ELSEVIER

REVIEWS: CURRENT TOPICS

Journal of Nutritional Biochemistry 13 (2002) 380–390

THE JOURNAL OF Nutritional Biochemistry

Antiangiogenic mechanisms of diet-derived polyphenols

Yihai Cao*, Renhai Cao, Ebba Bråkenhielm

Microbiology and Tumor Biology Center, Karolinska Institutet, S-171 77 Stockholm, Sweden

Received 12 March 2002; received in revised form 2 April 2002; accepted 12 April 2002

Abstract

Accumulating evidence demonstrates that polyphenols in natural products are beneficial against human lethal diseases such as cancer and metastasis. The underlying mechanisms of anti-cancer effects are complex. Recent studies show that several polyphenols, including epigallocatechin-3-gallate (EGCG) in green tea and resveratrol in red wine, inhibit angiogenesis when administrated orally. These polyphenols have direct effects on suppression of angiogenesis in several standard animal angiogenesis models. Because angiogenesis is involved in many diseases such as cancer, diabetic retinopathy and chronic inflammations, the discovery of these polyphenols as angiogenesis inhibitors has shed light on the health beneficial mechanisms of natural products, which are rich in these molecules. At the molecular level, recent studies have provided important information on how these molecules inhibit endothelial cell growth. Perhaps the greatest therapeutic advantage of these small natural molecules over large protein compounds is that they can be administrated orally without causing severe side effects. It is anticipated that more polyphenols in natural products will be discovered as angiogenesis inhibitors and that these natural polyphenols could serve as leading structures in the discovery of more potent, synthetic angiogenesis inhibitors.

Keywords: Flavonoids; EGCG; Resveratrol; Angiogenesis; Tumor

1. Introduction

Although the entire genome of humans have become public (30,000–40,000 genes), which will with no doubt provide a landmark information for development of therapeutic and preventive methods against genetic and non-genetic diseases, one should not neglect the power of natural compounds in the treatment of human diseases [1,2]. In fact, more than 60% of the world's population relies almost entirely on plants for medication [3]. Of the 520 new drugs approved between 1983 and 1994, 39% were natural products, of which 60–80% were antibiotics and anti-cancer drugs from natural products [4]. Of the 20 best-selling non-protein drugs in 1999, 9 were derived or developed as the result of leads generated by natural products. Among these natural products, "functional food" has become increasingly appreciated both by medical professionals and by the general public, due to its health benefits. The reason for this is simply because these "daily" consumed natural products have shown beneficial effects correlated with lower risks of developing lethal diseases such as cancer and heart diseases, and they can be obtained without prescriptions. Polyphenols, especially flavonoids, that are rich in fruits, soybeans, vegetables, herbs, roots and leaves, act as active components in prevention of cancer, heart diseases and diabetes [5]. For example, fresh green tea contains large amounts of catechin polyphenols, while resveratrol and quercetin are rich in grapes, red wine and other food products [6–9]. These compounds have been extensively studied for their effects in suppression of tumor growth and prevention of heart diseases in animal models. For example, a Medline search on green tea results in nearly 1,000 research articles. Although many of these studies are limited to epidemiological and anti-oxidative aspects of green tea and catechins in cancer prevention, some recent studies are aimed to elucidate the possible molecular mechanisms of how these compounds act on various systems in the body.

Angiogenesis, the process of new blood vessel growth, is involved in physiological and pathological processes such as embryonic development, wound healing, reproductive cycles, tumor growth and metastasis, diabetic retinopathy and chronic inflammations. Thus, suppression of abnormal angiogenesis may provide therapeutic strategies in the treatment of angiogenesis-dependent disorders. In the last de-

* Corresponding author. Tel.: +46-8-728-7596; fax: +46-8-31-94-70.
E-mail address: yihai.cao@mtc.ki.se (Y. Cao).

0955-2863/02/$ – see front matter
PII: S0955-2863(02)00204-8

cade, a number of potent endogenous angiogenesis inhibitors have been identified that produce dramatic effects in suppression of tumor growth and metastasis in animal models [10–12]. Encouraged by these animal studies, several protein angiogenesis inhibitors have been brought to clinical trials in the treatment of human cancers. Although their effects in human trials remain to be seen, protein therapy has several disadvantages. These include the requirement for high dosages, difficulties of manufacturing active molecules, high costs, risk of transmission of microorganism toxins in recombinant proteins, and the need for frequent, long-term injections. Thus, it seems impractical to apply protein angiogenesis inhibitors to a large number of patients. In contrast, angiogenesis inhibitors as small molecules derived from natural products have great advantages over protein inhibitors. As natural diet ingredients, they are proven non-toxic at physiological doses, can be easily manufactured or obtained, can be given orally and confer low cost. Therefore, it is of great importance to understand the underlying molecular mechanisms of these natural angiogenesis inhibitors in order to improve their antiangiogenic efficacies. In addition, the active components of these natural products could serve as structural bases for screening for more potent synthetic analogs.

2. The role of angiogenesis in tumor growth and metastasis

More than 30 years ago, Dr. Judah Folkman hypothesized that tumor growth and metastasis are dependent on the degree of neovascularization [13]. Although this principle has been proven by many elegant experiments, it is still worthwhile to mention the most simple and convincing evidence. When a piece of tumor tissue was implanted in the rabbit cornea, the tumor implant changed its growth rate from linear to exponential when newly formed blood vessels reached to the tumor tissue [14]. Recently, a similar corneal tumor implantation technique in smaller animals, such as mice, has been established. At the prevascular stage, a tiny tumor tissue consisting of several millions of cells remains in its dormant stage for about 10 days without growth. Survival of these cells in an avascular tumor is dependent on free diffusion of nutrients, O_2 and growth factors. The tumor implant is unable to grow beyond volumes of 2–3 $mm^{3.}$ However, these living tumor cells can still produce potent angiogenic factors, such as vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) and fibroblast growth factor-2 (FGF-2), which switch on an angiogenic phenotype of the tumor implant. Once newly formed blood vessels reach the tumor implant, the growth of the tumor is exponential. In fact, the tiny tumor implant grows beyond the size of the entire eye organ within a couple of days. In contrast, mechanical disruptions of tumor-induced vessels in the cornea can completely arrest tumor growth. This simple experiment not only provides compelling evidence that tumor growth is dependent on angiogenesis, but also demonstrates that intervention of tumor angiogenesis can become a powerful approach for cancer therapy. Using the same experimental set-up, single or mixtures of angiogenic factors such as VEGF and FGF-2, together with slow release polymers, have been implanted in avascular corneas. Like tumors, these pure angiogenic factors can induce rapid and robust angiogenic responses [15]. Today, many academic and industrial scientists are using this and other similar systems to screen for angiogenic compounds. It appears that tumor angiogenesis is not only critical for primary tumor growth but also for tumor metastasis. For example, overexpression of VEGF, an endothelial cell specific growth factor, in a murine fibrosarcoma resulted in metastases of all tumor-bearing mice [16]. The underlying mechanisms of a switch of angiogenic phenotype in tumors are complex and often require up-regulation of angiogenic factors and simultaneous down regulation of angiogenesis inhibitors.

