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REVIEW

Antiangiogenic Phytochemicals and Medicinal Herbs

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Medicinal herbs and their phytochemicals are potential novel leads for developing antiangiogenic drugs. This review aims to assess the current status of research with medicinal herbs and their phytochemicals for the development of antiangiogenic agents for cancer and other angiogenesis-related diseases including inflammation, diabetic retinopathy, endometriosis and obesity. Most studies reviewed have focused on vascular endothelial growth factor (VEGF)/vascular endothelial growth factor receptor 2 (VEGFR-2) signaling for endothelial response processes and have led to the identification of many potential antiangiogenic agents. Since human clinical trials with antiangiogenic modalities targeting VEGF/VEGFR-2 signaling have shown limited efficacy and occasional toxic side effects, screening strategies for herbal phytochemicals based on other signaling pathways important for cancer-endothelial and stromal crosstalks should be emphasized in the future. Copyright © 2010 John Wiley & Sons, Ltd.

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INTRODUCTION

Angiogenesis is the process involving the growth of new blood vessels from pre-existing vessels (Carmeliet and Jain, 2000). While physiological angiogenesis takes place mainly during wound healing and menstrual cycle events of the female reproductive tract (Gordon *et al.*, 1995; Risau, 1997), pathological angiogenesis occurs in diseases such as cancer, rheumatoid arthritis (RA), endometriosis and diabetic retinopathy. An abnormal or excessive level of angiogenesis also contributes to vascular malformation, obesity, chronic inflammation, whereas insufficient angiogenesis is related to Alzheimer's disease, coronary artery disease, stroke, myocardial infarction and ulcer formation (Carmeliet and Jain, 2000; Carmeliet, 2003).

Angiogenesis inhibitors and promoters are potential drugs for treating angiogenesis-related disorders. Because of the critical dependence of solid cancers on neo-angiogenesis for growth, progression and metastasis (Wary *et al.*, 2003), it was no surprise that major academic and industrial research and development efforts have been made in the past two decades for angio-therapy modalities for cancer worldwide. Clinical trials in the past few years with antiangiogenic modalities targeting the vascular endothelial growth factor A(VEGF-A)/vascular endothelial growth factor receptor 2 (VEGFR-2) using inactivating monoclonal antibodies or VEGFR-2 kinase inhibitor drugs as single agents or in combination with chemotherapy have shown survival benefit in cancer patients of an increasing number of advanced stage malignancies with many major challenges ahead (Ellis and Hicklin, 2008). With these recent clinical developments as a backdrop, we review the antiangiogenic properties of some medicinal herbs and phytochemicals to highlight the current status of natural products research and to provide guidance on the development of angiogenesis inhibitors from medicinal plants in the future.

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FUNDAMENTALS OF ANGIOGENESIS

The angiogenic responses involve many biochemical and molecular signaling events resulting in coordinated and complex cellular processes such as endothelial cell proliferation, directional migration, basement membrane degradation and remodeling by matrix metalloproteinase (MMP), especially MMP-2, capillary tube formation and differentiation, pericyte recruitment and maturation (Bussolino *et al.*, 1997). Overproduction of angiogenic factors and/or down-regulation of angiogenesis inhibitors usually accompany and drive tumor angiogenesis. VEGF is a potent pro-angiogenic factor crucial for tumor vascular development (Keck *et al.*, 1989; Ferrara and Davis-Smyth, 1997; Ellis and Hicklin, 2008) and strongly induced by hypoxia that is a common feature of solid cancer. Of the VEGF families of proteins, VEGF-A isoforms such as $VEGF_{165}$ and $VEGF_{121}$ exert mitogenic and pro-angiogenic actions on the

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endothelial cells via binding to membrane protein tyrosine kinase-receptors expressed on endothelial cells, including VEGFR-1 (also known as Flt-1 for Fms-like tyrosine kinase-1) and VEGFR-2 (also known as Flk-1 for fetal liver kinase-1 or KDR for kinase insert domain containing receptor) (Bernatchez *et al.*, 1999; Ellis and Hicklin, 2008; Fischer *et al.*, 2008). VEGFR-2 is by far the most important receptor for VEGF-A signaling in vascular endothelial cells (Takahashi and Shibuya, 1997).

