

# Angiogenesis: from plants to blood vessels

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**Angiogenesis is a major pathological component of diseases such as cancer and coronary heart disease. Although major advances have been made and encouraging clinical results obtained, safer and more effective approaches are required. The identification of new drugs from plants has a long and successful history, and certain proangiogenic and antiangiogenic plant components have been used in traditional Chinese medicine (TCM) for thousands of years. Similar to Western combination therapy, TCM uses mixtures of plant extracts, termed *fufang*, to maximize efficacy and minimize adverse effects or toxicity. More evidence-based research and chemical optimization of these compounds could further enhance the effectiveness of these plant-based medicines in angiotherapy.**

## Medicines from plants

Since antiquity, plants have been used to treat many ailments. However, it was not until the 1800s that pure compounds were isolated from plants, paving the way for modern pharmaceuticals. In 1805, morphine was isolated from the opium poppy (*Papaver somniferum*) by the German pharmacist Friedrich Serturner. Following the isolation of salicylic acid from the bark of the willow tree (*Salix alba*), Felix Hoffmann synthesized aspirin in 1897. Ephedrine was isolated from the Chinese herb mahuang (*Ephedra*) in 1887 and became popular with American physicians in 1924 for its bronchodilating and decongestant properties. Sodium cromoglycate, first used in 1968, is a khellin derivative that was isolated from Egyptian khella seeds (*Ammi visnaga*) by Roger Altounyan. The antimalarial drug artemisinin was developed in 1972 from the Chinese herb qinghao (sweet wormwood, *Artemisia annua* L.). These examples illustrate the rich history of plant-based medicines.

Angiogenesis is the growth of neovessels from existing vasculature. Usually, angiogenesis is tightly controlled by a balance of angiogenesis factors and inhibitors, and occurs only in embryonic development, wound healing and the

female reproductive cycle. Angiogenic diseases result from new blood vessels growing either excessively (e.g. cancer, diabetic retinopathy and psoriasis) or insufficiently (e.g. chronic wounds and ischaemic heart disease). To date, the stimulation of angiogenesis using angiogenesis peptides has produced encouraging clinical results in treating coronary artery disease. Blocking angiogenesis with antibodies of angiogenesis factors or with enzyme inhibitors is effective for treating malignancy but there is room for improvement. Of particular relevance to this article is the fact that some of the plant-derived anticancer drugs (e.g. Taxol<sup>®</sup>, camptothecin and combretastatin) are antiangiogenic. In traditional Chinese medicine (TCM), many herbs are used in the treatment of angiogenic diseases such as chronic wounds and rheumatoid arthritis. Thus, it is rational to explore these medicinal plants as a source of novel angiomodulators. In this article, we review plant-based angiotherapy and discuss potential future TCM therapies.

## Angiogenesis

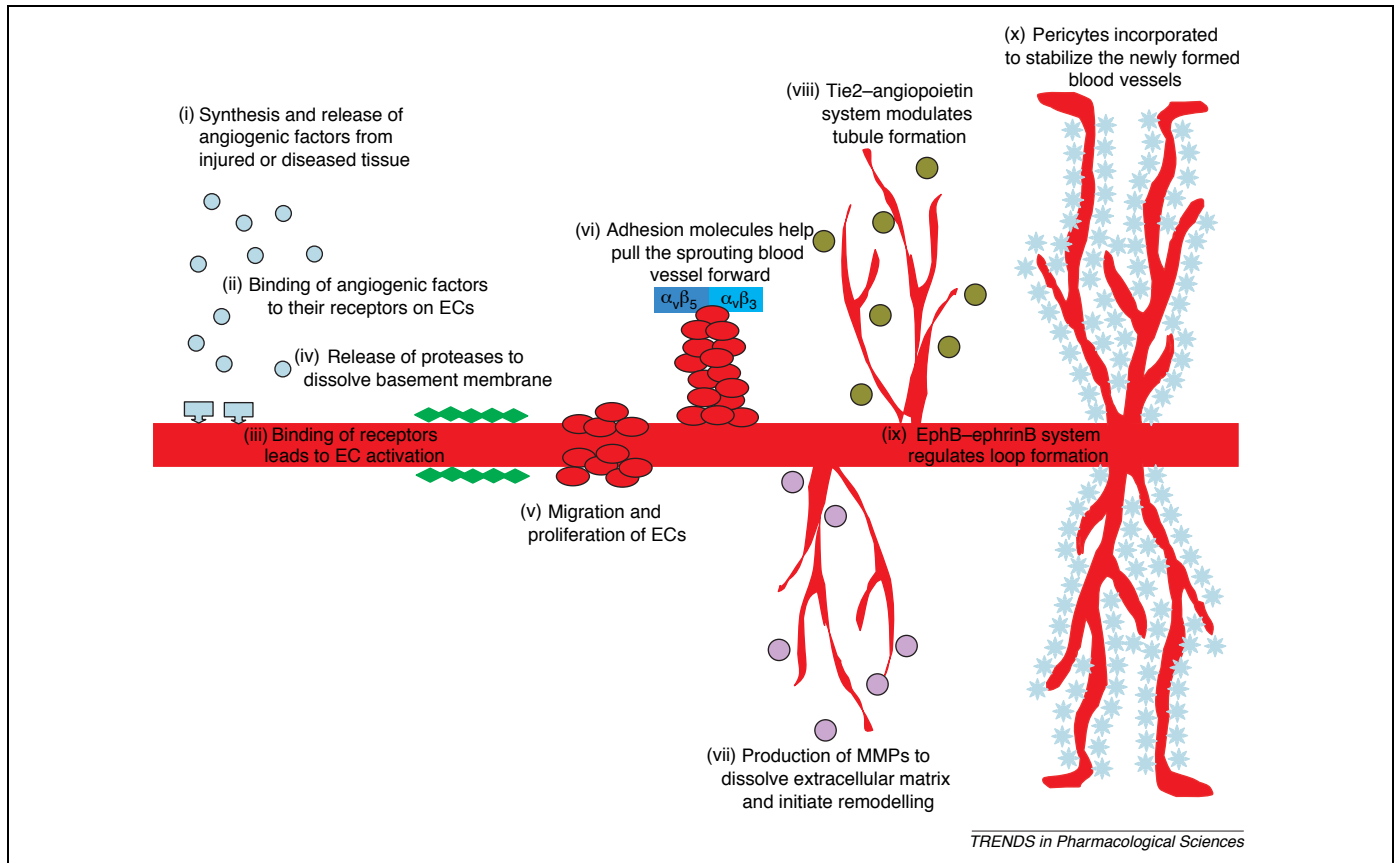
Since Judah Folkman's seminal article about tumour angiogenesis [1], numerous articles have been written about the aspects of angiogenesis (e.g. Ref. [2]; see also *Angiogenesis*: <http://www.kluweronline.com/issn/0969-6970/contents>).