3. Angiogenesis inhibitors in clinical trials

Encouraged by animal studies, many angiogenesis inhibitors are currently in various phases of human cancer trials. For example, a simple search in the Internet results in more than 40 protein or chemical compounds that are in clinical trials for the treatment of human cancers (Table 1). Most of these trials are registered by the National Cancer Institute, but sponsored by different pharmaceutical companies [17]. Preclinical studies show that most of these angiogenesis inhibitors effectively block tumor growth without causing toxicity. As shown in Table 1, these therapeutic molecules inhibit neovascularization at various steps of blood vessel formation. For example, several matrix metalloproteinase inhibitors block the degradation of the endothelial basement membrane, thus preventing capillary sprout formation. Development of antagonistic reagents by blocking angiogenic ligand/receptor-mediated signaling pathways has become one of the most attractive approaches in antiangiogenic therapy. These strategies are mainly aimed at inhibition of VEGF, one of the specific key angiogenic factors secreted by most tumors. However, these single angiogenic factor antagonists may encounter major problems in development of drug resistance, as tumor cells most likely may switch their angiogenic stimulators. As cancer masses consist of heterogeneous populations of tumor cells, the anti-VEGF reagents might stress tumor cells to select for colonies producing other angiogenic factors such as FGF-2. Thus, VEGF antagonists may not be effective in the treatment of all cancers. In contrast, general angiogenesis inhibitors that block common pathways of tumor angiogenesis can bypass drug resistance and be therapeutically effective against all cancer types. Angiostatin and endostatin are two such specific endothelial cell angiogenesis inhibitors, which have entered clinical trials. According to animal studies, rela-

Table 1
Angiogenesis inhibitors in clinical trials

Drug	Phase	Sponsor
Protease Inhibitors		
Captopril	I/II	Bristol-Myers S.
CGS 27023A	I/II	Novartis
Col-3 (metastat)	I/II	CollaGenex & NCI
BMS-275291	II/III	Bristol-Myers S.
Neovastat (Æ-941)	III	Aeterna Lab.
Growth Factor Antagonists		
SU-6668	I	Pharmacia Corp./Sugen
IMC-1C11	I	ImClone
CEP-7055	I	Sanofi-Synth & Cephalon
PI-88	II	Progen Industries
Mobist	II	Amgen/Imclone
Iressa (ZD 1839)	I/II	Astra Zeneca
PTK787 (ZK22584)	I/II	Novartis
Anti-VEGF Ab. (Avastin)	II/III	NCI, Genentech
SU-5416	II/III	Pharmacia Corp./Sugen
Angiozyme (Ribozyme)	II/III	Ribozyme Pharm.
Avicine	II/III	AVI Biopharma
IM862	II/III	Cytran
SU101	II/III	Pharmacia Corp, SUGEN
octreotide acetate (Sandostatin)	III	Novartis
Suramin (Metaret)	FDA	Warner-Lambert & NCI
Direct Endothelial Inhibitors		
Angiostatin	I	EntreMed
2-ME	I	EntreMed
Combretastatin (CA4P)	I/II	Oxigene
TNP-470	I/II	TAP Pharm.
Endostatin	II	EntreMed
Penicillamine	II	NCI, available
Squalamine (MSI 1256 F)	II/III	Genera & Magainin Pharm.
Thalidomide	II/III	NCI Celgene & EntreMed
Farnesyl Transferase Inhibitor		
-L-778,123	I	NCI, Merck
-SCH66336	I	Schering-Plough Corp.
Cytokines		
IL-12	I/II	Genetics
Interferon-αIII	Available	
Anti-Integrin Agents		
Vitaxin (medi-552, LM 609)	II	Medimmune Inc./Ixsys
MD 121974	I/II	Merck
Others		
CAI (Carboxyamido-triazole)	II/III	NCI
Celecoxib	I/II	Pharmacia
ImmTher	II	Endorex
Flavopiridol	II	Aventis & NCI

tively large dosages (20–100 mg/kg) of these angiogenesis inhibitors have to be delivered in order to reach maximal effects. When these amounts are translated into human trials, it seems unattractive to deliver huge amounts of biologically active recombinant proteins for long-term treatments of large number of cancer patients. In addition to high dosages, frequent injections of these angiogenesis inhibitors are also required. Therefore, discovery of small molecules in natural products as angiogenesis inhibitors is an important approach in improving or even replacing the current antiangiogenic therapy in the treatment of cancer and other diseases.

4. Mechanisms of antiangiogenic polyphenols

4.1. Antiangiogenic polyphenols

Consumption of plant-based diets could be beneficial in decreasing the risks of onset and progression of angiogenesis-related diseases, including cancer, diabetic retinopathy and rheumatoid arthritis. The known active components in natural food products are mainly attributed to the existence of beneficial polyphenols, which are abundant in tea, coffee, fruits (grape, apple, peach), vegetables (tomato, celery, potato), beans (soy), grains and seeds. There are close to 5000 different polyphenols described so far. They are divided into subgroups such as isoflavones, flavonoids and lignans. Recently, several polyphenols extracted from various plants have been found to be potent inhibitors of angiogenesis (Table 2). Further, many polyphenols have been found to inhibit experimental tumor growth in animals. These studies suggest that polyphenols could be an important group of therapeutic natural compounds in the treatment of angiogenesis dependent disorders.

Flavonoids, rich in soybeans, tea, fruits and leafy vegetables, are the most abundant polyphenols in our daily diets. The average intake of flavonoids by a human is in the range of 25–50 mg/day, including flavonols quercetin, myricetin and kaempferol, and the flavones luteolin and apigenin [18–20]. In regard to chemopreventive activity in cancers, it appears that several flavonoids have dual effects, as they suppress the growth of both tumor cells and endothelial cells. For example, luteolin, genistein, apigenin, quercetin and fisetin inhibit cultured tumor and endothelial cells at similar concentrations [21,22]. Many polyphenols act as natural estrogenic agonists, which compete with endogenous estrogen for the binding of type II estrogen binding sites, resulting in decreased activation of estrogenic signaling pathways. These compounds include isoflavones, flavonoids and lignans, such as kaempferol, quercetin, apigenin, genistein and resveratrol [23]. This weak estrogenic activity has been correlated with their anti-proliferative capacity in tumor cells [24,25].

A few polyphenols seem to selectively inhibit endothelial cell growth at lower concentrations as compared with tumor cells. Epigallocatechin-3-gallate (EGCG) and resveratrol are such examples [26,27]. It has been reported that flavones 3,4-dihydroxyflavone, resveratrol and luteolin are among the most potent anti-endothelial polyphenols in suppression of cell proliferation with IC_{50} of 1.4–2 μM [22, 26]. Some other polyphenols preferentially inhibit endothelial cell migration at low concentrations [22]. However, these anti-endothelial growth effects cannot be directly translated into the *in vivo* antiangiogenesis effects in ani-

Table 2
Antiangiogenic polyphenols

Compound	Source	Anti-Endothelial Effects	Antiangiogenesis	Ref
Isoflavones				
Daidzein	soybeans	prolif.	n.d	[79,82]
Genistein	soybeans	prolif., migr.	cornea, tumor	[21,76,79,81-83]
Resveratrol	grapes, peanuts	prolif., migr., tube form.	CAM, cornea, tumor	[26,58,63,66,71]
Flavnoids				
Flavonoles				
3-Hydroxyflavone	vegetables	prolif., migr.	n.d	[22]
Fisetin	fruits, vegetables	prolif., migr.	CAM, cornea	[22,74,96]
Quercetin	grapes, peanuts	prolif., migr., tube form.	n.d	[22,68,71]
Myricetin	cranberry, black currant	prolif.	n.d	[22,97,98]
Silibinin	milk thistle	prolif.	n.d	[30,109]
Flavones				
Flavone	vegetables, herbs	prolif.	n.d	[22]
3,4-Dihydroxyflavone	vegetables	prolif., migr.	n.d	[22]
Apigenin	celery roots	prolif., migr., tube form.	tumor lymph vessel	[22,87,93]
Luteolin	vegetables, fruit	prolif., migr.	cornea	[22,74,75]
Baicalein	*Scutellariae radix*	PROLIF.	n.d	[102]
Flavopiridol	semisynthetic	prolif.	Matrigel plug	[110,111]
Flavanones				
Eriodictyol	citrus fruits, beans	prolif.	n.d	[22]
Hesperetin	citrus fruits	prolif.	n.d	[22]
Catechins				
EC	tea	prolif.	n.d	[37]
ECG	tea	prolif.	n.d	[37]
EGC	tea	prolif.	n.d	[37]
EGCG	tea	prolif., tube form.	CAM, cornea	[27,36-38]

mals or humans, because absorption, tissue distribution, half-life of compounds and metabolic degradation must be taken into consideration. For example, EGCG in green tea is an effective inhibitor in suppression of distal angiogenesis in animals, although relatively high concentrations are required for endothelial cell inhibition *in vitro* [27]. At the molecular level, these polyphenol inhibitors suppress growth factor-triggered activation of receptors (EGCG), protein kinase C (luteolin, resveratrol, quercetin), tyrosine kinases (genistein), phosphoinositide 3-kinase (luteolin, quercetin) and epidermal growth factor receptor S6 kinase (genistein, kaempferol) [28,29]. They also block the function of cell cycle regulators such as cyclin dependent kinase (cdk) and thus keep tumor and endothelial cells at quiescent stages [22]. Other common inhibitory mechanisms of flavonoids include inhibition of mitogen-activated protein (MAP) kinases and upregulation of cell cycle repressors such as p21. Their inhibitory activities can also be extended to anti-inflammatory effects and inhibition of proteases such as matrix metalloproteinases (MMPs), which are coupled to antiangiogenesis [30]. Even the antioxidative effect has recently been linked to antiangiogenesis. For example, reduction of the oxidative stress by polyphenols leads to blockage of reactive oxygen species (ROS) formation and alterations in the cell redox equilibrium, resulting in activation of transcription factors such as AP-1, p53 and NFκB, which regulate the expression levels of one of the key angiogenic factors, VEGF [31]. Thus, the antiangiogenic mechanisms of polyphenols are complex.