In addition to VEGFs being direct ligands for activating endothelial cells, many other growth factors produced by cancer cells and other cell types act in paracrine fashion as well as by the activated endothelial cells in autocrine or paracrine fashion stimulate angiogenesis, many through upregulating VEGF production. Many endogenous inhibitory proteins have been identified such as endostatin, thrombospondin etc (Furumatsu *et al.*, 2002; Schuch *et al.*, 2005; Zhang and Lawler, 2007; Mahapatra, 2008). The following section reviews briefly endothelial response processes and signaling pathways to provide a framework for evaluating published work concerning how herbals and phytochemicals have been evaluated for antiangiogenic activities.

ENDOTHELIAL RESPONSES AND ASSAY MODELS FOR SCREENING POTENTIAL ANTIANGIOGENIC AGENTS

As mentioned above, angiogenesis is a complex process involving many factors such as proliferation, migration and differentiation of endothelial cells, degradation of extracellular matrix (ECM) and basement membrane, and maturation of the new blood vessels (Bussolino *et al*., 1997; Carmeliet and Jain, 2000; Griffioen and Molema, 2000; Bergers and Benjamin, 2003). Various *in vitro* and *in vivo* assays have been utilized to analyse angiogenesis experimentally. In general, basic fibroblast growth factor (bFGF) and VEGF are widely used as angiogenic activators in both *in vitro* and *in vivo* studies. *In vitro*, human umbilical vein endothelial cells (HUVECs) are isolated from fresh human umbilical cord veins by collagenase treatment (Jaffe *et al.*, 1973) and their proliferation is determined by viability assays such as 2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) (Jost *et al.*, 1992), 3-[4,5-dimethyl(thiazol-2yl)-3,5-diphery] tetrazolium bromide (MTT) (Carmichael *et al.*, 1987) or bromophenoluridine (BrdU) incorporation assay in the absence or presence of bFGF or VEGF. Chemotactic migration or motility of HUVECs is assayed by the wound-healing assay or microchemotaxis chambers (Choi *et al.*, 2007) or transwell (Pyun *et al.*, 2008). Differentiation is determined by tube formation assay using HUVEC seeded on Matrigel with or without angiogenic activator (Grant *et al.*, 1992). *In vivo*, chorioallantoic membrane (CAM) assay is performed to measure the number of newly formed blood vessels around angiogenic factor treated disc (Folkman, 1984) as exemplified in our study (Huh *et al.*, 2005). The Matrigel plug assay is widely performed in C57BL/6 mice subcutaneously inoculated with angiogenic factor-treated Matrigel and the content of hemoglobin is measured in the Matrigel plugs to indirectly quantify functional new blood vessel formation (Passaniti, 1992; Lee *et al.*, 2006a). Similarly, tumor-induced angiogenesis assay can be assessed by the detection of neovascularization around intradermally inoculated tumor cells (Runkel *et al.*, 1991). Taken together, these angiogenesis experimental tools can be useful for antiangiogenic activity screening with phytochemicals, herbal extracts and formulas.

