metabolic activation of quinones under hypoxic conditions occurs, in part, as a result of the bioreductive activation by the flavoprotein NAD(P)H:quinone-acceptor oxidoreductase (NQO1) (EC 1.6.99.2). GSH reacts with some quinones to form polyphenolic−GSH conjugates whose toxicity (neurotoxicity, hematotoxicity, and nephrotoxicity) sometime exceeds the reactivity of the parent quinone (*142*). Antioxidants

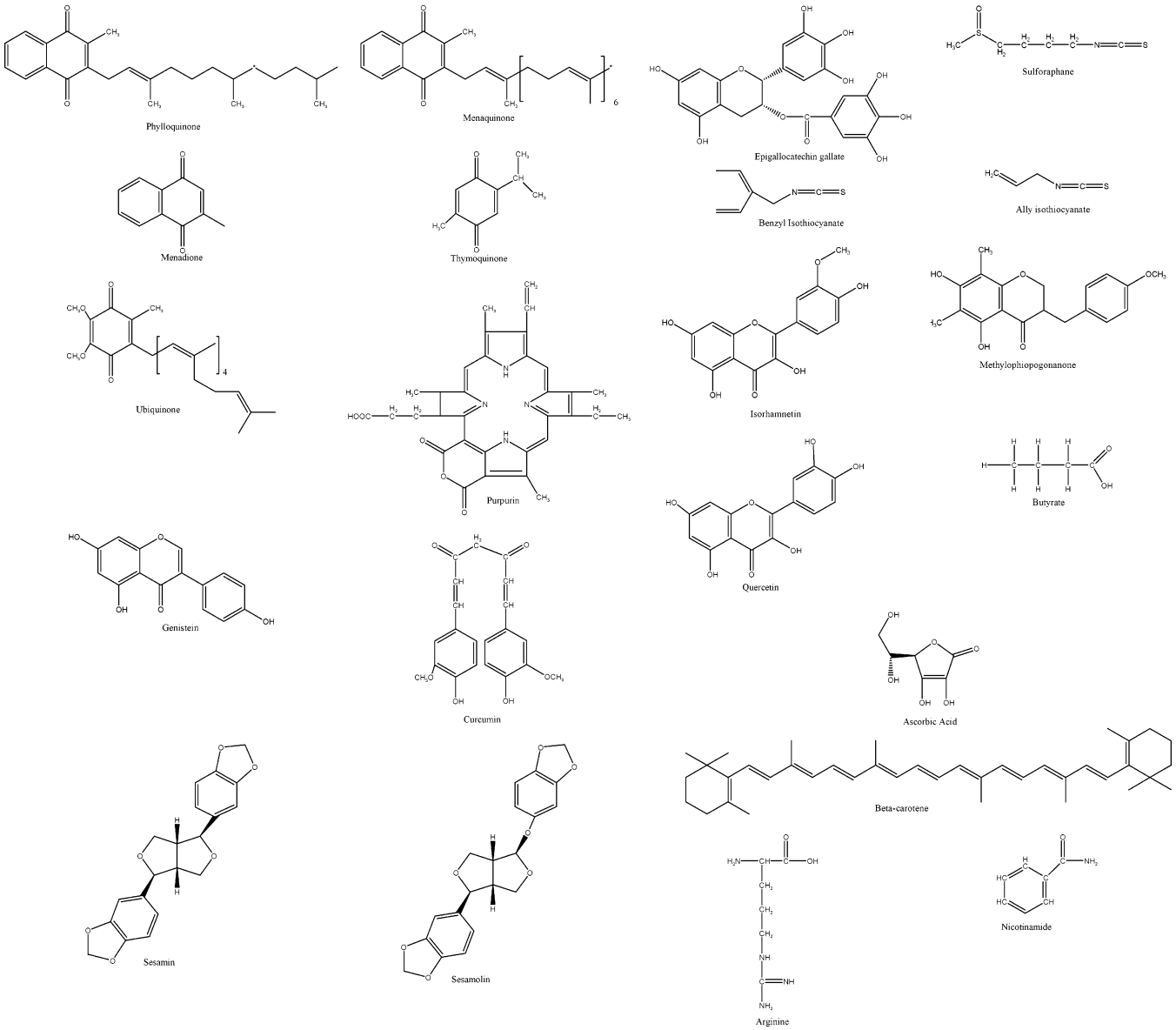

Figure 2. Structures of food-derived bioactive compounds with inhibitory activities against hypoxic tumor cells.