4.2. Green tea catechins

Tea, especially green tea and its polyphenols have been found to inhibit experimental tumorigenesis and tumor growth in animals. For instance, tea extracts inhibit most common cancers originating from skin, lung, esophagus, stomach, liver, duodenum, pancreas, colon, bladder, prostate and mammary gland [32]. The active anti-cancer compounds of tea are reported to be catechins, one of the main constituents in green tea. A typical tea beverage prepared with green tea contains 2.5–3.5 mg/ml tea solids, of which 30–42% are catechin compounds [33]. The most abundant tea catechin compound is EGCG. In humans drinking green tea according to Chinese traditions, serum levels of EGCG are in the range of 0.1–0.3 μM [31]. Catechins are also found in most fresh fruits, some legumes and in chocolate. In green tea-drinking populations, the lower incidence of esophageal and breast cancers has been attributed to flavonoids in tea, although epidemiological investigations on correlations of green tea consumptions and low risks of cancer incidences remain controversial [34]. EGCG is con-

sidered chemopreventive and has antitumor cell proliferative, invasive and metastasis effects, as well as anti-inflammatory characteristics [31,35,36]. Although the potential antitumor effects of green tea and its catechins had been investigated for a long time, little was known about their mechanisms. Several studies suggest that the antioxidative effect of catechins is involved in its antitumor activity. Other studies imply that tea catechins prevent carcinogen-induced tumorigenesis and tumor invasion [9,37]. One of the targets of EGCG has been shown to be the tissue plasminogen activator (t-PA), which is one of the critical proteases utilized by tumors to invade healthy tissues for metastasis [36]. However, these studies have not provided direct *in vivo* evidence of tumor suppression by catechins through these pathways.

Tumor growth and metastasis are dependent on the degree of neovascularization. A large body of evidence produced by various laboratories has shown that suppression of angiogenesis effectively block tumor growth and metastasis. Most angiogenesis inhibitors are isolated as protein molecules. As angiogenesis is critical for tumor growth, it was hypothesized that green tea and EGCG might also inhibit angiogenesis. Our laboratory was the first group to test this hypothesis. We have found that EGCG directly inhibits capillary endothelial cell proliferation at low concentrations, at which tumor cells were completely insensitive. *In vivo,* EGCG inhibits blood vessel formation and sprouting in developing chick embryos. Furthermore, mice drinking fresh green tea preparations showed significant inhibition of corneal neovascularization. This study demonstrates that green tea polyphenols are oral angiogenesis inhibitors. It is perhaps the first oral, natural angiogenesis inhibitor described. This study not only provides a mechanistic insight of how tea catechins inhibit tumor growth, but also points out that catechins as polyphenols is an important group of angiogenesis inhibitors [27].

Stimulated by this initial discovery, several other groups have confirmed that EGCG and other tea catechins inhibit endothelial growth and differentiation, and angiogenesis *in vivo* [38–40]. At the molecular level, antiangiogenic mechanisms include inhibition of MMP-2 and MMP-9, as well as urokinase plasminogen activator (u-PA) at higher concentrations (IC_{50} mM range), down-regulation of VEGF production in tumor cells, and repression of AP-1, NFκB and STAT-1 transcription factor pathways [41–43]. The targets of EGCG on blood vessels seem not only limited to endothelial cells. Recently, it is reported that EGCG inhibits epidermal growth factor receptor (EGFR) and platelet derived growth factor receptor (PDGFR)-β phosphorylations in vascular smooth muscle cells (VSMC), and inactivates phosphoinositide 3-kinase (PI3 kinase) and MAP kinase p42/p44 pathway [31]. Thus, EGCG and perhaps other catechins may have broad and complex impact on the suppression of blood vessel growth.

4.3. Resveratrol and quercetin

The "French paradox" has been described as low mortality rates of arteriosclerosis, heart disease and perhaps certain cancer forms, despite high fat caloric intake by the French population. This cardio-protective effect has been attributed to moderate intake of alcohol and antioxidant components in wine [44–47]. It appears that red wine, but not white wine, is the most beneficial beverage against cardiovascular diseases. More recently, red wine has been reported to reduce endothelin-1 production in endothelial cells and thus lead to vasodilation and lower blood pressure [48]. Polyphenols seem to be the active ingredients in wine, and red wine contains on average 10 mM polyphenols, including flavonoids, isoflavones and tannins [49]. Among the most abundant ones, resveratrol and quercetin have attracted much attention and have been extensively studied both *in vitro* and *in vivo.* Resveratrol is a compound found in grapes and its related products and also in some nuts. The concentrations of resveratrol in red wine range from 8–25 μM, depending on the types of grapes used and their culture conditions [50,51]. Resveratrol is enriched in the seeds and skin of the grapes, and has relatively poor solubility in water. However, resveratrol is soluble in low percentages of alcohol and is thus effectively absorbed by the body from wine [52,53]. It has been found to protect against ischemic reperfusion injury, cardiovascular diseases such as thrombosis and arteriosclerosis, and more recently also against cancer [54–56].

The heart protective effects of resveratrol are at least in part related to the anti-inflammatory and antioxidant activities, including reduction of LDL oxidation, platelet aggregation and inhibition of cyclooxygenase (COX) enzymes [49,57]. For example, one of the possible mechanisms could be inhibition of phosphorylation of IκB via blockage of induction of IκB kinases (IKK) by resveratrol in human monocytes, leading to inactivation of NFκB [58]. Thus resveratrol indirectly inhibits the activation of genes, such as proinflammatory cytokines regulated by NFκB. Further, resveratrol has been shown to induce vasodilation by up-regulation of nitric oxide synthase (NOS) in VSMC [59]. Resveratrol induces endothelial NOS (eNOS) and quercetin stimulates NOS in VSMC [49,60,61]. Further, wine polyphenols, such as catechin, epicatechin, quercetin, and resveratrol increase the levels of surface-localized fibrinolytic activity, such as t-PA and u-PA, in endothelial cells [62]. These effects of red wine polyphenols may provide a potential mechanism for the prevention of cardiovascular diseases.

Resveratrol have been shown to affect tumorigenesis and tumor growth [63]. It inhibits both normal and malignant cell proliferation *in vitro* at relatively high concentrations (20–100 μM). These cell types include fibroblasts, mouse epithelial cells and breast, colon and prostate carcinoma cells, and inhibitory mechanisms of resveratrol involve cell cycle G_1/S-phase arrest [64,65]. In animal studies, subcuta-

neous Lewis lung carcinomas and their lung metastases were inhibited by systemic administration of resveratrol [66]. The antitumor effect seems to correlate with reduction of tumor neovascularization. The antitumor mechanisms of resveratrol are complex and involve inhibition of MAP kinase phosphorylations, 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced AP-1 activation, c-Src, and COX-2 activity [67,68]. At high concentrations, resveratrol induces apoptosis in endothelial and tumor cells by stimulation of release of mitochondrial cyt-c, which subsequently activates the caspase 3-mediated apoptotic pathway [69,70]. Further more, resveratrol has been shown to induce accumulation of p53 in endothelial cells [61]. Thus, resveratrol-induced cellular apoptosis may involve both p53- and caspase-mediated pathways. Quercetin has been reported to suppress the *in vitro* proliferation of colorectal, breast, gastric, ovarian and lymphoid tumor cells [71]. It induces apoptosis in leukemic tumor cells by reduction of heat-shock protein (HSP70) production, which leads to stress response-induced cell death [72].

Quercetin also affects mitotic cell cycles in tumor cells, gene expressions, immune responses, free radical formation and angiogenesis [73]. *In vivo*, it is an oral antitumor agent in mouse tumor models [73].