There are ten sequential steps of angiogenesis (Figure 1). Recent studies have shown the importance of leucocytes as providers of cytokines, chemokines and enzymes that are involved in angiogenesis. Angiogenesis stimulators and inhibitors target one or more of these steps.

- (i) In response to hypoxia, injured or diseased tissues synthesize and release angiogenic factors;
- (ii) angiogenic factors bind to their receptors on endothelial cells (ECs);
- (iii) receptor binding leads to EC activation;
- (iv) proteases are released to dissolve the basement membrane;
- (v) ECs migrate and proliferate;
- (vi) adhesion molecules (e.g. integrin  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ ) help to pull the sprouting blood vessel forward;

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Available online 11 May 2006



**Figure 1.** The ten sequential steps of angiogenesis. Only the key cellular and molecular events are depicted. Depending on the microenvironment (e.g. oxygen tension, leucocyte infiltration and release of antiangiogenic factors such as transforming growth factor- $\beta$  and platelet factor 4), the newly developed vasculature either undergoes maturation into a functional network or regresses to maintain the original vascular density.

- (vii) matrix metalloproteinases (MMPs) are produced to dissolve the extracellular matrix and to initiate remodelling;
- (viii) angiopoietin-Tie-2 interaction modulates tubule formation;
- (ix) the EphB-ephrinB system regulates loop formation;
- (x) pericytes are incorporated to stabilize the newly formed blood vessel.

### Angiogenesis is a common denominator of many diseases

In 1994, The Angiogenesis Foundation (<http://www.angio.org>) declared angiogenesis a 'common denominator' in the most important diseases of society. In many serious diseases states, the body loses control of angiogenesis.

#### Excessive angiogenesis

In diseases such as cancer (Figure 2), age-related macular degeneration, psoriasis and endometriosis, excessive angiogenesis occurs when diseased cells produce abnormally large amounts of angiogenesis factors [e.g. vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2 and hepatocyte growth factor], overwhelming the effects of natural angiogenesis inhibitors (e.g. angiostatin, endostatin and thrombospondin). More than 70 other conditions (e.g. obesity and asthma) are associated with excessive angiogenesis. In these conditions, new blood vessels feed diseased tissues and destroy normal tissues;

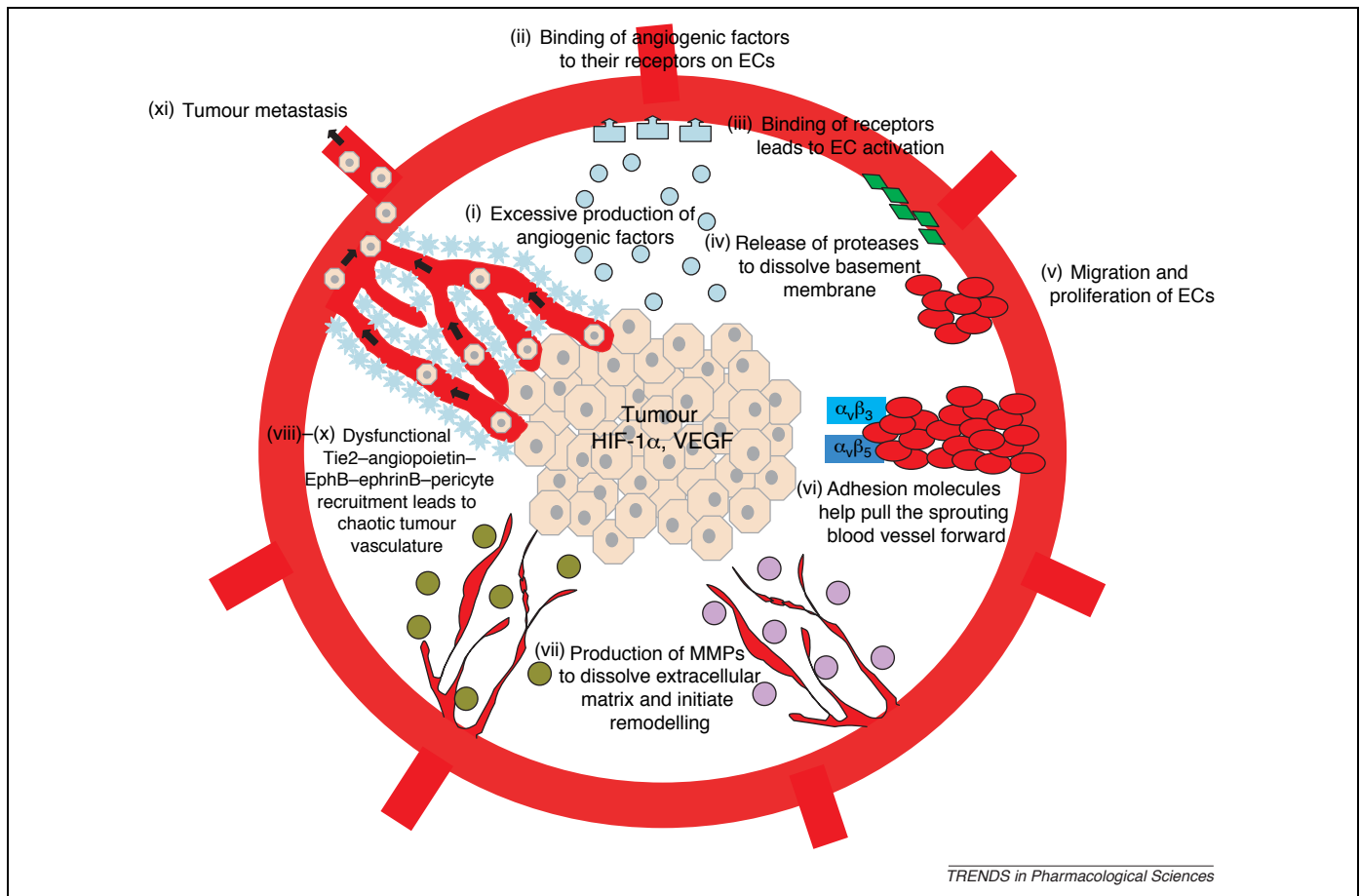
in cancer, tumour cells use the new vessels to escape into the circulation and lodge in other organs (tumour metastases). Antiangiogenic therapies, which are aimed at suppressing new blood vessel growth, are being developed to treat these chronic diseases.