such as desferrioxamine and GSH reductase protect against the cytotoxicity of quinones (*142*). NQO1 reduces quinones to hydroquinones, which can be glucuronidated or sulfated for cellular excretion. NQO1 is lowly expressed in normal cells but is expressed at high levels in organs such as the kidney and liver that have high detoxifying activities. Individuals with low or no NQO1 activity had a higher risk of lung, urological, and hematological cancers than individuals with wild type (*142*−*146*). Cruciferous vegetables such as broccoli and Brussels sprouts up-regulate the activity of NQO1 (*147*). The aerobic cytotoxicity of quinones increases with an increase of their potential of single-electron reduction potential at pH 7.0 (*142*). However, the role of single- and two-electron reduction in the cytotoxicity of quinones is not well-known. Naturally occurring quinones are extensively used as components of multivitamin formulation, dyes, platelet antiaggregation, inhibitors of thromboxane A_2, and clinically as antitumor drugs and antiallergens (*148*).

5.2.1. Naphthoquinones. Naphthoquinones constitute the largest group of naturally occurring quinones (*149*). Among the naphthoquiones, 2-methyl-1,4-naphthoquinones constitute a family of vitamin K, which includes phylloquione (vitamin K_1), menaquinone (vitamin K_2), and menadione (vitamin K_3). Phylloquinone occurs in fish, algae, and foods of plant and animal origin (*150*). Vitamin K_1 protects cells against oxidative stress induced by GSH depletion (*151*, *152*) (**Figure 1**). Reduced phylloquinone is a cofactor for γ-glutamylcarboxylase, an enzyme that catalyzes the posttranslational synthesis of γ-carboxyglutamic acid (Gla) from glutamic acid in vitamin K-dependent proteins. The γ-carboxylation reaction generates phylloquinone epoxide, which in turn is reduced back to phylloquinone by thiols and epoxide reductase. However, warfarin inhibits the cycling and interrupts the activation of blood coagulation factors (*153*). Menaquinones (**Figure 1**) occur naturally in bacteria and occur in mammals as a product of conversion of phylloquinone by the intestinal flora (*154*, *155*). K vitamins are liposoluble. Vitamin K_1 is better absorbed as phylloquinone-fortified oil than from vegetables, and the average daily intake is 90−120 μg/day for younger or older people (*156*). Vitamin K bioavailability is suppressed by vitamins A and E (*157*). Vitamin K_1 was less cytotoxic than vitamins K_2 and K_3 against HSC-2 and HSG human oral tumor and promyelocytic HL-60 leukemic cell lines under aerobic conditions (*158*). The bioreductive and nontoxic nature of some dietary quinones such

as vitamin K_1 (phylloquinone) suggests that these bioactive compounds may have antihypoxic and antiangiogenic activities. The antitumor action of vitamin K has been investigated for many years (*159*). Pretreatment of rat carotid rings with vitamin K_1 at 5×10^{-8}, 5×10^{-7}, and 5×10^{-6} mol/L prevented the inhibitory effect of hypoxia-induced reduction in *E*(max) in intact rings (*160*).

Vitamin K supplementation has been hypothesized to protect against neuronal damage associated with Alzheimer's disease and vascular dementia (*161*). Carrie et al. (*161*) provided in vivo evidence of the positive relationship between vitamin K (phylloquinone and menaquinone-4) supplementation and delayed age-related degeneration in the nervous system.

5.2.2. Terpenoid Quinones. Thymoquinone (**Figure 2**), a terpenoid quinone, is the major component (28−57%) of black seed (*Nigella sativa*) oil (*162*). Black seeds or its oils are commonly used in Southeast Asia, the Middle East, and Europe for culinary purposes and as antioxidant, antiinflammatory, analgesic, anticarcinogenic, antimutagenic, antihepatotoxic, protection against colitis, antidiabetic, antimicrobial, antiulcer, oxidative stress inhibitor, radical scavenger, and as cyto- and cardiovascular protective dietary compounds (*163*). Thymoquinone easily dimerizes in water to form dithymoquinone. The daily plasma concentration of thymoquinone, the concentration at which thymoquinone becomes toxic to normal cells, and the activity of thymoquinone under hypoxia are not known.

5.2.3. Ubiquinone. Ubiquinone (coenzyme Q_{10}, CoQ_{10}) is a lipid soluble essential cofactor of the electron transport chain where it accepts electrons from complexes I and II and transfers them one at a time to complex III in the inner mitochondrial membrane (**Figure 2**). CoQ_{10} is important in ATP synthesis and acts in its reduced form (ubiquinol) as an important antioxidant for cardiolipin in the mitochondria (*164*). CoQ_{10} is synthesized de novo, increases with exercise and cold adaptation, but decreases with aging (*164*). Antioxidation, immune stimulation, prevention of heart damage in patients on chemotherapy for cancer, diabetes complications, and potential treatment for early Parkinson's disease have all been ascribed to CoQ_{10}. Organ meat (heart, liver, and kidney), fish, nuts, soy, spinach, and yeast are good sources. The maximum daily dose recommended by Q_{10} producers is 30 mg, and levels of up to 200 mg of Q_{10} were associated with 6.1-fold increase in plasma Q_{10} levels and decreased lipid peroxidation in vivo (*165*). However, the bioavailability of Q_{10} supplements is not equally the same (*166*). Food fortification with Q_{10} may continuously protect the mitochondria against respiration-linked oxygen stress, with preservation of the genomic and structural integrity of these energy-producing organelles and concomitant increase in functional life span. However, the potential bioreductive and antiangiogenic activity of Q_{10} has never been documented. Similarly, the utilization of Q_{10} as a functional food, not as a dietary supplement, has never been suggested.