Recently, resveratrol has been reported to be an angiogenesis inhibitor that is sufficiently potent to suppress FGF-2- and VEGF-induced neovascularization *in vivo* [26]. It directly inhibits capillary endothelial cell proliferation, migration and tube formation *in vitro* [26,74]. Quercetin exhibits similar inhibitory effects albeit at somewhat higher concentrations [74]. It appears that the MAP kinase pathway is at least one of the targets of resveratrol in endothelial cells, as it effectively inhibits phosphorylations of MAP kinases [26]. It has been found that resveratrol inhibits the binding of VEGF to human umbilical vein endothelial cells [66]. Resveratrol upregulates $p21^{WAF}$ in endothelial cells, which down-regulates cyclin D1, -D2 and -E as well as cdk -2, -4 and -6, leading to cell cycle arrest at the G_1-phase [61]. In developing chick embryos, resveratrol inhibits neovascularization in the chorioallantoic membrane (CAM) in a dose dependent manner. In a mouse tumor model, oral administration of resveratrol inhibits tumor growth and the antitumor effect is well correlated with reduction of vascular density [26]. Thus, antiangiogenesis is a part of the mechanism of tumor suppression by resveratrol. However, the antiangiogenic feature of resveratrol is not only limited to pathological angiogenesis, such as tumor neovascularization, but also affects physiological angiogenesis. For example, the dosages used in suppression of tumor growth in mice effectively delayed wound repair in normal mice [26]. Thus, resveratrol cannot distinguish physiological angiogenesis from pathological blood vessel growth. Unlike single angiogenic factor antagonists, the therapeutic value of this molecule is that it blocks a common angiogenic pathway triggered by several angiogenic factors. Another advantage is that this small molecule can be given orally.

4.4. Genistein and daidzein

The intake of soybeans has been epidemiologically correlated with the lower incidence of breast, prostate and colon cancers in China and Japan as compared with Western European and American populations. For instance, the intake of soy proteins by Chinese and Japanese populations is considerably higher (30–35 g/day) than by Western populations (5–10 g/day) [75,76]. Soybeans contain many polyphenol compounds, of which the most abundant ones, genistein (1–3 mg/g soy protein) and daidzein have been investigated most thoroughly [77]. Oriental populations consume 20–80 mg genistein/day, whereas in the USA intake is only 1–3 mg/day [75]. The concentration of genistein in the urine of humans eating a plant-based diet is 30-fold higher as compared with those consuming a traditional Western diet [78]. Soy proteins have been reported in animal studies to inhibit the growth of murine bladder carcinoma by mechanisms of inhibiting cell proliferation, induction of tumor cell apoptosis, and inhibition of angiogenesis [75,79]. Genistein was discovered as an inhibitor of tyrosine kinase EGFR phosphorylation [80]. Several *in vitro* and *in vivo* studies describe the chemopreventive effects of this isoflavone. Genistein and daidzein inhibits tumor cell proliferation *in vitro* [81]. In comparison to genistein, daidzein has weaker inhibitory effect [82]. In prostate cancer cells, genistein was shown to induce G_2/M cell cycle arrest by increasing p21 and inhibiting cyclin B expression [83]. However, various tumor cell lines show different sensitivities to genistein treatment [76]. Most tumor cells only responded to concentrations above 10 μM, which surmounts the presumed upper limit for serum concentrations of genistein even in humans on a high soy diet. In contrast, the anti-endothelial proliferative effects was seen with an IC_{50} of 3.7–18 μM, suggesting that genistein at physiological concentrations (<18 μM) would be more likely to affect angiogenesis rather than tumor cells [84].

Genistein, quercetin, apigenin, and fisetin inhibit endothelial cell proliferation *in vitro* [21,22,85]. Genistein has also been found to inhibit endothelial cell migration and tube formation. *In vivo*, genistein inhibits corneal neovascularization induced by FGF-2 in rabbits [86]. In support of this finding, genistein suppresses tumor growth *in vivo*. Its antitumor effect is correlated with increased apoptosis, reduction in tumor cell proliferation, and decreased vessel density [79]. Genistein is currently in clinical trials as an angiogenesis inhibitor for the treatment of breast and prostate cancer [76,87,88]. Among the many described effects of genistein, the antiangiogenic mechanisms involve down-regulation of MMP-9 and VEGF, as well as upregulation of tissue inhibitor of metalloproteinases (TIMP)-1, which lead to reduction of tumor cell invasion and blood vessel growth [31,89].

4.5. Apigenin and luteolin

The flavone apigenin is commonly found in leafy vegetables, fruits and tea leaves [7]. It is one of the most widely occurring plant flavonoids found in natural products. For example, the concentration of apigenin in celery roots is about 75 mg/kg [90]. Apigenin inhibits the proliferation of breast cancer cells and is a chemopreventive agent in mouse tumor models [91–94]. In keratinocytes, apigenin induces G_1 cell-cycle arrest by inhibition of cdk2 kinase activity, hypophophorylation of Rb and by induction of the cdk inhibitor $p21^{WAF1}$ [95]. It also inhibits lymphatic vessel growth in chemically induced intestinal rat adenocarcinomas [96]. *In vitro,* apigenin inhibits endothelial cell proliferation, migration and tube formation [22,90]. Antiangiogenic mechanisms have been suggested to involve inhibition of hyaluronidase, resulting in decreased production of cleaved hyaluronic acids, which may stimulate angiogenesis. A direct antiangiogenic mechanism of apigenin is inhibition of endothelial cell proliferation by inducing hyperphosphorylation of Rb, leading to G_2/M cell cycle arrest [90]. In addition, apigenin inhibits migration of endothelial cells and capillary formation *in vitro,* independently of its inhibition of hyaluronidase activity. In contrast, in VSMC apigenin stimulates cdk2 activity by repression of p21 and p27 expression, resulting in a stimulation of proliferation in these cells [90].

Luteolin is found in many vegetables and fruits, such as olives, apples and citrus fruits. Together with apigenin, luteolin is considered as one of the most potent anti-tumor proliferative flavonoids. In human tumor cells, luteolin inhibits both EGFR tyrosine autophosphorylation and phosphorylation of its downstream MAP kinases, and decreases c-myc protein levels, leading to apoptosis [97]. Apigenin and luteolin upregulates p21 expression in tumor cells, leading to cell cycle G_2/M arrest [83]. Luteolin have been shown to inhibit endothelial cell proliferation and migration [22,78]. Furthermore, luteolin and quercetin potently inhibited the secretion of tumor cell MMP-2 and MMP-9 [98]. *In vivo,* it inhibits FGF-2 induced corneal neovascularization in rabbits [77]. In this experiment, the flavonoid fisetin was the most potent inhibitor, followed by genistein and luteolin.

4.6. Other flavonoids

4.6.1. Fisetin

Fisetin is widely distributed in fruits and vegetables. It weakly inhibits endothelial cell proliferation and migration although the mechanisms are still unclear [22]. However, this flavonoid seems to be a potent angiogenesis inhibitor *in vivo.* In the CAM assay, fisetin was shown to induce avascular zone formation at the dose of 100 ng/egg [99]. Further, fisetin was shown to potently inhibit rabbit corneal neovascularizationl [77].

4.6.2. Myricetin

Myricetin is found in vegetables, fruits and berries, such as in black currants and cranberry juice. It inhibits endothelial cell proliferation *in vitro,* with mechanism suggested to involve in inhibition of IKKs [100,101].

4.6.3. Baicalein

Baicalein, purified from *Scutellaria baicalensis Georgi* (Huangqi), is identified as the active ingredient in oriental herbal medicine, Qing-Fei-Tang. It has traditionally been used for treatment of chronic respiratory diseases with long-lasting cough, and have recently been investigated for its anti-asthmatic effects [102]. Its antitumor mechanisms include inhibition of cdk-4 and cdk-6, leading to growth arrest at cell cycle G_0/G_1 [103]. At higher concentrations it induced apoptosis in tumor cells by upregulation of Fas ligand. Baicalein was reported to inhibit 15-lipooxygenase in endothelial cells at low concentrations [104]. It has also been found to inhibit NOS and to block endothelial proliferation by induction of growth arrest [105,106].