#### Insufficient angiogenesis

In chronic wound, coronary artery disease, stroke and non-union fracture, inadequate (in size and/or number) blood vessels grow and circulation is not properly restored, leading to the risk of tissue death and, in the case of alopecia, hair loss. Insufficient angiogenesis is caused by the inadequate production of angiogenesis growth factors and/or excessive amounts of angiogenesis inhibitors. Therapeutic angiogenesis, which is aimed at stimulating neovascularization with growth factors, is being developed to reverse these conditions.

#### Angiotherapy

Angiotherapy is an operational term introduced to encompass several treatments that are aimed at 'turning on' or 'turning off' angiogenesis in disease [3]. Stabilization and maturation of the neovasculature is also crucial to the successful outcome of angiotherapy (Table 1). In diabetic retinopathy, the new blood vessels are immature and prone to haemorrhage. Instead of inhibiting the growth of these leaky vessels totally, it might be more beneficial to induce the proper incorporation of pericytes and the formation of



**Figure 2.** Tumour angiogenesis and metastasis. In response to high metabolic demand and low oxygen tension, tumour cells express HIF-1 $\alpha$ , leading to the overproduction of angiogenic factors (e.g. VEGF-A, FGF-2 and angiopoietins) and their receptors on ECs. The concurrent underproduction of endogenous angiogenic inhibitors (e.g. angiostatin and thrombospondin) and their receptors leads to an imbalance in favour of the angiogenic switch. Following the breakdown of basement membrane, ECs migrate and proliferate, resulting in uncontrolled neovascularization. Dysfunctional interactions of the Tie2-angiopoietin and EphB-ephrinB systems, and pericyte recruitment by PDGF-B produced by ECs contribute to the chaotic tumour vasculature. Tumour cells continue to grow in response to growth or survival factors (e.g. hepatocyte growth factor and heregulin) generated by tumour-associated fibroblasts. Eventually, tumour cells escape into neovessels and metastasize to distant organs.

basement membrane to create a functional vascular network.

During the past decade, major advances have been made in the field of angiogenesis, including the elucidation of the signalling pathways of several angiogenesis factors and the discovery of several natural and synthetic angiogenesis stimulators and inhibitors, leading to the translation of experimental drugs into clinical use.

In 1997, the first angiogenesis-stimulating drug – becaplermin (Regranex<sup>™</sup>), a recombinant human platelet-derived growth factor (PDGF) – was approved by the Food and Drug Administration (FDA: <http://www.fda.gov/>) for treating diabetic foot ulcers. This was followed in 1999 by a huge wave of angiogenesis-stimulating factors entering clinical trials for coronary artery disease [4], peripheral vascular disease, stroke and chronic wound. In parallel, antiangiogenic drugs were tested in patients with cancer, age-related macular degeneration, diabetic retinopathy or psoriasis. In 2003, bevacizumab (Avastin<sup>®</sup>), a humanized monoclonal antibody against VEGF-A, became the first antiangiogenic drug in large-scale clinical trials to inhibit tumour blood vessel growth and prolong survival in patients with metastatic colorectal cancer [5] and other malignancies. When used in combination with fluorouracil-based chemotherapy, Avastin<sup>®</sup> improves overall response

rates, time to progression, and survival. This achievement renewed confidence in targeted antiangiogenic approaches for constituting a complementary therapeutic modality in addition to surgery, radiotherapy and chemotherapy. Thus, angiotherapy has the potential to become a major adjuvant for managing a large number of diseases characterized by inadequate or excessive angiogenesis.