5.2.4. Anthraquinones. Purpurin (1,2,4-trihydroxyanthraquinone) is a dietary quinone red pigment isolated from madder root (*Rubia tinctorium*). Several biological activities including antimutagenicity, inhibition of human cytochrome P450 1B1, 1A1, and 1A2, antigenotoxicity, inhibition of DNA damage induced by carcinogens, inhibition of xanthine oxidase, and inhibition of trypanozome cruzi have been ascribed to purpurin (*167*). Purpurin (**Figure 2**) was a better inhibitor of Trp-P-2(NHOH) mutagenicity than EGCG or chlorophyllin, both of which are well-known antimutagenic and antiangiogenic dietary compounds (*167*).

Anthraquinones such as emodin, chrysophanol, and physcion were identified in vegetables (peas, cabbage, lettuce, and beans) at concentrations between 0.04 and 36 mg/kg (*168*). Whereas emodin was genotoxic, chrysophanol and physcion were not. However, the authors suggested that the average daily intake of these anthraquinones did not pose a health threat in a balanced human diet because of the protective effects of the food matrix.

5.3. Challenges to Dietary Quinones as Bioreductive Functional Foods. The ability of quinones to redox cycle and create ROS and their reactive electrophile characteristics, which cause them to form covalent adducts with cellular macromolecules, are the basis of their potential pro-oxidant effects. The relative contribution of oxidant and electrophilic properties to quinones is influenced by chemical structure, in particular substituent effects (*169*). Lame et al. (*170*) demonstrated, using human bronchial epithelial cells, that 1,4-benzoquinone and 1,4-naphthoquinone caused direct arylation of critical cellular macromolecules such as proteins or DNA with potential for cellular toxicity. Protein and nonprotein sulfhydryls are major targets of reaction with quinones. The reaction is often considered protective to the body because the thiol function in GSH serves as a sacrificial nucleophile that spares critical nucleophilic sites on cellular macromolecules from irreversible modification (*140*). The capacity for GSH synthesis is insufficient to maintain GSH concentrations when tissues are exposed to redox cycling compounds such as quinones. GSH depletion may enhance quinone toxicity that may cause morbidity or death (*171*). GSH deficiency is a characteristic of most chronic angiogenic diseases. Dietary antioxidants such as vitamin C are known to down-regulate the toxicity of quinones. Quinone cosupplementation with vitamin C, GSH, or cruciferous vegetables may negate the toxic effects of certain quinones on normal cells. GSH supplementation from fruits and vegetables, and not GSH from meat, was reported to have an inverse relationship with the risk of oral and pharyngeal cancer (*172*, *173*). GSH has a short plasma half-life, and oral bioavailability is poor, suggesting that a constant replenishment and methods to enhance bioavailability are needed.

Cardiotoxicity is the major side effect observed when alkylating agents such as quinones are used at high dose in combination with other cytotoxic agents against cancer and other angiogenic diseases such as multiple sclerosis (*174*, *175*). Toxicity may be associated with total dose, rate of administration, individual predisposition to cardiovascular disorder, and combination with cytotoxic agents. However, cardiotoxicity by quinones can be prevented by oral administration of taurine, which exerts an opposing effect on myocardial calcium content and lipid peroxidation (*176*). Vitamin K, thymoquinone, and purpurin are not cardiotoxic (*159*, *163*, *167*).

6. FLAVONOID AND PHENOLIC INHIBITORS OF HYPOXIA

6.1. Genistein. Genistein (**Figure 2**), which has demonstrated effectiveness in vitro and in clinical trials, has a structure similar to semiquinones (*177*). The antiangiogenic activity of genistein in pancreatic cancer was hypothesized to be mediated by the inhibition of HIF-1 and down-regulation of VEGF (*178*). Genistein, orally administered at 2, 4, or 8 mg/kg to cancer patients, was well-tolerated, bioavailable with a plasma concentration equivalent to a concentration that is associated with antimetastatic activity in vitro (*179*). A rash was the only side effect reported in this study. Sasamura et al. (*180*) suggested that genistein may be effective for chemoprevention in individuals at risk of developing renal cell carcinoma because genistein blocked hypoxia-induced VEGF by inhibiting *src* tyrosine kinase

in the cancer cells. Kumi-Diaka and Towsend reported that higher doses of genistein (100 μg/mL) on Sprague−Dawley rat sperm cells could potentially suppress male fertility via suppression of acrosome reaction while low doses could enhance fertility by promoting acrosome reaction (*181*).