CLINICAL EXPERIENCE WITH CURRENT ANTIANGIOGENICS

Clinical trials in the past few years with antiangiogenic modalities such as angiostatin, endostatin, solimastat, bevacizumab and angiozyme targeting the VEGF-A/VEGFR-2 with inactivating monoclonal antibodies or VEGFR-2 kinase inhibitor drugs as single agents or in combination with chemotherapy have shown survival benefit in cancer patients of an increasing number of advanced stage malignancies (Ellis and Hicklin, 2008). For instance, the addition of bevacizumab to chemotherapy of metastatic colorectal cancer prolonged median progression free survival (PFS; HR 0.61, 95% CI 0.51–0.73) in five trials including 3101 patients (Wagner *et al.*, 2009). However, major hurdles for clinical implementation include limited efficacy, rapid development of resistance to the antiangiogenic modalities and in rare cases severe toxicity, arising from VEGF-A/VEGFR-2 ablation-induced severe hypoxia and its complications (Ellis and Hicklin, 2008). On the other hand, VEGFR-1 is expressed in not only endothelial cells, but also in many other cell types, such as macrophages, stromal cells, pericytes, smooth muscle cells, tumor cells, dendritic cells, bone marrow progenitors and leukemic cells (Hoeben *et al*., 2004; Fischer *et al*., 2008). VEGFR-1 binds placental growth factor (PlGF) and VEGF-B in addition to VEGF-A. Naturally occurring soluble non-signaling VEGFR1 or genetically engineered VEGFR1 serve as traps for VEGF-A due to the much tighter binding than VEGFR-2 for VEGF-A (Hoeben *et al*., 2004; Fischer *et al.*, 2008). As more mechanistic studies reveal that the ligand specificity and signaling consequences for the VEGFRs are not equivalent and important cross-talks among different ligand-VEGFR signaling cascades within and among endothelial and many other cell types comprise the tumor angiogenesis environment, approaches targeting other VEGFRs and ligand signaling may complement the existing VEGF/VEGFR-2 antiangiogenesis modalities to improve the cancer treatment efficacy and patient safety (Ellis and Hicklin, 2008; Fischer *et al*., 2008). While monoclonal antibodies and other biological agents are being developed and tested against these un-conventional targets (Fischer *et al*., 2008), small molecular compounds that target different VEGFRs may add to the arsenal of novel antiangiogenesis drug leads.

Although many medicinal herbals and phytochemicals have recently been evaluated for antiangiogenic properties (Table 1), most of the screening and investigations focused on VEGF-A/VEGFR-2- or bFGF-induced signaling and processes. Screening assays based on other targets (Fischer *et al*., 2008) are necessary to complement existing approaches.

Table 1. Angiogenesis-inhibiting phytochemicals

Compound classification	Compound	Scientific name	Crude Drugs	Efficacy	IC_{50}	References
POLYPHENOLIC COMPOUNDS						
Flavonoid polyphenolics						
Flavonol	Quercetin	*Rosa multiflora*	Rosae Fructus	Tumor angiogenesis	>100 μm	Chen *et al.*, 2008
	Fisetin	*Gleditsia japonica*	Gleditsiae Spina	Inflammatory angiogenesis	~2.0 μM	Lee, 2003
				Endometriosis-related angiogenesis		Laschke *et al.*, 2008; Xu *et al.*, 2009
				Inflammatory angiogenesis		Lee *et al.*, 2009b
Flavone	Apigenin	*Hydnocarpus anthelminthia*	Hydnocarpi Semen	Corneal neovascularization	~5.0 μM	Joussen, 2000
				Tumor angiogenesis		Liu *et al.*, 2005
	Morelloflavone	*Hovnia dulcis Garcinia dulcis*	Hoveniae Semen Cum Fructus Garciniae Fructus	Tumor angiogenesis	<20 μM	Jeon *et al.*, 2005
Flavanol	Epigallocatechin gallate	*Thea sinensis*	Theae Follium	Tumor angiogenesis	6.5–25 μM	Dona *et al.*, 2003; Xu *et al.*, 2009;
Isoflavone	Genistein	*Pueraria lobata* *Punica granatum*	Puerariae Radix Granati Cortex	Tumor angiogenesis	≈10 μM	Büchler, 2004; Wang, 2005
Phenolic acids	Gallic acid	*Euphorbia pekinensis* *Sanguisorba officinalis*	Euphorbiae Radix Sanguisorbae Radix	Tumor angiogenesis	≈100 μM	Liu, 2006
	Ellagic acid	*Geranium thunbergii*	Geranii Herba	Tumor angiogenesis	0.18 μM	Labrecque *et al.*, 2005
	1,2,3,4,6-penta-O-galloyl-β-D-glucose (PGG)	*Euphorbia pekinensis* *Paeonia lactiflora*	Euphorbiae Radix Paeoniae Radix	Tumor angiogenesis	≈4 μM	Lee, 2004; Huh *et al.*, 2005; Zhang *et al.*, 2009