### Plants as a source of angiogenesis-modulating compounds

As highlighted by Szymkowski [6] and Lindsay [7], recent genomics-focused drug-discovery efforts have failed to produce the expected large number of compounds aimed at ‘novel’ targets. The multifactorial nature of chronic diseases is probably the most significant problem in relation to target discovery. By contrast, the identification of efficacious drugs from plants has a long, albeit difficult,

**Table 1. The three aspects of angiotherapy**

Angiostimulation (therapeutic angiogenesis)	Angiosuppression (antiangiogenesis)
Chronic wound	Tumour, arthritis, psoriasis
Myocardial infarction	Atherosclerosis, endometriosis
Non-union fracture	Diabetic retinopathy
Alopecia	Obesity
<b>Angiostabilization</b>	

precedent. Thus, there is always merit in supplementing rational design and high-throughput screening in drug development by paying attention to traditional remedies. In a recent programme on BBC2, Kathy Sykes investigated the ancient practice of herbalism (<http://www.open2.net/alternativemedicine/>). Different countries have distinct herbal traditions, each with their indigenous plants and unique practices. But one claim underlies them all – herbs have remarkable properties that make them potentially powerful medicines. In South Africa, *Sutherlandia frutescens* is being developed as a new treatment for HIV–AIDS. In Germany, medicinal herbs are being broken down into their constituent parts and submitted to rigorous clinical trials.

Plants contain many active ingredients. They are complex chemical cocktails with medicinal properties that modern pharmaceuticals cannot reproduce. A wide range of plants contains compounds with angiogenesis-modulating properties (Figure 3, Table 2).

### Some plant-derived anticancer drugs are antiangiogenic *Taxol*<sup>®</sup>

In 1962, scientists at the National Cancer Institute (<http://www.cancer.gov/>) determined that an extract from the bark of the Pacific yew tree (*Taxus brevifolia*) possessed anticancer properties. In 1971, the active compound in this extract was identified by Wani *et al.* [8] as *Taxol*<sup>®</sup>, a complex polyoxygenated diterpene (Figure 3). However, it was estimated that between three and ten 100-year-old trees are required to produce enough natural *Taxol*<sup>®</sup> to treat one cancer patient. Fortunately, the needles and twigs of the European yew tree (*Taxus Baccata*) contain 10-deacetyl baccatin III, which is a close relative of *Taxol*<sup>®</sup>. Because the needles are quickly replenished, harvesting large quantities has little effect on the population of yew trees. To produce a sustainable source, 10-deacetyl baccatin III was chemically modified by Bristol-Myers Squibb (<http://www.bms.com/landing/data/index.html>) and, in 1995, a semi-synthetic version of *Taxol*<sup>®</sup> – paclitaxel – was made available to the public.

*Taxol*<sup>®</sup> kills proliferating cancer cells by disrupting their microtubule cytoskeletons [9–11]. Of particular interest is the recent discovery that, at low picomolar concentrations, *Taxol*<sup>®</sup> is antiangiogenic, inhibiting VEGF production [12] and hypoxia-inducible factor (HIF)- $\alpha$  protein expression [13]. The data support a clinical application of continuous ultra-low-dose *Taxol*<sup>®</sup> to treat cancer [14].

### *Camptothecin*

Using bioactivity-directed fractionation, Wall *et al.* [15] isolated camptothecin (Figure 3) from the Chinese tree *Camptotheca acuminata*. In this technique, the crude plant extract is purified in an iterative manner and the ‘fractions’ are tested for bioactivity at each stage. Those fractions showing the most potent activity progress to the next stage of purification. The process is repeated many times until the compound (or compounds) responsible for the bioactivity observed with the crude extract is isolated.

The use of camptothecin as an anticancer agent languished for almost 15 years until its unique mode of action for killing tumour cells was determined. Camptothecin traps topoisomerase I in complexes with DNA, thus preventing DNA replication and resulting in the death of the cancer cell. The discovery of this rekindled the interest in developing analogues of camptothecin that are water soluble and retain their anticancer activity. In the mid-1990s, two camptothecin analogues, topotecan and irinotecan, received FDA approval for use against ovarian, lung, breast and colon cancers [16,17].

In 1999, Clements *et al.* [18] showed that camptothecin and topotecan inhibit human EC growth *in vitro* in a noncytotoxic manner and that this inhibition lasts >96 h after drug removal. They also showed that these two compounds, unlike the nonspecific cytotoxic agent cisplatin, are as effective as TNP-470 [19] at inhibiting angiogenic growth in the *in vivo* disc angiogenesis model. Thus, in addition to their tumoricidal activities, camptothecins might have an indirect *in vivo* antitumour effect that is mediated through the inhibition of angiogenesis.