6.2. Curcumin. Pharmacologically, curcumin (**Figure 2**) has been found to be safe; human clinical trials indicated no dose-limiting toxicity when administered at doses up to 10 g/day and enormous potential in the prevention and therapy of cancer (*121*). Prolonged incubation (18 h) of bovine aortic endothelial cells with curcumin (5−15 μM) in normoxic or hypoxic conditions resulted in enhanced cellular resistance to oxidative damage (*182*). Hypoxia was found to be an important factor in aggravating the inflammatory lesion in RA, through increased production of cyclooxygenase-2 (COX-2)-derived nociceptive eicosanoids and increased release of tissue-damaging MMPs (*183*). Induction of COX-2 by inflammatory cytokines or hypoxia-induced oxidative stress can be mediated by nuclear factor κB (NF-κB). NF-κB is released following phosphoryltion of IκB. Curcumin prevents phosphorylation of IκB and prevents the release of NF-κB; as a result, curcumin inhibits the induction of COX-2, an important angiogenic modulator in colon cancer (*184*).

6.3. Lignans. Cell viability and lactate dehydrogenase (LDH) activity data of neuronal and PC12 cells under hypoxia indicated that sesamin and sesamolin (**Figure 2**), two antioxidant lignans from sesame seeds, dose dependently reduced the activity of LDH and inhibited MAPKs and caspase-3 (*185*). It was suggested that the activity of sesamin and sesamolin may have been related to the suppression of ROS generation and MAPK activation.

6.4. EGCG. Hypoxic rats treated with high doses of EGCG (25 or 50 mg/kg) had lower levels of NADPH-d/nNOS expression than control rats, and it was suggested that EGCG may attenuate oxidative stress following acute hypoxia (*186*). Hydrocephalus is a progressive brain disorder characterized by abnormalities in the flow of cerebrospinal fluid and ventricular dilatation and associated with increased expression of VEGF, HIF, TGF-β1, and MMP-9 that leads to cerebral atrophy and, if left untreated, can be fatal. A single daily dose of 50 mg/kg of EGCG injected into the peritoneum of hydrocephalus-induced infantile rats for 15 days significantly reduced periventricular white matter malondialdehyde (MDA) levels when compared with nontreated hydrocephalic animals (*187*). The antiangiogenic activities of EGCG have been reported using in vitro and in vivo models (*188*, *189*). Green tea extract AR25 was evaluated in moderately obese patients for 3 months and was associated with a 4.6% decrease in body weight and 4.48% decrease in waist circumference (*190*). The mechanism of inhibition was reported to involve, among others, inhibition of lipases and stimulation of thermogenesis. Catechins, in general, are bioavailable, but the bioavailability is very low (*191*). These authors reported that approximately 1.68% of ingested catechins were present in the plasma, urine, and feces, and the apparent bioavailability of the gallated catechins was lower than the nongallated forms.

6.5. Isothiocyanates. Sulforaphane (4-methylsulfinylbutyl-isothiocyanate), the major isothiocyanate released upon hydrolysis of broccoli glucoraphanin, induces detoxification enzymes such as quinone reductase, which in turn can amplify the effectiveness of quinones under hypoxic conditions. Sulforaphane down-regulates COX-2 and NF-κB at the translational level (*192*). Sulforaphane and other isothiocyanates such as benzyl isothiocyanate and allylisothiocyanate provide powerful protection against carcinogenesis, mutagenesis, and other forms of toxicity by electrophiles and reactive forms of oxygen (*147*, *192*). Broccoli, broccoli sprouts, and broccoli seeds are dietary sources of glucosinolate and are reported to be functional foods because of their contents of sulforaphanes (*193*−*195*). *H. pylori* can stabilize HIF-1 under aerobic conditions (*72*). Sulforaphane might be beneficial in the treatment of *H. pylori*-infected individuals. It is known that *H. pylori* colonization of the gastrointestinal epithelial cells is associated with gastric adenocarcinoma. *H. pylori*-infected gastric cells produce ROS, which stabilize HIF-1 protein in human gastric cancer cells under normoxic conditions (*72*). Sulforaphane temporally eradicated *H. pylori* in three patients out of nine who consumed 14, 28, or 56 g of broccoli twice daily for 7 days (*196*). *H. pylori* was completely eradicated from 8 out of 11 mice bearing human gastric xenografts after 5 day administration of sulforaphane at a dose of 1.33 mg a day in each xenograft (*197*). It was suggested that the average daily intake of glucosinolate, which is estimated at 100 mg of sulforaphane, can provide similar results to humans (*198*).