4.6.4. Silibinin

Silibinin is the main active component of silymarin (380 mg silibinin/g silymarin), a flavonoid extract from the milk thistle (*Silybum marianum*) [107]. After consumption of 140 mg silymarin the plasma concentration of silibinin in humans was reported to range from 40–180 ng/ml, with a half-life of several hours [107]. The plant has been widely used for its antihepatotoxicity effects. Silymarin exhibits antiproliferative effects in prostate, breast and cervical tumor cell lines, as well as cancer preventive efficacy in mouse skin tumorigenesis models [108–111]. Some of silymarin's anticancer effects could be due to its antiangiogenic properties including preventing VEGF and MMP-2 secretion in breast and prostate tumor cells, and induction of endothelial apoptosis [31,112].

5. Future perspectives

Polyphenols isolated from natural products have become an important group of small molecules in prevention and treatment of diseases. It is believed that this group of health beneficial molecules will be further expanded as novel compounds will be discovered and tested in experimental systems. Many natural polyphenols have inhibitory effects on tumor growth *in vivo.* Although their activities are nonspecific for tumor cells, and the underlying mechanisms are complex, these natural compounds have several great advantages over other anticancer therapeutic agents. 1) Our daily diet is rich in many of these polyphenols, and they can be easily obtained in grocery stores without prescriptions. 2) These polyphenol-enriched natural products are usually available at low costs. 3) Ingestion of these natural products does not require FDA approvals. 4) Polyphenolic small

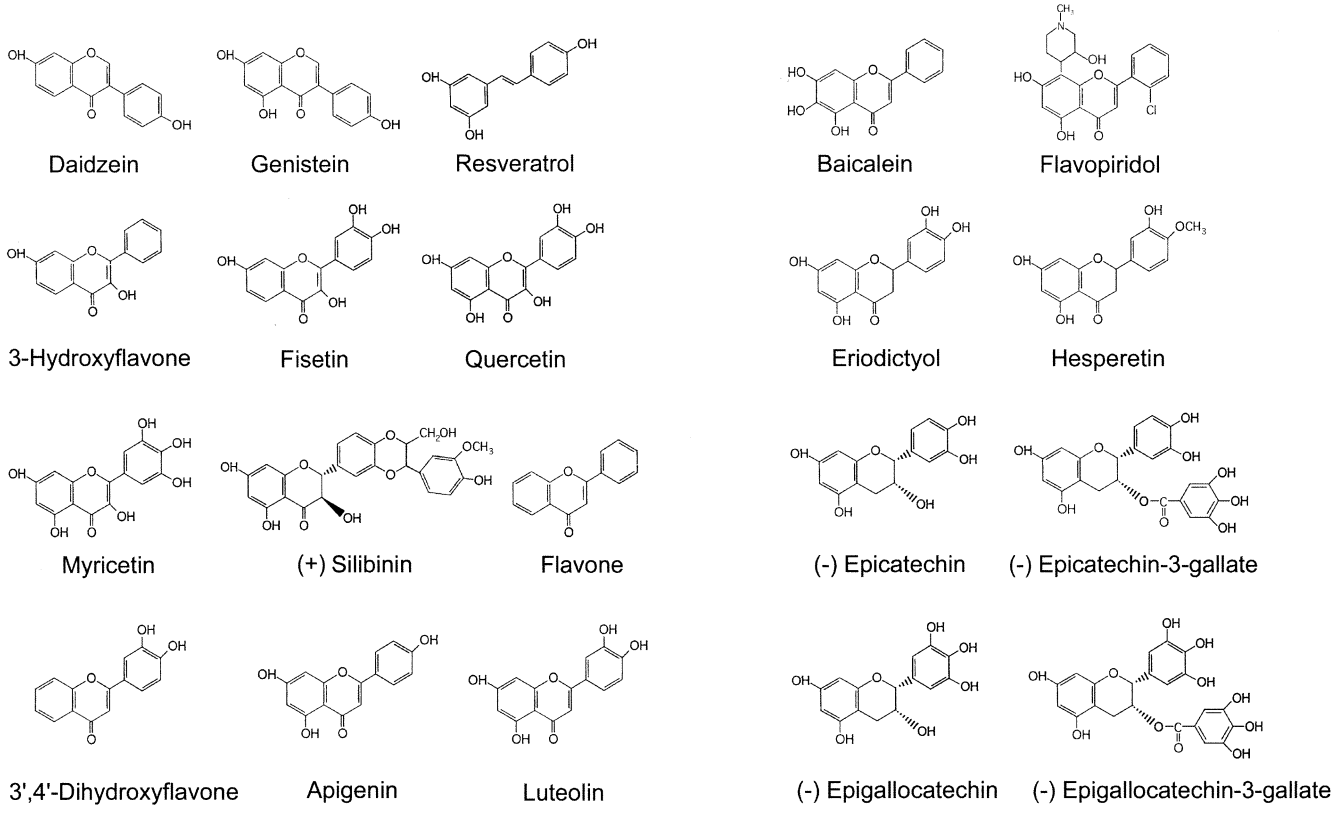

Fig. 1. Chemical structures of antiangiogenic polyphenols.

molecules can usually be sufficiently absorbed after oral ingestion. 5) They rarely have any side effects, as many have been used as food products for centuries. 6) The half-life of many polyphenols in the body is relatively long. 7) They are ideal therapeutic agents for long-term prevention and treatment of diseases such as angiogenesis dependent diseases. For example, antiangiogenesis compounds may have to be delivered for the rest of life of a cancer patient. As oral angiogenesis antagonists, we would like to emphasize that these compounds are non-specific angiogenesis inhibitors. They generally inhibit the growth of a wide spectrum of cell types. However, endothelial cells seem to be more sensitive to these polyphenols than other cell types, because relatively low concentrations of these polyphenols can result in endothelial cell inhibition. Thus, selective inhibition of angiogenesis can be achieved by using low dosages.

The other important anticancer feature of these polyphenols is that they have a broad spectrum of effects on various tumors derived from different tissues. This broad effect may be partially due to their antiangiogenic activity as all tumor growth is angiogenesis dependent. It appears that these polyphenols target common angiogenic pathways triggered by various angiogenic factors. For example, resveratrol blocks both FGF-2- and VEGF-induced angiogenesis. This broad effect has significant therapeutic implications in the treatment of cancer. It is known that tumor cells are frequently mutated and they switch their proangiogenic factors during tumor progression [113]. Thus, antiangiogenic agents targeting single angiogenic factor, including VEGF antagonists, may encounter drug resistance in cancer treatment. In contrast, intervention of common angiogenic pathways by polyphenols and other antiangiogenesis compounds should have great advantage over the mono-factorial angiogenesis antagonists.

Although many characterized polyphenols have antiangiogenic features, they are generally not potent angiogenesis inhibitors. Thus, oncologists and patients should not consider these compounds as “miracle” anti-cancer drugs, especially within short-term consumption. However, based on the chemical structures of these natural antiangiogenic polyphenols (Fig. 1), it most likely seems possible to identify more potent and specific angiogenesis inhibitors from their synthetic analogs. For example, flavopiridol is a synthetic flavone derivative with potent anti-endothelial, antiangiogenic and antitumor effects [114,115]. Currently, flavopiridol is in phase II clinical trials for the treatment of various cancer forms in humans [116]. We speculate that both natural and synthetic polyphenolic compounds will become important therapeutic agents in cancer and other angiogenesis dependent diseases. These compounds, either alone or in combinations with other current therapeutic strategies, will produce beneficial effects against most common human diseases including cancer and cardiovascular diseases.

References

[1] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, et al., Initial sequencing and analysis of the human genome, Nature 409 (2001) 860–921.

[2] J.C. Venter, M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G. Sutton, H.O. Smith, M. Yandell, C.A. Evans, R.A, Holt, et al., The sequence of the human genome, Science 291 (2001) 1304–1351.

[3] A. Harvey, Strategies for discovering drugs from previously unexplored natural products, Drug Discov Today 5 (2000) 294–300.

[4] G.M. Cragg, D.J. Newman, K.M. Snader, Natural products in drug discovery and development, J Nat Prod 60 (1997) 52–60.

[5] C.J. Dufresne, E.R. Farnworth, A review of latest research findings on the health promotion properties of tea, J Nutr Biochem 12 (2001) 404–421.

[6] A. Damianaki, E. Bakogeorgou, M. Kampa, G. Notas, A. Hatzoglou, S. Panagiotou, C. Gemetzi, E. Kouroumalis, P.M. Martin, E. Castanas, Potent inhibitory action of red wine polyphenols on human breast cancer cells, J Cell Biochem 78 (2000) 429–441.