Table 1. ***Continued***

Compound classification	Compound	Scientific name	Crude Drugs	Efficacy	IC_{50}	References
Other non-flavonoid polyphenolics	Resveratrol (Stilbene)	*Veratrum album* *Morus alba*	Veratrumae Radix	Tumor angiogenesis Inflammatory angiogenesis	0.7 ± 0.1 μM	Chen *et al.*, 2006; Bishayee, 2009; Bertelli, 2001
	Curcumin	*Curcuma longa* *Curcuma zedoaria* *Alpinia oxyphylla*	Curcumae Longae Radix Zedoariae Rhizoma Alpiniae Fructus	Tumor angiogenesis Adipokine-induced angiogenesis Inflammatory angiogenesis	≈40 μM	Lin, 2007; Kunnumakkara, 2008; Binion, 2008 Ejaz *et al.*, 2009 Jackson, 2006
TERPENES	Campesterol (Phytosterol)	*Gastrodia elata*	Gastrodiae Rhizoma	Tumor angiogenesis	≈25 μM	Choi *et al.*, 2007
INDOLES	Sulforaphane	*Raphanus sativus*	Raphani Semen	Tumor angiogenesis	≈5 μM	Jackson, 2007; Yao *et al.*, 2008
PYRANOCOUMARINS AND SIMPLE COUMARINS	Decursin	*Angelica gigas*	Angelicae Gigantis Radix	Tumor angiogenesis	<10 μM	Lee *et al.*, 2009b; Jung *et al.*, 2009; Son, 2009
	Decursinol angelate			Tumor angiogenesis		Jung *et al.*, 2009; Son, 2009
	Decurisnol			Tumor angiogenesis	≈1 μM	
	Scopolin	*Scopolia japonica* *Morus alba*	Scopolilae Rhizoma Mori Folium	Inflammatory angiogenesis	N/A	Pan *et al.*, 2009
MISCELLANEOUS	11,11′-dideoxyverticillin	*Shiraia bambusicola*		Tumor angiogenesis	≈1 μM	Chen *et al.*, 2005
	Celastrol	*Tripterygium wilfordii*	Tripterygiumae Radix	Tumor angiogenesis	≈100 nM	Huang, 2003; Tao, 2003; He, 2009
	Erianin	*Dendrobium chrysotoxum*	Dendrobia Stipes	Tumor angiogenesis	≈100 nM	Gong, 2004
	Pedicularioside G	*Pedicularis striata*	Pedicularis Herba	Tumor angiogenesis	<100 μM	Mu, 2008
	Shiraiachrome A	*Shiraia bambusicola*		Inflammatory angiogenesis	≈2.5 μM	Tong *et al.*, 2004
	Thymoquinone	*Nigella sativa*	Nigellae Semen	Tumor angiogenesis	<100 nM	Arbiser, 2007

FARNESIFEROL C (FC) REPRESENTS A NOVEL CLASS OF COMPOUNDS THAT TARGET BOTH VEGFR-1 AND VEGFR-2 SIGNALING

The hypothesis was examined that novel antiangiogenic activities of FC from *Ferula assafoetida* contribute to anticancer efficacy (Lee *et al*., 2010). In HUVECs, exposure to the 10–40 μmol/L concentration range of FC inhibited VEGF-induced cell proliferation, migration, invasion, tube formation and the expression of MMP-2. In addition, FC inhibited the angiogenic sprouting of VEGF-treated rat aorta in an *ex vivo* model. Furthermore, FC inhibited the *in vivo* growth of mouse Lewis lung cancer (LLC) allograft model by 60% ($p < 0.001$) at a daily i.p. dosage of 1 mg/kg body weight without any negative effect on the weight of the host mice. Immunohistochemistry staining showed decreased microvessel density (CD34) and proliferative index (Ki-67) without affecting the apoptotic (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) index. Mechanistically, FC decreased the binding of VEGF to VEGFR-1/Flt-1, but not to VEGFR-2/KDR/Flk-1. In terms of early signaling, FC exerted a rapid inhibitory action (examined within 10 min) on VEGF-induced autophosphorylation of VEGFR-1 without affecting that of VEGFR-2. Nevertheless, FC decreased the phosphorylation of most of the kinases downstream of VEGFR-2: focal adhesion kinase (FAK), Src, ERK 1/2, p38 MAPK and JNK without affecting Akt. Computer simulation suggests that FC may inhibit Src or FAK protein activities directly through its docking to their ATP-binding sites. Taken together, the multitargeting actions of FC, particularly VEGFR-1 inhibition, suggest a novel drug candidate to complement current VEGF/VEGFR-2-targeting antiangiogenic modalities for cancer.