6.6. Other Phenolic Inhibitors of Hypoxia. The VEGF gene is enhanced under hypoxic conditions, and its transcription is dependent on HIF-1 levels. Flavonoid compounds such as isorhamnetin, luteolin, quercetin, and methyl ophiopogonanone B at concentrations of above 10 μg/mL inhibited the accumulation of VEGF mRNA and HIF-1 in HepG2 cells under hypoxic conditions or in $CoCl_2$-treated HepG2 cells (*199*).

Quercetin significantly inhibited hypoxia-induced functional and structural tubular injury in addition to lipid peroxidation but did not alter hypoxia-induced ATP depletion in freshly isolated rat renal proximal tubules (*200*). However, Wilson and Poellinger (*201*) reported that dietary flavonoid quercetin also activates HIF-1α in all steps of its activation pathway, in a manner similar to hypoxia. These authors found that quercetin, an inhibitor of Ser/Thr kinases, stabilizes HIF-1α and causes nuclear localization of the protein in a transcriptionally active state. These results indicate that quercetin regulates HIF-1 function under aerobic conditions and suggest further studies into the role of quercetin as a functional food.

6.7. Short Chain Fatty Acids. The short chain fatty acid butyrate, a product of bacterial fermentation of dietary fiber in the large bowel, repressed HIF-1α nuclear sequestration through inhibition of HIF-1α nuclear translocation in 2 mM butyrate-treated Caco-2 cells (*40*). The authors demonstrated that diminished HIF-1α nuclear presence was associated with reduced VEGF levels in butyrate-treated Caco-2 cells. However, butyrate has very limited bioavailability (*202*). The limited bioavailability of butyrate may limit its usefulness in the circulation.

6.8. Vitamins. Sodium ascorbate (**Figure 2**) at a physiological concentration range (25−50 μM) suppressed HIF-1α levels and HIF transcriptional targets in PC3 prostate, OVCAR3 ovarian, and Hs 578T breast cancer cells under normoxic conditions for at least 24 h (*203*). Under normoxic conditions, vitamin C at 25 μM inhibited HIF-1α expression, induced by 25 nM of IGF-I and 100 nM of insulin, in serum-deprived MCF-7 cells. Chronic hypoxia enhances agonist-evoked rises of $[Ca^{2+}]i$ in cortical astrocytes and increases production of amyloid β-peptides (Aβ Ps) of Alzheimer's disease (*204*). Vitamin C at 200 μM prevented the rises of $[Ca^{2+}]i$ in hypoxic cells. Ascorbic acid at 0.5−2.5 μM demonstrated angiostatic activity in vitro (inhibition of tube formation on Matrigel), in vivo using the CAM assay (through both its antioxidant properties and the stimulation of collagen synthesis), and was suggested to be

useful as a supplementary therapy in various angiogenic diseases (*205*). Hypoxia decreased the ascorbate content, which implies physiological activity of ascorbate carried alongside the lipophilic ascorbyl palmitate (AP) molecule (*206*). AP was able to cross biological barriers and satisfied the tissue demand for ascorbate better than the hydrophilic form. Sodium ascorbate is a mineral salt of ascorbic acid, buffered, and therefore less acidic than ascorbic acid. Sodium ascorbate and ascorbic acid are chemically identical and equally bioavailable. There are no known differences in their biological activity. Other mineral salts of ascorbic acid include calcium, potassium, molybdenum, zinc, and magnesium ascorbate. Mineral ascorbates are often recommended to people with gastrointestinal problems (abdominal pain or diarrhea) who avoid plain ascorbic acid. Sodium ascorbate generally provides 131 mg of sodium per 1000 mg of ascorbic acid and may not be advisable for individuals following low sodium diets (e.g., for high blood pressure). Other ascorbates may be preferred because they provide less than the upper level of mineral intake for adults per day. For instance, pure calcium ascorbate provides 114 mg of calcium per 1000 mg of ascorbic acid and the RDA for calcium is 1000−1200 mg/day (*207*). Nicotinamide or vitamin B3 is described in section 7 as a vasoactive compound.

6.9. Carotenoids. Hypoxia induces oxidative stress in organisms leading to tissue injury and, as a result, causes an increase in MDA levels in plasma and tissues and a concurrent decrease in blood GSH and glutathione peroxidase (GPx). β-Carotene supplementation at 10 mg/kg body weight to male albino rats induced a significant decrease ($P < 0.05$) in MDA and an increase in plasma and tissue GSH levels in animals exposed to hypoxia (*208*).