### *Combretastatin*

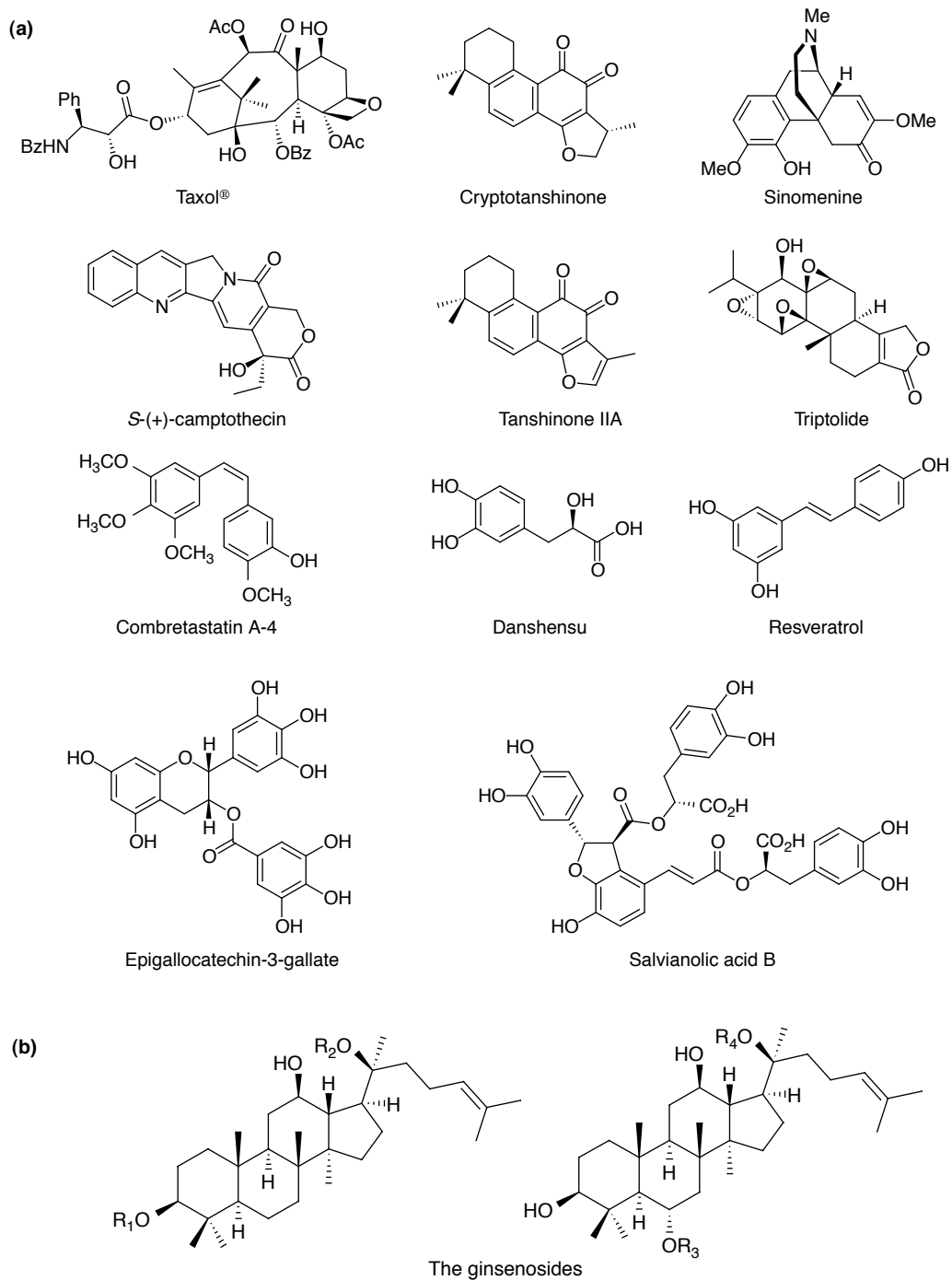
Combretastatin is a microtubule-targeting agent found in the bark of the African bush willow tree (*Combretum caffrum*), identified in 1987 by Pettit *et al.* [20]. Further work led to the production of water-soluble phosphate derivatives with enhanced bioavailability [21,22]. Vincent *et al.* [23] showed that combretastatin A4 phosphate (CA4P) (Figure 3) selectively targets ECs, but not smooth muscle cells, and induces the regression of unstable nascent tumour neovessels in mice by rapidly disrupting the molecular engagement of the EC-specific junctional molecule vascular endothelial (VE) cadherin *in vitro* and *in vivo*. CA4P increases EC permeability and inhibits EC migration and capillary tube formation, predominantly through the disruption of the VE-cadherin– $\beta$ -catenin–Akt signalling pathway, resulting in rapid vascular collapse and tumour necrosis. CA4P synergizes with low and nontoxic doses of neutralizing monoclonal antibodies to VE cadherin by blocking the assembly of neovessels, thereby inhibiting tumour growth. These data indicate that CA4P selectively induces the regression of unstable tumour neovessels, in part through the disruption of VE cadherin signalling. A combined treatment of anti-VE-cadherin agents in conjunction with microtubule-disrupting agents provides a novel synergistic strategy to disrupt assembly and induce regression of tumour neovessels selectively, with minimal toxicity and without affecting normal stabilized vasculature.

### Antiangiogenic functional foods

Because angiogenesis can be overcome with a systemic nontoxic low dose of angiogenesis inhibitors over a long period of time, antiangiogenic functional foods provide a good strategy for the prevention of degenerative diseases [24,25]. Here, we focus on three of these foods.

### *Soybeans*

The incidence of breast cancer and prostate cancer is much lower in Asian countries such as Japan and China



Ginsenoside	R1	R2	R3	R4
Rb <sub>1</sub>	Glc <sup>1-2</sup> Glc-	Glc <sup>1-6</sup> Glc-	n/a	n/a
Rb <sub>2</sub>	Glc <sup>1-2</sup> Glc-	Ara(p) <sup>1-6</sup> Glc-	n/a	n/a
Rg <sub>3</sub>	Glc <sup>1-2</sup> Glc-	H-	n/a	n/a
Re	n/a	n/a	Rha <sup>1-2</sup> Glc-	Glc-
Rg <sub>1</sub>	n/a	n/a	Glc-	Glc-

Glc,  $\beta$ -D-glucopyranosyl; Xyl,  $\beta$ -D-xylopyranosyl; Ara(p),  $\alpha$ -L-arabinopyranosyl; Rha,  $\alpha$ -L-rhamnoopyranosyl.

Figure 3. Chemical structures of angiomodulators derived from medicinal plants and functional foods.