[7] K. Griffiths, M.S. Morton, L. Denis, Certain aspects of molecular endocrinology that relate to the influence of dietary factors on the pathogenesis of prostate cancer, Eur Urol 35 (1999) 443–455.

[8] G.J. Soleas, D.M. Goldberg, L. Grass, M. Levesque, E.P. Diamandis, Do wine polyphenols modulate p53 gene expression in human cancer cell lines?, Clin Biochem 34 (2001) 415–420.

[9] C.S. Yang, J.M. Landau, M.T. Huang, H.L. Newmark, Inhibition of carcinogenesis by dietary polyphenolic compounds, Annu Rev Nutr 21 (2001) 381–406.

[10] Y. Cao, Endogenous angiogenesis inhibitors and their therapeutic implications, Int J Biochem Cell Biol 33 (2001) 357–369.

[11] Y. Cao, Endogenous angiogenesis inhibitors: angiostatin, endostatin, and other proteolytic fragments, Prog Mol Subcell Biol 20 (1998) 161–176.

[12] M.S. O'Reilly, The preclinical evaluation of angiogenesis inhibitors, Invest New Drugs 15 (1997) 5–13.

[13] J. Folkman, Tumor angiogenesis: therapeutic implications, N Engl J Med 285 (1971) 1182–1186.

[14] M.A. Gimbrone Jr., R.S. Cotran, S.B. Leapman, J. Folkman, Tumor growth and neovascularization: an experimental model using the rabbit cornea, J Natl Cancer Inst 52 (1974) 413–427.

[15] Y. Cao, P. Linden, J. Farnebo, R. Cao, A. Eriksson, V. Kumar, J.H. Qi, L. Claesson-Welsh, K. Alitalo, Vascular endothelial growth factor C induces angiogenesis in vivo, Proc Natl Acad Sci USA 95 (1998) 14389–14394.

[16] A. Eriksson, R. Cao, R. Pawliuk, S.M. Berg, M. Tsang, D. Zhou, C. Fleet, K. Tritsaris, S. Dissing, P. Leboulch, Y. Cao, Placenta growth factor-1 antagonizes VEGF-induced angiogenesis and tumor growth by the formation of functionally inactive PlGF-1/VEGF heterodimers, Cancer Cell 1 (2002) 99–108.

[17] http://www.nci.nih.gov/clinical_trials/.

[18] F. Casagrande, J.M. Darbon, Effects of structurally related flavonoids on cell cycle progression of human melanoma cells: regulation of cyclin-dependent kinases CDK2 and CDK1, Biochem Pharmacol 61 (2001) 1205–1215.

[19] M.G. Hertog, P.C. Hollman, M.B. Katan, D. Kromhout, Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands, Nutr Cancer 20 (1993) 21–29.

[20] P.C. Hollman, M.B. Katan, Health effects and bioavailability of dietary flavonols, Free Radic Res 31 (1999) S75–80.

[21] T. Fotsis, M. Pepper, H. Adlercreutz, T. Hase, R. Montesano, L. Schweigerer, Genistein, a dietary ingested isoflavonoid, inhibits cell proliferation and in vitro angiogenesis, J Nutr 125 (1995) 790S–797S.

[22] T. Fotsis, M.S. Pepper, E. Aktas, S. Breit, S. Rasku, H. Adlercreutz, K. Wahala, R. Montesano, L. Schweigerer, Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis, Cancer Res 57 (1997) 2916–2921.

[23] M. Segasothy, P.A. Phillips, Vegetarian diet: panacea for modern lifestyle diseases?, QJM 92 (1999) 531–544.

[24] F.O. Ranelletti, R. Ricci, L.M. Larocca, N. Maggiano, A. Capelli, G. Scambia, P. Benedetti-Panici, S. Mancuso, C. Rumi, and M. Piantelli, Growth-inhibitory effect of quercetin and presence of type-II estrogen-binding sites in human colon-cancer cell lines and primary colorectal tumors, Int J Cancer 50 (1992) 486–492.

[25] Z.M. Shao, M.L. Alpaugh, J.A. Fontana, S.H. Barsky, Genistein inhibits proliferation similarly in estrogen receptor-positive and negative human breast carcinoma cell lines characterized by P21WAF1/CIP1 induction, G2/M arrest, and apoptosis, J Cell Biochem 69 (1998) 44–54.

[26] E. Brakenhielm, R. Cao, Y. Cao, Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes, FASEB J 15 (2001) 1798–1800.

[27] Y. Cao, R. Cao, Angiogenesis inhibited by drinking tea, Nature 398 (1999) 381.

[28] G. Peterson, Evaluation of the biochemical targets of genistein in tumor cells, J Nutr 125 (1995) 784S–789S.

[29] J.R. Stewart, N.E. Ward, C.G. Ioannides, and C.A. O'Brian, Resveratrol preferentially inhibits protein kinase C-catalyzed phosphorylation of a cofactor-independent, arginine-rich protein substrate by a novel mechanism, Biochemistry 38 (1999) 13244–13251.

[30] S. Garbisa, S. Biggin, N. Cavallarin, L. Sartor, R. Benelli, A. Albini, Tumor invasion: molecular shears blunted by green tea, Nat Med 5 (1999) 1216.

[31] F. Tosetti, N. Ferrari, S. De Flora, A. Albini, Angioprevention: angiogenesis is a common and key target for cancer chemopreventive agents, FASEB J 16 (2002) 2–14.

[32] C.S. Yang, J.Y. Chung, G. Yang, S.K. Chhabra, M.J. Lee, Tea and tea polyphenols in cancer prevention, J Nutr 130 (2000) 472S–478S.

[33] D.A. Balentine, S.A. Wiseman, L.C. Bouwens, The chemistry of tea flavonoids, Crit Rev Food Sci Nutr 37 (1997) 693–704.

[34] Y. Tsubono, Y. Nishino, S. Komatsu, C.C. Hsieh, S. Kanemura, I. Tsuji, H. Nakatsuka, A. Fukao, H. Satoh, S. Hisamichi, Green tea and the risk of gastric cancer in Japan, N Engl J Med 344 (2001) 632–636.

[35] L. Sartor, E. Pezzato, S. Garbisa, (-)Epigallocatechin-3-gallate inhibits leukocyte elastase: potential of the phyto-factor in hindering inflammation, emphysema, and invasion, J Leukoc Biol 71 (2002) 73–79.

[36] J. Jankun, S.H. Selman, R. Swiercz, E. Skrzypczak-Jankun, Why drinking green tea could prevent cancer, Nature 387 (1997) 561.

[37] C.S. Yang, M.J. Lee, L. Chen, G.Y. Yang, Polyphenols as inhibitors of carcinogenesis, Environ Health Perspect 105 (1997) 971–976.

[38] Y.D. Jung, L.M. Ellis, Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea, Int J Exp Pathol 82 (2001) 309–316.

[39] S. Lamy, D. Gingras, R. Beliveau, Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation, Cancer Res 62 (2002) 381–385.

[40] R. Swiercz, E. Skrzypczak-Jankun, M.M. Merrell, S.H. Selman, J. Jankun, Angiostatic activity of synthetic inhibitors of urokinase type plasminogen activator, Oncol Rep 6 (1999) 523–526.

[41] S. Garbisa, L. Sartor, S. Biggin, B. Salvato, R. Benelli, A. Albini, Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate, Cancer 91 (2001) 822–832.

[42] Y.D. Jung, M.S. Kim, B.A. Shin, K.O. Chay, B.W. Ahn, W. Liu, C.D. Bucana, G.E. Gallick, L.M. Ellis, EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells, Br J Cancer 84 (2001) 844–850.

[43] J.K. Lin, Y.C. Liang, S.Y. Lin-Shiau, Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade, Biochem Pharmacol 58 (1999) 911–915.