6.10. Selenium. Selenium is a cofactor for GPx, and selenium-containing GPx catalyzes the oxidation of reduced GSH to GSH disulfide, thereby reducing various peroxides to nontoxic compounds. Sprague−Dawley rats exposed to hypoxia in a hypobaric chamber 6 h daily for 1 week showed an increase in MDA levels in plasma and tissues and a decrease in blood GSH, GPx, and selenium (*209*). Sodium selenite supplementation at 10 μg per kg BW reversed the trend. When the rats were supplemented with Se and exposed to hypoxic stress, Se significantly inhibited the increase in MDA levels in plasma and other tissues and enhanced GSH levels in all tissues studied. Supplementation with selenoselenite and selenocysteime at 1 μM each enhanced the activity of Se-dependent GPx in cultured normoxic rat cardiomycytes and cardiomycytes subjected to hypoxia/reoxygenation (*210*). Between 50 and 60% of cancer patients have tumors harboring mutations or deletions of *p53*, and these patients in general have a poorer prognostic than patients with tumors harboring wild-type *p53* (*211*). Selenium confers protection against cancer because it spares an important tumor suppressor gene *p53* (*212*). Inorganic selenate and selenite predominate in water, whereas organic selenomethionine and selenocysteine are found in cereals and vegetables.

6.11. Amino Acids. Nitric oxide, a bioproduct of arginine hydrolysis, in hypoxia prevented the accumulation and stabilization of HIF-1α as a result of an increase in prolyl hydroxylase. It also allowed redistribution of oxygen toward nonrespiratory oxygen-dependent targets such as prolyl hydroxylases so that they did not register hypoxia (*213*). Arginine as an amino acid has not yet been suggested as a functional food. Taurine (2-amino ethane sulfonic acid) is an intracellular amino acid with various biological and physiological functions such antioxidant, inhibition of advanced glycation end products, and prevention of diabetes neuropathy and retinopathy (*214*−*217*). Taurine is added in sport drinks but has not yet been suggested as a functional food (*217*−*220*).

7. VASOACTIVE FUNCTIONAL FOODS

Nicotinamide or vitamin B3 (**Figure 2**), a blood flow modifier and inhibitor of transient blood flow fluctuation, is commonly used to decrease perfusion-limited hypoxia and improve tumor oxygenation (*60*, *221*−*223*). Nicotinamide enhances the effects of radiotherapy and improves delivery of chemotherapeutic agents to the tumors (*224*). Nicotinamide, thought to act by suppressing the transient closure of small blood vessels that cause intermittent tumor hypoxia, induced a small increase in blood oxygenation but no detectable change in perfusion/flow (*225*). Nicotinamide used in combination with normobaric carbogen (95% O_2 + 5% CO_2) increased the radiation sensitivity of CaNT tumors under 21, 95, and 100% oxygen in animal models (*226*). ARCON (accelerated radiotherapy with carbogen and nicotinamide) is a new therapeutic strategy that combines radiation treatment modifications, with the aim of counteracting the resistance mechanisms associated with tumor cell repopulation and hypoxia (*224*). ARCON has produced promising results in terms of tumor control in phase I and II clinical trials. Nicotinamide at 80 mg/kg was reported to show side effects such as nausea, acute skin and mucous membrane toxicity, gastrointestinal toxicity, and vomiting (*227*). However, these side effects significantly decreased at a dose of 60 mg/kg (*228*). Nicotinamide is a vitamin with a GRAS status. Studies to investigate the effect of long-term ingestion of higher dose ($\leq$60 mg/kg) of nicotinamide on hypoxia-induced angiogenesis are needed.