**Table 2. Plant-derived angiogenic and antiangiogenic compounds and their mechanisms**

Plant species	Compound	Mechanism	Refs
<b>Angiogenic compounds</b>			
<i>Aloe vera</i>	$\beta$ -Sitosterol	Stimulates HUVEC motility; enhances expression of von Willebrand factor, VEGF, VEGF receptor Flk-1 and laminin	[64,65]
<i>Vitis</i> spp. (grape)	Resveratrol	Upregulates VEGF and Flk-1	[38]
<i>Panax ginseng</i> (ginseng)	Ginsenoside Rg1	Upregulates eNOS expression, which activates the PI3K–Akt pathway	[42]
<i>P. ginseng</i>	Ginsenoside Re	Stimulates EC proliferation, migration and tube formation	[43]
<i>Astragalus membranaceus</i> (huangqi)	Unknown	Unknown	[46–49]
<i>Angelica sinensis</i> (danggui)	Unknown	Unknown	[46–49]
<i>Salvia miltiorrhiza</i> (danshen)	Salvianolic acid B	Upregulates the genes encoding MMP-2, VEGF, VEGF receptor 2 and Tie-1	[50]
<b>Antiangiogenic compounds</b>			
<i>Glycine max</i> (soybean)	Genistein	Suppresses VEGF and FGF-2 expression; inhibits receptor tyrosine kinase; inhibits activation of NF- $\kappa$ B and Akt signalling pathways	[26–30]
<i>Camellia sinensis</i> (green tea)	EGCG	Abrogates VEGF signalling by interfering with formation of VEGF receptor 2 complex	[31–34]
<i>Vitis</i> spp. (grape)	Resveratrol	Disrupts Src-dependent VE cadherin tyrosine phosphorylation	[35,36]
<i>Taxus brevifolia</i> (Pacific yew tree)	Taxol <sup>®</sup>	Disrupts microtubule cytoskeleton; inhibits VEGF production; inhibits HIF-1 $\alpha$ protein	[8–14]
<i>Vinca rosea</i> (periwinkle)	Vincristine	Disrupts microtubule cytoskeleton; inhibits VEGF production	[10–12]
<i>Camptotheca acuminata</i>	Camptothecin	Blocks topoisomerase I; inhibits EC proliferation and tube formation; decreases HIF-1 $\alpha$ and VEGF expression	[15–18]
<i>Combretum caffrum</i> (African bush willow tree)	Combretastatin	Inhibits tubulin assembly	[20–23]
<i>Glycyrrhiza uralensis</i> (liquorice)	Isoliquiritin	Inhibits tube formation	[66,67]
<i>P. ginseng</i>	Ginsenosides Rb1, Rb2 and Rg3	Inhibit VEGF production by tumour cells	[41,44,45]
<i>S. miltiorrhiza</i> (danshen)	TIIA Cryptotanshinone	G <sub>1</sub> –G <sub>0</sub> arrest of ECs Apoptosis of ECs	[51,52]
<i>Sinomenium acutum</i>	Sinomenine	G <sub>1</sub> –G <sub>0</sub> arrest of ECs	[53,54]
<i>Tripterygium wilfordii</i> Hook.f.	Triptolide	Inhibits VEGF expression and secretion from ECs; inhibits COX-1, COX-2 and 5-lipoxygenase; decreases transcription of the gene encoding inducible nitric oxide synthase	[53,55–58]
<i>Cordyceps militaris</i>	Unknown	Inhibits FGF-2 expression in ECs and MMP-2 expression in tumour cells	[68]
<i>Ganoderma lucidum</i>	Polysaccharide peptide	Causes EC apoptosis by reducing Bcl-2 expression and increasing Bax expression; decreases VEGF secretion from tumour cells	[69]

than in the USA and European countries. Epidemiological studies indicate that the significant difference in cancer incidence among different ethnic groups is partly attributable to dietary habits. Indeed, one of the major differences in diet among these populations is that the Japanese and the Chinese consume a diet that is traditionally high in soy products. Among the predominant isoflavones in soy, genistein (Figure 3) is the most potent at inhibiting EC proliferation and *in vitro* angiogenesis at an IC<sub>50</sub> of 150  $\mu$ M, and inhibits carcinogenesis in animal models [26]. Moreover, genistein suppresses VEGF and FGF-2 expression, inhibits receptor tyrosine kinase phosphorylation and activation of Akt and inhibits the activation of NF- $\kappa$ B, resulting in apoptosis in otherwise apoptosis-resistant cancer cells. As a phytoestrogen, genistein also targets oestrogen- and androgen-mediated signalling pathways in carcinogenesis and has antioxidant properties. Overall, both *in vivo* and *in vitro* studies have shown that this isoflavone is a promising reagent for cancer chemoprevention and/or treatment. [27–30].

#### Green tea

In 1999, Cao and Cao [31] reported that both green tea and one of its components, epigallocatechin-3-gallate

(EGCG) (Figure 3), significantly prevent angiogenesis. Further work showed that EGCG suppresses the oxidant-induced production of the proangiogenic cytokine IL-8 and inhibits VEGF-induced Akt activation and VE cadherin phosphorylation at physiological doses [32,33]. The amount of green tea in the drinking water of the animals was 1.25% (4.69 mg/ml), containing 708  $\mu$ g/ml of EGCG. The concentration of EGCG in the plasma was reported to be 0.1–0.3  $\mu$ M, which is similar to levels in humans after drinking two or three cups of green tea. These data indicate that drinking green tea could be beneficial for preventing and treating angiogenic diseases such as cancer and diabetic retinopathy. To confirm this, proper pharmacokinetics studies should be carried out in humans [34].

#### Red grapes

Resveratrol (Figure 3), which is present in red wine, peanuts, mulberries and medicinal plants such as *Polygonum cuspidatum*, inhibits angiogenesis without causing severe side-effects when administered orally [35]. It has attracted considerable attention as one of the most promising cancer chemopreventive agents in recent years [36]. Thus, it is likely that more polyphenols in natural products will be discovered as angiogenesis

inhibitors and that they could serve as leading structures in the discovery of more-potent, synthetic angiogenesis inhibitors [37]. However, Fukuda *et al.* [38] reported that resveratrol ameliorates myocardial damage by inducing VEGF angiogenesis and the tyrosine kinase receptor Flk-1. The impact of this latest finding awaits further evaluation.