ANTIANGIOGENETIC ACTIVITIES OF MEDICINAL HERBS AND PHYTOCHEMICALS IN CANCER

Some medicinal herbs and various compounds have been evaluated for antiangiogenic as well as anticancer activities. Naturally occurring gallotannin, penta-1,2,3,4,6-O-galloyl-beta-D-glucose (PGG) isolated from Gallnut of *Rhus chinensis* MILL was reported to inhibit the proliferation, migration and tube formation of endothelial cells (Huh *et al*., 2005; Zhang *et al.*, 2009) and also induce apoptosis in prostate cancer cells (Hu *et al.*, 2008; Hu *et al.*, 2009). Antiangiogenic activities have been reported for paeonol (Kim *et al.*, 2009c), shikonin (Lee *et al.*, 2008), campesterol (Choi *et al*., 2007), heyneanol A (Lee *et al*., 2006a), 6-(1-oxobutyl)-5,8-dimethoxy-1,4-naphthoquinone (Lee *et al.*, 2007), Korean *Angelica gigas* (Lee *et al.*, 2009a) and herbal formulations such as Kamikaekyuktang (Lee *et al.*, 2006b), Shiquandabutangjiaweibang (Kim *et al.*, 2005) and Bojungbangdocktang (Kim *et al.*, 2009b). In addition, Wang and colleagues demonstrated that feiyanning decoction (FD), a traditional Chinese herbal mixture, has inhibitory effects on angiogenesis by down-regulating the transcription factor nuclear factor kappa B (NF-κB), survival protein Akt and hypoxia inducible factor 1-alpha (HIF-α) in A549 lung cancer cells (Zhang *et al.*, 2008; Wang *et al.*, 2009). Resveratrol, a natural polyphenol, has been reported to suppress angiogenesis in rat glioma and other cancers (Chen *et al.*, 2006; Bishayee, 2009). Furthermore, the inhibitory effect of morelloflavone on tumor angiogenesis was found to occur through the targeting of Rho guanosine triphosphatases (GTPases) and extracellular signal-regulated kinase (ERK) (Pang *et al.*, 2009). Yi and colleagues reported that thymoquinone and gambogic acid decreased cell proliferation, migration, invasion and tube formation by suppressing the Akt pathway in human HUVECs and human prostate cancer cells (PC-3) (Yi *et al.*, 2008a, 2008b). Significantly, vascular endothelial growth factor (VEGF) and/or its receptor were down-regulated or inhibited by numerous phytochemicals, including sulforaphane (Yao *et al.*, 2008), celastrol (Huang *et al.*, 2008), aplidin (Straight *et al.*, 2006), meisoindigo (Xiao *et al.*, 2006), 4-O-methylgallic acid (Jeon *et al.*, 2005), ellagic acid (Labrecque *et al.*, 2005), delphinidin (Lamy *et al.*, 2008), curcumin (Ejaz *et al.*, 2009) and philinopside A (Tong *et al.*, 2005).

Although it has been well established that angiogenesis plays a significant role in the tumorigenensis of solid tumors, recent studies have shown the critical role of angiogenesis in suspension leukemic cells (Letilovic *et al.*, 2006; Xu *et al.*, 2006). There is growing evidence that angiogenic factors such as bFGF, VEGF and VEGF-R are increased in leukemic cell lines and patients (Leung *et al.*, 1989; Katoh *et al.*, 1995; Perez-Atayde *et al.*, 1997) and also are involved in hematopoietic regulation (Hattori *et al.*, 2001). In this regard, He and his colleagues reported that antisense-VEGF decreased VEGF protein expression and inhibited the growth of chronic myelogenous leukemia (CML) K562 cells and injection of antisense-VEGF expressing K562 cells into nude mice decreased the microvessel density of the bone marrow compared with untreated control (He *et al.*, 2003).