8. INHIBITORS OF HIF-1 PATHWAY UNDER AEROBIC CONDITIONS

Cells expressing *src* and *ras* oncogenes, as well as compounds such as desferrioxamine and $CoCl_2$, enhance and stabilize HIF-1α in vivo. Dietary compounds that inhibit HIF-1α pathway in vivo would be effective chemopreventive functional foods. Isoprenoids, a diverse class of volatile phytochemicals present in minute concentrations in many fruits and vegetables and cereal grains may be of great interest in functional foods because isoprenoids inhibit farnesyl protein transferase (FPTase) (*229*). FPTase stabilizes HIF under aerobic conditions. There are about 23 000 isoprenoid types in food and vegetables. Isoprenoids in edible fruits and vegetables include D-limonene, perillyl alcohol, menthol, γ-tocotrienol, β-ionone, perillaldehyde, carvacrol, thymol, β-ionone, geraniol, geranylgeraniol, perillylamine, farnesol, and DL- α-tocopherol, oryzanol-free tocotrienol-rich fraction of rice bran oil, and tocotrienols from palm oil, to name a few (*230*−*232*). Their actions are synergistic at very low doses (*231*, *232*). D-Limonene and gallotannin had a strong inhibition on FPTase (*233*). No clinical benefits were observed in patients with metastatic breast and colon cancers and advanced ovarian cancer using 2025 mg of perillyl alcohol four times daily (*234*). It was concluded that the potentials of perillyl alcohol and other isoprenoids on metastatic cancer had little prospect because of the dose-limiting gastrointestinal toxicities. Reviews of experimental and population studies suggest that the appropriate position for dietary isoprenoids may be as chemopreventive agents (*235*). The National Cancer Institute has an ongoing study that assesses disease recurrence in patients previously treated for stages I−IIIA breast cancer. Farnesyl transferase inhibitors that modify Ras proteins have shown remarkable antitumor activity in preclinical models and are currently under phase II

and III clinical evaluation (*236*). Phorbol esters, such as phorbol-12-myristate-13-acetate, a known tumor promoter, was reported to stabilize a novel HIF-1α isoform, HIF-1α (785), under aerobic conditions (*237*). Overexpression of HIF-1α (785) enhanced tumor growth in vivo.

9. DEVELOPING HIF-1 PATHWAY-CONTROLLING FOOD PRODUCTS: OPPORTUNITIES AND CHALLENGES

Complete or irreversible inhibition of HIF-1 pathway may have a dramatic repercussion on the progression of tumor angiogenesis. However, irreversible inhibition of HIF-1 pathway may not be desirable since HIF-1 is also critical in embryogenesis. Down-regulation of HIF-1 pathway may be a more appropriate approach to disease prevention. The episode of thalidomide needs to be remembered and not repeated. Feeding 200 mg of chocolate rich in catechins and theobromine to 2 month old Balb/c mice (an equivalent of 200 g of chocolate per person daily) decreased the relative length of limbs and thigh bones in 4 week old progeny and decreased VEGF content of offspring femoral bones (*238*). Therefore, ingestion of large amounts of antiangiogenic functional foods by pregnant women or other individuals may require medical supervision.

Down-regulation of HIF-1 pathway may also become important for diabetic retinopathy, pulmonary hypertension, obesity, and atherosclerosis since VEGF is a major up-regulator in these diseases. Functional food activators of HIF-1 pathway may show potentials in diseases such as myocardial ischemia and diabetes wound healing, which are characterized by insufficient angiogenesis.

Most dietary inhibitors of angiogenic diseases are found in minute concentrations in their natural sources. The identification of dietary compound inhibitors of hypoxia-induced angiogenesis provides tremendous research justification for plant and animal geneticists, food scientists, and nutritionists in order to improve the yield, design appropriate technologies for processing, and conduct nutritional evaluation studies. The challenge posed by finding ways to optimize the in vivo effectiveness of these compounds should not be underestimated. Some antiangiogenic functional foods as well as functional foods that down-regulate HIF-1 pathways will require formulation in special carriers to prevent their hydrolysis in the gastrointestinal tract or binding to unintended serum proteins. For instance, protamine inhibits excessive angiogenesis and blocks endothelial, fibroblast, and platelet growth factors, while protamine sulfate inhibits insufficient angiogenesis by accelerating gastric ulcer healing (*239*, *240*). However, protamine is easily hydrolyzed by chymotrypsin (*241*). Naturally occurring coumarins such as 6′,7′-dihydroxybergamottin and bergamottin from grapefruit juice are known to simultaneously inactivate several carcinogen metabolizing enzymes CYP 450s and inhibit drug transporter MDR1 P-glycoprotein (Pgp) and multidrug resistance protein 2, both of which are expressed at apical membranes (*242*, *243*). Curcumin, ginsenosides, piperine, some catechins from green tea, and silymarin were also found to be reversible inhibitors of Pgp (*244*). Innovative training and research that strengthen our fundamental understanding of the relationships between functional foods and cell growth in health and disease before and after the angiogenic switch is needed.