### Chinese medicinal herbs as a source of angiogenesis modulators

It is clear that plants have the potential to be a rich source of angiogenesis modulators and it is noteworthy that cancer chemotherapeutic strategies commonly require multiple agents. The relatively recent practice of combining drugs for synergistic effects in Western medicine has long been exploited in TCM in the form of *fufang* (Box 1), in which different herbs are used in combination as a composite formula.

What is the difference between Western and Eastern approaches? In the West, the search is always for the active component, even if this means that the efficacy is lower compared with the full plant extract. The goal is to identify a lead compound that can be chemically modified to produce more-potent analogues with better water solubility and bioavailability. This is illustrated by the development of Taxol<sup>®</sup>, camptothecin and combretastatin.

In the East, the oral administration of highly effective combinations of plants is often used. In London in the 1990s, for example, Ding-Hui Luo used a combination of ten herbs to treat steroid-resistant eczema in children. This oral *fufang* has been effective in clinical studies [39], and Phytopharm (<http://www.phytopharm.co.uk/>) has been developing a simplified *fufang* with four herbs. However, a multitude of challenges results from trying to apply a Western approach to therapies originating from the East and, indeed, to any plant-based system of therapy: (i) many active compounds are present in each herb; (ii) the synergistic and antagonistic interactions between active compounds from different herbs are largely unknown; (iii) individual active compounds are usually less potent than the total herbal extract from which they are isolated; (iv) some compounds are prodrugs and are active only after absorption and metabolism; and (v) slower action compared with Western medicine. Against this background, we review the angiomodulatory effects and mechanisms of action of some pure compounds isolated in TCM. With rigorous evaluation of their individual and combinatorial activities, it is possible to create a new paradigm for charting the future of TCM drug development for angiotherapy.

### Angiogenic stimulators from TCM

#### *Bidirectional effects of Panax ginseng*

*Panax ginseng* has been prized as a panacea in TCM for at least 2000 years. It has become a commonly used nutraceutical, with annual sales in excess of US\$0.2 billion. Intriguingly, existing literature reports both wound-healing [40] and antitumour [41] effects of ginseng extract through opposing activities on the vascular system. To elucidate this, we merged a chemical fingerprinting

approach with a deconstructional study of the effects of pure molecules from ginseng extract on angiogenesis [42].

Mass-spectrometric compositional analysis of American, Chinese and Korean, and Sanqi ginseng revealed distinct 'sterol ginsenoside' fingerprints, especially in the ratio between the panaxatriol ginsenoside Rg1 and the panaxadiol ginsenoside Rb1 (Figure 3), the two most prevalent constituents of ginseng. Using a matrigel implant model and reconstituting the extracts using distinct ratios of the two ginsenosides, we demonstrated that the dominance of Rg1 leads to angiogenesis, whereas Rb1 dominance exerts an opposing effect.

Rg1 also promotes functional neovascularization into a polymer scaffold *in vivo* and the proliferation of, chemoinvasion of and tubulogenesis by human umbilical vein ECs (HUVECs) *in vitro*: an effect mediated by the expression of endothelial nitric oxide synthase (eNOS) and the phosphatidylinositol-3 kinase (PI3K)–Akt pathway. By contrast, Rb1 inhibits the earliest step in angiogenesis, the chemoinvasion of ECs.

This study explains, for the first time, the ambiguity regarding the effects of ginseng in vascular pathophysiology, which is based on the existence of opposing active principles in the extract. We also discovered a specio-geographic variation impinging on the compositional fingerprint that could modulate the final phenotype. This emphasizes the need for regulations that standardize herbal therapy, which is currently under the dietary supplement and health education act. These data also offer an opportunity for drug development. For example, another angiogenic ginsenoside, Re, also increases vascularization in neoconnective tissue fibrils filled with extracellular matrix [43]. Thus, Re and Rg1 represent a prototype for non-peptide molecules that can induce therapeutic angiogenesis: for example, in wound healing and tissue regeneration.

By contrast, two metabolites of Rb1 – Rb2 and Rg3 (Figure 3) – can inhibit tumour angiogenesis and metastasis [41], probably by inhibiting the release of VEGF from tumour cells [44]. A recent study demonstrated that concurrent administration of Rg3 enhances the therapeutic effects of a continuous low-dose regimen of cyclophosphamide in inhibiting angiogenesis and metastasis of Lewis lung carcinoma [45]. Such an approach has the advantage of a reduced susceptibility to drug-resistance mechanisms, in addition to improved length of animal survival.

#### *Astragalus membranaceus and Angelica sinensis*

For ~800 years, *danggui buxue tang* (DBT) has been used in Chinese medicine to treat menopausal irregularity and menstrual disorders by raising the *qi* and nourishing the blood of the patients. It is a simple *fufang* decoction of two herbs: *Astragalus membranaceus* (huangqi) and *Angelica sinensis* (danggui) in the ratio 5:1 [46]. Recent studies indicate that DBT can stimulate circulation, enhance haematopoietic function, prevent osteoporosis and exert an antioxidant activity [47,48].