Several natural products were reported to exert antiangiogenic effects against leukemia cells. For instance, mastic oil from *Pistacia lentiscus* var. chia attenuated angiogenesis by inhibiting VEGF expression and inhibited the cell growth and survival via the downregulation of ERK1/2 in K562 cells (Loutrari *et al.*, 2006). Meisoindigo, a compound from the Indigo plant *Isatis* also showed an antileukemic effect on CML through decreasing VEGF secretion as well as inhibiting tube formation of HUVECs in an *in vitro* Matrigel model (Xiao *et al*., 2006). Similarly, artesunate, a derivative of artemisinin extracted from *Artemisia annua*, inhibited angiogenesis and VEGF expression in K562 cells *in vitro* and *in vivo* (Zhou *et al.*, 2007). Overall, many herbs and their compounds have the potential of antiangiogenic activity in leukemia cells.

ANGIOGENESIS AND INFLAMMATION

Inflammatory proteins are closely linked to angiogenesis (Krupinski *et al.*, 2008). Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, IL-8 and monocyte chemoattractant protein (MCP)-1 strongly promote chronic inflammation of rheumatoid arthritis (RA) and inflam-

mation-related angiogenesis (Koch *et al.*, 1992; Harris, 1994; Feghali and Wright, 1997; Maruotti *et al.*, 2006). Specifically, RA is characterized by endothelial cell proliferation and angiogenesis, leukocyte activation and pannus formation (Feldmann, 1996; Firestein, 2003). Therefore, angiogenesis inhibitors could be indicated to treat inflammatory arthritis (Schoettler and Brahn, 2009). Indeed, scopolin isolated from the stems of herbal plants such as *Scopolia japonica* is one of several coumarin constituents used for the therapy of RA (Silvan *et al.*, 1996). Recent studies support the antiangiogenic and antirheumatic effects of scopolin on adjuvant-induced arthritis in rats (Pan *et al.*, 2009). Scopolin reduced the expression levels of IL-6, VEGF and basic fibroblast growth factor (bFGF)-2 in rat synovial tissue. Similarly, fisetin and paeoniflorin showed inhibitory effects on inflammation and angiogenesis (Zheng *et al.*, 2007; Lee *et al.*, 2009b), and decreased the levels of IL-1β-induced inflammatory cytokines (TNF-α, IL-6)/chemokines (IL-8, MCP-1) and VEGF during RA in fibroblast-like synovial cells (FLS). Fisetin also reduced the levels of phospho-ERK, c-jun *N*-terminal kinase (JNK) and activated p38 mitogen-activated protein kinase (MAPK) along with the incidence and severity of collagen-induced arthritis (Lee *et al.*, 2009b). Additionally, paeoniflorin inhibited inflammation and decreased the expression of IL-1β, IL-6, VEGF and granulocyte macrophage colony stimulating factor (GM-CSF) (Zheng *et al.*, 2007). Dell'Aica and colleagues reported that hyperforin, a polyphenol-derivative of St John's wort (*Hypericum perforatum*) extract, reduced IL-8-induced angiogenesis and bleomycin-induced inflammation in a murine model (Dell'Aica *et al.*, 2007). Similarly, epigallocatechin-3-gallate (EGCG), the most abundant catechin of green tea, blocked neutrophil-mediated angiogenesis in an *in vivo* inflammatory angiogenesis model (Dona *et al.*, 2003). Shiraiachrome A from the Chinese bamboo fungus *Shiraia bambusicola*, which has been used for the treatment of RA as well as cancer in traditional Chinese medicine, exerted antiangiogenic effects on human microvascular endothelial cells (Tong *et al.*, 2004). Collectively, this evidence suggests that many herbs and their phytochemicals may exert both antiangiogenic effects and antiinflammatory activity.