[44] M. Gronbaek, A. Deis, T.I. Sorensen, U. Becker, P. Schnohr, G. Jensen, Mortality associated with moderate intakes of wine, beer, or spirits, BMJ 310 (1995) 1165–1169.
[45] S. Renaud, M. de Lorgeril, Wine, alcohol, platelets, and the French paradox for coronary heart disease, Lancet 339 (1992) 1523–1526.
[46] M.H. Criqui, B.L. Ringel, Does diet or alcohol explain the French paradox?, Lancet 344 (1994) 1719–1723.
[47] M. Serafini, A. Ghiselli, A. Ferro-Luzzi, Red wine, tea, and antioxidants, Lancet 344 (1994) 626.
[48] R. Corder, J.A. Douthwaite, D.M. Lees, N.Q. Khan, A.C. Viseu Dos Santos, E.G. Wood, M.J. Carrier, Endothelin-1 synthesis reduced by red wine, Nature 414 (2001) 863–864.
[49] J.M. Wu, Z.R. Wang, T.C. Hsieh, J.L. Bruder, J.G. Zou, Y.Z. Huang, Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in red wine (Review), Int J Mol Med 8 (2001) 3–17.
[50] A.A. Bertelli, L. Giovannini, R. Stradi, S. Urien, J.P. Tillement, A. Bertelli, Evaluation of kinetic parameters of natural phytoalexin in resveratrol orally administered in wine to rats, Drugs Exp Clin Res 24 (1998) 51–55.
[51] Y. Wang, F. Catana, Y. Yang, R. Roderick, R.B. van Breemen, An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and in wine, J Agric Food Chem 50 (2002) 431–435.
[52] A.A. Bertelli, L. Giovannini, R. Stradi, A. Bertelli, J.P. Tillement, Plasma, urine and tissue levels of trans- and cis-resveratrol (3,4′,5-trihydroxystilbene) after short-term or prolonged administration of red wine to rats, Int J Tissue React 18 (1996) 67–71.
[53] A. Bertelli, A.A. Bertelli, A. Gozzini, L. Giovannini, Plasma and tissue resveratrol concentrations and pharmacological activity, Drugs Exp Clin Res 24 (1998) 133–138.
[54] L.M. Hung, J.K. Chen, S.S. Huang, R.S. Lee, M.J. Su, Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes, Cardiovasc Res 47 (2000) 549–555.
[55] A.A. Bertelli, L. Giovannini, D. Giannessi, M. Migliori, W. Bernini, M. Fregoni, A. Bertelli, Antiplatelet activity of synthetic and natural resveratrol in red wine, Int J Tissue React 17 (1995) 1–3.
[56] Z. Wang, Y. Huang, J. Zou, K. Cao, Y. Xu, J.M. Wu, Effects of red wine and wine polyphenol resveratrol on platelet aggregation in vivo and in vitro, Int J Mol Med 9 (2002) 77–79.
[57] O. Saijonmaa, T. Nyman, R. Kosonen, F. Fyhrquist, Upregulation of angiotensin-converting enzyme by vascular endothelial growth factor, Am J Physiol Heart Circ Physiol 280 (2001) H885–H891.
[58] M. Holmes-McNary, A.S. Baldwin Jr., Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase, Cancer Res 60 (2000) 3477–3483.
[59] E.K. Naderali, S.L. Smith, P.J. Doyle, G. Williams, The mechanism of resveratrol-induced vasorelaxation differs in the mesenteric resistance arteries of lean and obese rats, Clin Sci (Lond) 100 (2001) 55–60.
[60] M. Chiesi, R. Schwaller, Inhibition of constitutive endothelial NO-synthase activity by tannin and quercetin, Biochem Pharmacol 49 (1995) 495–501.
[61] T.C. Hsieh, G. Juan, Z. Darzynkiewicz, J.M. Wu, Resveratrol increases nitric oxide synthase, induces accumulation of p53 and p21(WAF1/CIP1), and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G2, Cancer Res 59 (1999) 2596–2601.
[62] L.H. Abou-Agag, M.L. Aikens, E.M. Tabengwa, R.L. Benza, S.R. Shows, H.E. Grenett, F.M. Booyse, Polyphyenolics increase t-PA and u-PA gene transcription in cultured human endothelial cells, Alcohol Clin Exp Res 25 (2001) 155–162.
[63] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beecher, H.H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G, Mehta, et al., Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275 (1997) 218–220.
[64] A. Sgambato, R. Ardito, B. Faraglia, A. Boninsegna, F.I. Wolf, A. Cittadini, Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage, Mutat Res 496 (2001) 171–180.
[65] N. Ahmad, V.M. Adhami, F. Afaq, D.K. Feyes, H. Mukhtar, Resveratrol causes WAF-1/p21-mediated G(1)-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells, Clin Cancer Res 7 (2001) 1466–1473.
[66] Y. Kimura, H. Okuda, Resveratrol isolated from Polygonum cuspidatum root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice, J Nutr 131 (2001) 1844–1849.
[67] M. MacCarrone, T. Lorenzon, P. Guerrieri, A.F. Agro, Resveratrol prevents apoptosis in K562 cells by inhibiting lipoxygenase and cyclooxygenase activity, Eur J Biochem 265 (1999) 27–34.
[68] K. Subbaramaiah, P. Michaluart, W.J. Chung, T. Tanabe, N. Telang, A.J. Dannenberg, Resveratrol inhibits cyclooxygenase-2 transcription in human mammary epithelial cells, Ann N Y Acad Sci 889 (1999) 214–223.
[69] B. Szende, E. Tyihak, Z. Kiraly-Veghely, Dose-dependent effect of resveratrol on proliferation and apoptosis in endothelial and tumor cell cultures, Exp Mol Med 32 (2000) 88–92.
[70] J.W. Park, Y.J. Choi, S.I. Suh, W.K. Baek, M.H. Suh, I.N. Jin, D.S. Min, J.H. Woo, J.S. Chang, A, Passaniti, et al., Bcl-2 overexpression attenuates resveratrol-induced apoptosis in U937 cells by inhibition of caspase-3 activity, Carcinogenesis 22 (2001) 1633–1639.
[71] D.M. Morrow, P.E. Fitzsimmons, M. Chopra, H. McGlynn, Dietary supplementation with the anti-tumour promoter quercetin: its effects on matrix metalloproteinase gene regulation, Mutat Res 480–481 (2001) 269–276.
[72] Y.Q. Wei, X. Zhao, Y. Kariya, H. Fukata, K. Teshigawara, A. Uchida, Induction of apoptosis by quercetin: involvement of heat shock protein, Cancer Res 54 (1994) 4952–4957.
[73] A. Hayashi, A.C. Gillen, J.R. Lott, Effects of daily oral administration of quercetin chalcone and modified citrus pectin, Altern Med Rev 5 (2000) 546–552.
[74] K. Igura, T. Ohta, Y. Kuroda, K. Kaji, Resveratrol and quercetin inhibit angiogenesis in vitro, Cancer Lett 171 (2001) 11–16.
[75] S. Barnes, T.G. Peterson, L. Coward, Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer, J Cell Biochem Suppl 22 (1995) 181–187.
[76] M.J. Messina, V. Persky, K.D. Setchell, and S. Barnes, Soy intake and cancer risk: a review of the in vitro and in vivo data, Nutr Cancer 21 (1994) 113–131.
[77] A.M. Joussen, K. Rohrschneider, J. Reichling, B. Kirchhof, F.E. Kruse, Treatment of corneal neovascularization with dietary isoflavonoids and flavonoids, Exp Eye Res 71 (2000) 483–487.
[78] T. Fotsis, M.S. Pepper, R. Montesano, E. Aktas, S. Breit, L. Schweigerer, S. Rasku, K. Wahala, H. Adlercreutz, Phytoestrogens and inhibition of angiogenesis, Baillieres Clin Endocrinol Metab 12 (1998) 649–666.
[79] J.R. Zhou, P. Mukherjee, E.T. Gugger, T. Tanaka, G.L. Blackburn, S.K. Clinton, Inhibition of murine bladder tumorigenesis by soy isoflavones via alterations in the cell cycle, apoptosis, and angiogenesis, Cancer Res 58 (1998) 5231–5238.
[80] T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukami, Genistein, a specific inhibitor of tyrosine-specific protein kinases, J Biol Chem 262 (1987) 5592–5595.
[81] D. Dixon-Shanies, N. Shaikh, Growth inhibition of human breast cancer cells by herbs and phytoestrogens, Oncol Rep 6 (1999) 1383–1387.
[82] J.R. Zhou, E.T. Gugger, T. Tanaka, Y. Guo, G.L. Blackburn, S.K. Clinton, Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice, J Nutr 129 (1999) 1628–1635.