Biomarkers are important measurable phenotypic molecular signatures of a cell that aid in early disease detection, risk assessment, or response to a particular therapeutic intervention. Numerous direct or indirect biomarkers of angiogenesis have been identified, and in some cases, biomarkers are similar across several angiogenic diseases (*17*). Studies using transgenic mice overexpressing angiogenic stimulators have demonstrated a crucial role for these markers in pathological angiogenesis development. However, most of these biomarkers have been identified in the invasive and proliferative stages of the angiogenic disease; are related to cell proliferation, invasion, migration, hormone dependence, apoptosis, and metastasis; and few of these factors are proving clinically useful for healthy life survival (*14*). Most antiangiogenic compounds are protease inhibitors since angiogenesis is mostly a cascade of proteolytic activities (*16*). Antiangiogenic functional foods have an advantage over synthetic inhibitors in that most of these compounds are regularly consumed by some populations around the world, have shown to be nontoxic at physiological concentrations when taken orally, and can be taken out when not in need (*16*, *245*, *246*). Reversible protease inhibition is an advantage over irreversible inhibition because most enzymes associated with angiogenesis have been described to be important for the formation of new blood vessels in both physiological and pathological conditions. For instance, MMP-3 deficient mice (irreversible inhibition) exhibited impaired wound contraction and collagenase resistant mice showed severe delay in wound healing, indicating that antiangiogenic compounds that are reversible on withdrawal may be beneficial to the host because of the functional overlap between the functions of enzymes involved in angiogenesis (*134*).

10. FUTURE PROSPECTS

The promise and completion of the Human Genome Project have raised expectations that the knowledge of all genes and proteins in the human body will lead to the identification of better biomarkers for angiogenic diseases. The complex array of factors associated with chronic angiogenic diseases makes it difficult to accurately predict the outcome for each individual. Individuals may react differently to the same antiangiogenic food. In the future, functional foods may be personalized depending on individual reactions to a dietary bioactive compound. In the future, it will be necessary to categorize functional foods as agents directed against hypoxia, HIF, HIF-dependent genes, or indirect agents that are directed against angiogenesis pathways.

Preoteases initiate, modulate, terminate, and control important cellular functions such as DNA replication, cell cycle progression, cell proliferation and migration, tissue remodeling, hemostasis, wound healing, immunity, angiogenesis, and apoptosis. Proteases, protease substrates, and protease inhibitors will be an important focus for functional foods research. Reversible protease inhibition is a feature of most functional foods and will be an advantage over many synthetic irreversible inhibitors of angiogenesis (*16*).

Degradomics is the application of genomic and proteomic techniques to identify the degradomes (the complete set of proteases that are expressed at a specific time by a cell, tissue, or organism), degradome substrates, and degradome inhibitors that are present in an organism (*247*). The degradomic approach could provide a powerful tool in finding physiological substrates of many proteolytic enzymes whose functions remain to be determined. A dedicated and complete human protease and inhibitor microarray, called the CLIP−CHIP oligonucleotides (70-mers) for identifying all 715 human proteases, inactive homologues and inhibitors revealed the elevated expression of a number of proteases in invasive ductal cell carcinoma including ADAMTS17, carboxypeptidases A5 and M, tryptase-γ and matriptase-2 (*248*). The concept of degradomic may help to identify proteases that functional foods can selectively inhibit,

thereby influencing the onset and/or progression of an angiogenic disease. Successful functional foods can then advance to clinical trials.

In conclusion, diet continues to have great potential to prevent the progression of several chronic angiogenic diseases. There is a long list of functional foods that can affect HIF-1 pathway-induced angiogenesis. Because hypoxia precedes angiogenesis in some cases and appears to be a major contributor to the up-regulation of angiogenesis transcription factors, metronomic use of bioreductive or anti-HIF-1 pathways functional foods may provide long-term preventive effects against the progression of chronic angiogenic diseases. Functional foods that enhance the activity of endogenous regulators of HIF-1 pathways such as the tumor suppressor genes *p53* and *VHL*, which are known to inhibit HIF-1 expression, must be identified and evaluated in vitro and in vivo. Functional foods that enhance the activities of endogenous inhibitors of angiogenesis, such as endostatin, angiostatin, TSP-1, and TIMPs should be identified and evaluated. Identifying functional foods that can stimulate rapid ubiquitination of HIF-1α, even under hypoxic conditions, is desirable. Metronomic consumption of foods that inhibit HIF-1α accumulation under both hypoxic and normoxic conditions and functional foods that regulate other pathways that lead to angiogenesis may offer significant protection against the unwanted progression of chronic angiogenic diseases.

ABBREVIATIONS USED

VEGF, vascular endothelial growth factor; Ang2, angiopoietin-2; PI3K/AKT/mTOR, phosphatidyl-3-inositol kinase/Akt kinase/mammalian target of rapamycin; SOD, superoxide dismutase.

ACKNOWLEDGMENT

We are grateful to Dr. Greg Semenza (Johns Hopskins School of Medicine), Dr. Giovanni Melillo (NCI/NIH), Dr. Brad Wouters (University of Maastricht), and Dr. Ken McMillin (Louisiana State University Agricultural Center) for their reviews and suggestions.

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Received for review December 2, 2004. Revised manuscript received March 14, 2005. Accepted March 16, 2005.

JF0479719