ANGIOGENESIS AND DIABETIC RETINOPATHY

Proliferative diabetic retinopathy is a vision-related complication of diabetes mellitus caused by too much angiogenesis (Jardeleza and Miller, 2009). Recently, caffeic acid, a phenolic compound present in vegetables, fruits and coffee, was reported to inhibit VEGF-induced proliferation, migration and tube formation of retinal endothelial cells by suppressing reactive oxygen species (ROS)-induced VEGF expression (Kim *et al.*, 2009a). Chen and colleagues reported that quercetin (3,3′,4′,5,7-penthydroxy flavone), found in various fruits and vegetables, inhibits proliferation, migration and tube formation of rhesus choroids-retina endothelial cell line RF/6A (Chen *et al.*, 2008). Similarly, hypericin, an active ingredient in St John's Wort, inhibited retinal neovascularization through the down-regulation of ERK in a mouse model of oxygen-induced retinopathy (Higuchi *et al.*, 2008). Collectively, these data support the potential therapeutic effects of diverse herbal compounds on diabetic retinopathy.

ANGIOGENESIS AND ENDOMETRIOSIS

Endometriosis, the outgrowth of endometrium-like tissue into the uterine cavity, is a common disease among women of reproductive age. It has recently become apparent that angiogenesis plays a pivotal role in its pathophysiology (Groothuis *et al.*, 2005; Becker and D'Amato, 2007; Taylor *et al.*, 2009). EGCG inhibited the estrogen-induced activation of endothelial cells (Laschke *et al.*, 2008). It also increased apoptosis in an endometriosis mouse model by reducing the mRNA levels of VEGF and enhancing the mRNA levels of NF-κB and MAPK1 (Xu *et al.*, 2009). Curcumin, a phenolic isolated from the rhizome of *Curcuma longa* (turmeric), arrested endometriosis by down-regulating MMP-9 activity (Swarnakar and Paul, 2009). Recently, many reports have confirmed the association of NF-κB with angiogenesis in endometriosis and suggest the potential of medicinal herbals and phytochemicals in targeting NF-κB to treat endometriosis (Gonzalez-Ramos *et al.*, 2008; Liu *et al.*, 2009).

ANGIOGENESIS AND OBESITY

Latest evidence suggests that obesity is associated with the substantial modulation of adipose tissue structure, a process which involves adipogenesis, angiogenesis and extracellular matrix remodeling. In the early stages of adipose tissue development, blood vessel formation and pre-adipocyte differentiation are triggered by adipose tissue explants and endothelial cells, respectively. Therefore, modulation of angiogenesis and of proteolysis may impair adipose tissue development (Liu and Meydani, 2003; Christiaens and Lijnen, 2006; Lijnen, 2008).

Based on this concept, curcumin with its antiangiogenic activity and inhibition of adipogenesis in 3T3-L1 pre-adipocytes has been suggested for the prevention of obesity (Ejaz *et al.*, 2009). Since adipogenesis requires the recruitment of new blood vessels (Rupnick *et al.*, 2002), mice fed a high-fat diet and treated with curcumin (500 mg/kg) had a lower body weight gain, adiposity and microvessel density in adipose tissue, which coincided with reduced expression of VEGF and its receptor. Guggulsterone isolated from *Commiphora wightii* (syn C. mukul) was reported to suppress obesity, cancer and cardiovascular disease via regulation of various transcription factors, including NF-κB, signal transducer and activator of transcription 3 (STAT3) and CCAAT-enhancer-binding protein-alpha (C/EBP-α), and of various steroid receptors, such as androgen and glucocorticoid receptors (Deng, 2007; Shishodia *et al.*, 2008). Similarly, 2-(8-hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl) propionic acid (NM-3), a small isocoumarin molecule with antiangiogenic activity, was shown to attenuate renal alterations in obese type 2 diabetic db/db mice. These examples suggest the potential of antiangiogenic compounds from herbal plants as

preventive or therapeutic agents for metabolic diseases including obesity and diabetes (Ichinose *et al.*, 2006).

MOLECULAR TARGETS OF ANTIANGIOGENIC HERBAL COMPOUNDS

VEGF expression is often stimulated by other indirect angiogenic factors, such as platelet-derived growth factor (PDGF), bFGF, TGF-α (Glade Bender *et al.*, 2004). Labrecque and colleagues reported that ellagic acid mediated the inhibition of VEGF-induced phosphorylation of VEGFR-2 in bovine aortic endothelial cell (BAEC) as well as the PDGF-induced phosphorylation of PDGF receptor (PDGFR) in pulmonary aortic smooth muscle cell (PASMC) (Labrecque *et al.*, 2005). Delphinidin and philinopside A also showed inhibitory effects on PDGFR in smooth muscle cell (SMC) and human mammary epithelial cells, respectively (Tong *et al.*, 2005; Lamy *et al.*, 2008).