[83] T. Kobayashi, T. Nakata, T. Kuzumaki, Effect of flavonoids on cell cycle progression in prostate cancer cells, Cancer Lett 176 (2002) 17–23.
[84] S. Barnes, Effect of genistein on in vitro and in vivo models of cancer, J Nutr 125 (1995) 777S–783S.
[85] T. Fotsis, M. Pepper, H. Adlercreutz, G. Fleischmann, T. Hase, R. Montesano, L. Schweigerer, Genistein, a dietary-derived inhibitor of in vitro angiogenesis, Proc Natl Acad Sci USA 90 (1993) 2690–2694.
[86] F.E. Kruse, A.M. Joussen, T. Fotsis, L. Schweigerer, K. Rohrschneider, H.E. Volcker, [Inhibition of neovascularization of the eye by dietary factors exemplified by isoflavonoids], Ophthalmologe 94 (1997) 152–156.
[87] Comment, Clinical development plan: genistein, J Cell Biochem Suppl 26 (1996) 114–126.
[88] T.P. Fan, R. Jaggar, R. Bicknell, Controlling the vasculature: angiogenesis, anti-angiogenesis and vascular targeting of gene therapy, Trends Pharmacol Sci 16 (1995) 57–66.
[89] Z.M. Shao, J. Wu, Z.Z. Shen, S.H. Barsky, Genistein exerts multiple suppressive effects on human breast carcinoma cells, Cancer Res 58 (1998) 4851–4857.
[90] V. Trochon, E. Blot, F. Cymbalista, C. Engelmann, R.P. Tang, A. Thomaidis, M. Vasse, J. Soria, H. Lu, C. Soria, Apigenin inhibits endothelial-cell proliferation in G(2)/M phase whereas it stimulates smooth-muscle cells by inhibiting P21 and P27 expression, Int J Cancer 85 (2000) 691–696.
[91] D.F. Birt, D. Mitchell, B. Gold, P. Pour, and H.C. Pinch, Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid, Anticancer Res 17 (1997) 85–91.
[92] B. Li, H. Pinch, D.F. Birt, Influence of vehicle, distant topical delivery, and biotransformation on the chemopreventive activity of apigenin, a plant flavonoid, in mouse skin, Pharm Res 13 (1996) 1530–1534.
[93] H. Wei, L. Tye, E. Bresnick, D.F. Birt, Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice, Cancer Res 50 (1990) 499–502.
[94] D.F. Birt, B. Walker, M.G. Tibbels, E. Bresnick, Anti-mutagenesis and anti-promotion by apigenin, robinetin and indole-3-carbinol, Carcinogenesis 7 (1986) 959–963.
[95] D.M. Lepley, J.C. Pelling, Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin, Mol Carcinog 19 (1997) 74–82.
[96] A. Tatsuta, H. Iishi, M. Baba, H. Yano, K. Murata, M. Mukai, H. Akedo, Suppression by apigenin of peritoneal metastasis of intestinal adenocarcinomas induced by azoxymethane in Wistar rats, Clin Exp Metastasis 18 (2000) 657–662.
[97] F. Yin, A.E. Giuliano, A.J. Van Herle, Signal pathways involved in apigenin inhibition of growth and induction of apoptosis of human anaplastic thyroid cancer cells (ARO), Anticancer Res 19 (1999) 4297–4303.
[98] Y.T. Huang, J.J. Hwang, P.P. Lee, F.C. Ke, J.H. Huang, C.J. Huang, C. Kandaswami, E. Middleton Jr., M.T. Lee, Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor, Br J Pharmacol 128 (1999) 999–1010.
[99] T. Oikawa, M. Shimamura, H. Ashino, O. Nakamura, T. Kanayasu, I. Morita, S. Murota, Inhibition of angiogenesis by staurosporine, a potent protein kinase inhibitor, J Antibiot (Tokyo) 45 (1992) 1155–1160.
[100] M.F. Melzig, R. Loose, G. Schonherr, Effect of flavonoids on daunomycin-induced toxicity in cultivated endothelial cells, Pharmazie 52 (1997) 793–796.
[101] S.H. Tsai, Y.C. Liang, S.Y. Lin-Shiau, J.K. Lin, Suppression of TNFalpha-mediated NFkappaB activity by myricetin and other flavonoids through downregulating the activity of IKK in ECV304 cells, J Cell Biochem 74 (1999) 606–615.
[102] C. Taniguchi, M. Homma, O. Takano, T. Hirano, K. Oka, Y. Aoyagi, T. Niitsuma, T. Hayashi, Pharmacological effects of urinary products obtained after treatment with saiboku-to, a herbal medicine for bronchial asthma, on type IV allergic reaction, Planta Med 66 (2000) 607–611.
[103] W. Liu, M. Kato, A.A. Akhand, A. Hayakawa, M. Takemura, S. Yoshida, H. Suzuki, I. Nakashima, The herbal medicine sho-saiko-to inhibits the growth of malignant melanoma cells by upregulating Fas-mediated apoptosis and arresting cell cycle through downregulation of cyclin dependent kinases, Int J Oncol 12 (1998) 1321–1326.
[104] T. Schewe, C. Sadik, L.O. Klotz, T. Yoshimoto, H. Kuhn, H. Sies, Polyphenols of cocoa: inhibition of mammalian 15-lipoxygenase, Biol Chem 382 (2001) 1687–1696.
[105] S.L. Hsu, Y.C. Hsieh, W.C. Hsieh, C.J. Chou, Baicalein induces a dual growth arrest by modulating multiple cell cycle regulatory molecules, Eur J Pharmacol 425 (2001) 165–171.
[106] Z.Y. Chen, Y.L. Su, C.W. Lau, W.I. Law, Y. Huang, Endothelium-dependent contraction and direct relaxation induced by baicalein in rat mesenteric artery, Eur J Pharmacol 374 (1999) 41–47.
[107] B. Rickling, B. Hans, R. Kramarczyk, G. Krumbiegel, R. Weyhenmeyer, Two high-performance liquid chromatographic assays for the determination of free and total silibinin diastereomers in plasma using column switching with electrochemical detection and reversed-phase chromatography with ultraviolet detection, J Chromatogr B Biomed Appl 670 (1995) 267–277.
[108] J. Zhao, M. Lahiri-Chatterjee, Y. Sharma, R. Agarwal, Inhibitory effect of a flavonoid antioxidant silymarin on benzoyl peroxide-induced tumor promotion, oxidative stress and inflammatory responses in SENCAR mouse skin, Carcinogenesis 21 (2000) 811–816.
[109] J. Zhao, R. Agarwal, Tissue distribution of silibinin, the major active constituent of silymarin, in mice and its association with enhancement of phase II enzymes: implications in cancer chemoprevention, Carcinogenesis 20 (1999) 2101–2108.
[110] X. Zi, R. Agarwal, Silibinin decreases prostate-specific antigen with cell growth inhibition via G1 arrest, leading to differentiation of prostate carcinoma cells: implications for prostate cancer intervention, Proc Natl Acad Sci USA 96 (1999) 7490–7495.
[111] M. Lahiri-Chatterjee, S.K. Katiyar, R.R. Mohan, R. Agarwal, A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model, Cancer Res 59 (1999) 622–632.
[112] C. Jiang, R. Agarwal, J. Lu, Anti-angiogenic potential of a cancer chemopreventive flavonoid antioxidant, silymarin: inhibition of key attributes of vascular endothelial cells and angiogenic cytokine secretion by cancer epithelial cells, Biochem Biophys Res Commun 276 (2000) 371–378.
[113] J. Folkman, Looking for a good endothelial address, Cancer Cell 1 (2002) 113–115.
[114] J.S. Kerr, R.S. Wexler, S.A. Mousa, C.S. Robinson, E.J. Wexler, S. Mohamed, M.E. Voss, J.J. Devenny, P.M. Czerniak, A. Gudzelak Jr., A.M. Slee, Novel small molecule alpha v integrin antagonists: comparative anti-cancer efficacy with known angiogenesis inhibitors, Anticancer Res 19 (1999) 959–968.
[115] S. Brusselbach, D.M. Nettelbeck, H.H. Sedlacek, R. Muller, Cell cycle-independent induction of apoptosis by the anti-tumor drug flavopiridol in endothelial cells, Int J Cancer 77 (1998) 146–152.
[116] R.M. Lush, M.A. Rudek, W.D. Figg, Review of three new agents that target angiogenesis, matrix metalloproteinases, and cyclin-dependent kinases, Cancer Control 6 (1999) 459–465.