MMP is another major molecule that contributes to angiogenesis (Stetler-Stevenson, 1999). Hyperforin strongly inhibited MMP-2 in bovine aortic endothelial cells (Martinez-Poveda *et al.*, 2005) as well as MMP-9 in polymorphonuclear neutrophils (PMNs) (Dell'Aica *et al.*, 2007).

Increased levels of proteins associated with the cell survival pathway, including Akt, NF-κB and MAPKs are known to play a major role in angiogenesis (Barthomeuf, 2007). In addition, recent papers have demonstrated that HIF-1α is associated positively with angiogenesis in tumors (Tsuzuki *et al.*, 2000; Bos *et al.*, 2005). Thymoquinone from *Eupatorium ayapana* inhibited Akt and extracellular signal-regulated kinase signaling pathways and suppressed tumor angiogenesis and tumor growth (Yi *et al.*, 2008a). Likewise, apigenin from *Dendranthema indicum* var. aromaticum or *Lobelia chinensis* inhibited VEGF and angiogenesis through suppression of HIF-1α expression and Akt activation in human lung cancer cells, suggesting a possible mechanism to prevent lung cancer (Liu *et al.*, 2005). Furthermore, a variety of natural compounds have been reported to inhibit angiogenesis via down-regulation of the MAPK signaling pathway: morelloflavone (Pang *et al.*, 2009), thymoquinone (Yi *et al.*, 2008a), sulforaphane (Yao *et al.*, 2008), epoxyquinol B (Kamiyama *et al.*, 2008), delphinidin (Lamy *et al.*, 2008), decursin (Jung *et al.*, 2009; Lee *et al.*, 2009a) and 11,11′-dideoxyverticillin (Chen *et al.*, 2005) in cancer. Our group reported that PGG diminished ERK 1/2 and JNK phosphorylation and increased phospho-p38 MAPK in a dose-dependent manner in bFGF-treated HUVECs (Huh *et al.*, 2005). Moreover, p38 MAPK inhibitor blocked PGG-mediated antiproliferative activity as well as the expression of COX-2 and VEGF in bFGF-stimulated HUVECs. This suggests that p38 MAPK plays a critical role in the inhibition of COX-2 and angiogenesis by PGG. Considering that p38 MAPK is generally activated by genotoxic agents or apoptosis, activation of p38 MAPK by PGG may mediate the apoptosis of endothelial cells during antiangiogenesis (Huh *et al.*, 2005).

NF-κB promotes angiogenesis and metastasis by regulating VEGF and MMP expression in tumors. For instance, octacosanol isolated from *Tinospora cordifolia* down-regulated VEGF gene expression by inhibiting MMPs and translocation of transcription factor NF-κB to the nucleus (Thippeswamy *et al.*, 2008). Likewise, several natural compounds such as scopolin, fisetin and paeoniflorin reduced the pro-inflammatory cytokines IL-6, IL-8 and MCP-1 that are known to be closely associated with angiogenesis (Zheng *et al.*, 2007; Lee *et al.*, 2009b; Pan *et al.*, 2009).

PERSPECTIVES ON PHYTOCHEMICALS AND MEDICINAL HERBS AS ANTIANGIOGENIC MODALITIES

Angiogenesis plays a critical role in the physiopathology of normal and pathological cells. Abnormal and excessive angiogenesis is causally linked to cancer and other diseases including RA, diabetic retinopathy, endometriosis and obesity. Although existing inhibitors of angiogenesis targeting VEGF/VEGFR-2 signaling have contributed to the development of potential drug candidates for the treatment of the various angiogenesis-related diseases, their side effects and low efficacy are major challenges. From this review, it can be concluded that antiangiogenic activities can be derived from medicinal herbals and phytochemicals, but their usefulness is not proven clinically. Future search for novel candidates should include additional targets as exemplified by FC that targets not only VEGF-2 signaling, but also VEGFR-1 signaling.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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