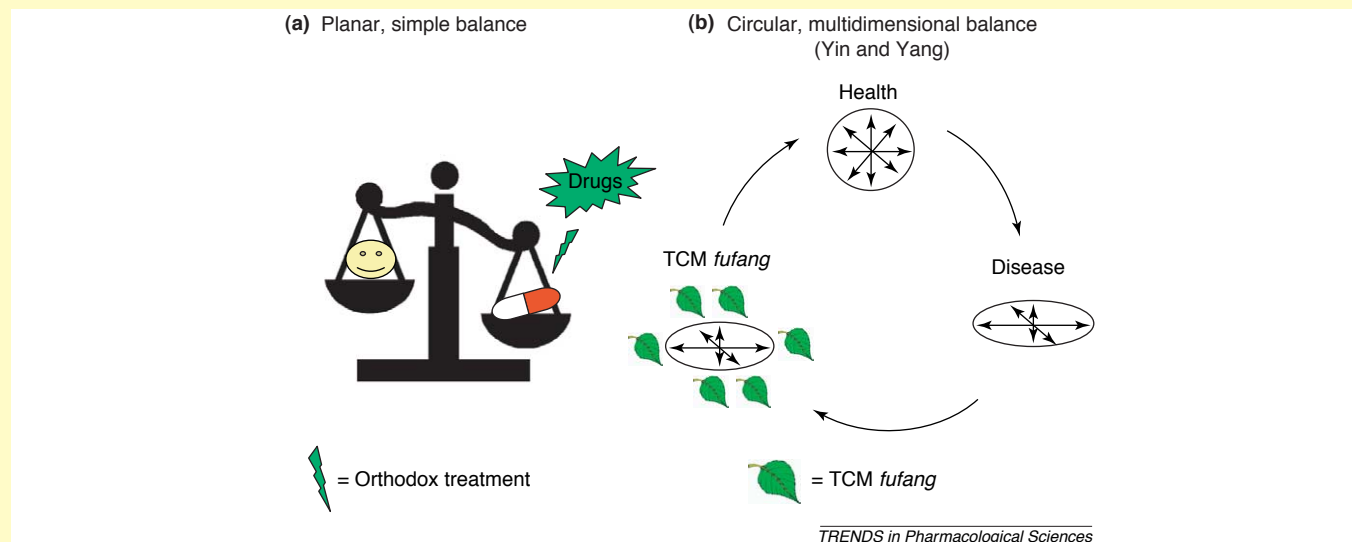
To explore the angiogenic potential of DBT, rats were treated with different ratios of these two herbs and their serum samples were applied daily to the chick

### Box 1. TCM *fufang*: the principle of *Jun-Chen-Zuo-Shi*

Unlike Western medicine, TCM prescriptions are rarely in the form of single tablets (Figure 1). Instead, they comprise several herbs and minerals to produce 'composite formulae' (*fufang*). Originating from *Huang Di Nei Jing* (Yellow Emperor's Classic [70]), the formulation of a TCM *fufang* is based on the principle of *Jun-Chen-Zuo-Shi* (Table I). The aim is to use a *Jun* herb (or herbs) to treat the main cause or the main symptom of a disease. The pharmacological actions of the *Jun* herb are enhanced by a *Chen* herb (or herbs), which treats accompanying symptoms. A *Zuo* herb (or herbs) is used as an adjuvant to modulate the effects of the *Jun* and/or *Chen* herbs, to reduce or eliminate the toxic or side effects of these herbs. As its Chinese name suggests, a *Shi* herb helps to deliver or guide the other

herbs in the prescription to the target organs. It also modulates or harmonizes the properties of other herbs in the *fufang*. For example, *shi-wu-tang* is often prescribed for gynaecological disorders. It contains *Rehmannia glutinosa* (*Jun*), *Angelica sinensis* (*Chen*), *Paeonia lactiflora* (*Zuo*) and *Ligusticum chuanxiong* (*Shi*). The proangiogenic and antiangiogenic properties of two TCM *fufang* are shown in Table II.

In every medical consultation, an experienced TCM practitioner is guided by this principle to modify the ingredients, dosage and dosage form by considering the physique of the patient and the severity of the disease, aiming to use this versatile approach to produce a personalized prescription for the best therapeutic outcome.



**Figure 1.** Distinction between Western medicine and TCM. In Western medical practice, diseases are diagnosed in a planar and discrete manner and are treated with specific drugs, either alone or in combination to achieve synergism. In TCM, diseases are diagnosed by considering the body as a whole. Such a holistic approach is based on the theory of Yin and Yang. Combinations of herbs are used in TCM *fufang*, offering a multitarget therapy to restore the 'imbalance' caused by the disease. Modified, with permission, from Ref. [71].

**Table I.** The *Jun-Chen-Zuo-Shi* principle of combining different herbs in TCM *fufang*

Class of herb	Function
<b>Jun</b> (emperor)	Main curative role aimed at the major symptom of a disease
<b>Chen</b> (minister)	Synergizes with <i>Jun</i> herb (or herbs) to strengthen the therapeutic effects, or treats accompanying symptoms
<b>Zuo</b> (adjuvant)	Reduces or eliminates possible toxic effects of the <i>Jun</i> and/or <i>Chen</i> herbs but also treats accompanying symptoms
<b>Shi</b> (courier)	Guides synergized prescription to target location (organs or channels); it can also harmonize the whole formula

**Table II.** Effects of two TCM *fufang* on angiogenesis

TCM <i>fufang</i>	Proangiogenesis	Antiangiogenesis	Refs
<i>Danggui buxue</i> decoction ( <i>Astragalus membranaceus</i> , <i>Angelica sinensis</i> )	Stimulates angiogenesis in CAM		[49]
QLY ( <i>Sophora flavescens</i> Ait., <i>Phellodendron amurense</i> Rupr., <i>Sinomenium acutum</i> Rehd. et Wils., <i>Dioscorea hypoglauca</i> Palib.)		Inhibits angiogenesis in a rat model of CIA; reduces <i>MMP-3</i> mRNA and increases <i>TIMP-1</i> protein, restoring the balance of <i>MMP-3</i> and <i>TIMP-1</i>	[53]

chorioallantoic membrane (CAM). CAM angiogenesis was observed 72 h after incubation. *A. membranaceus* and *A. sinensis* in a 5:1 ratio produced the most significant amount of angiogenesis, confirming the effectiveness of DBT [49]. More work is required to identify the angiogenic compounds in DBT.

#### Angiogenic inhibitors from TCM

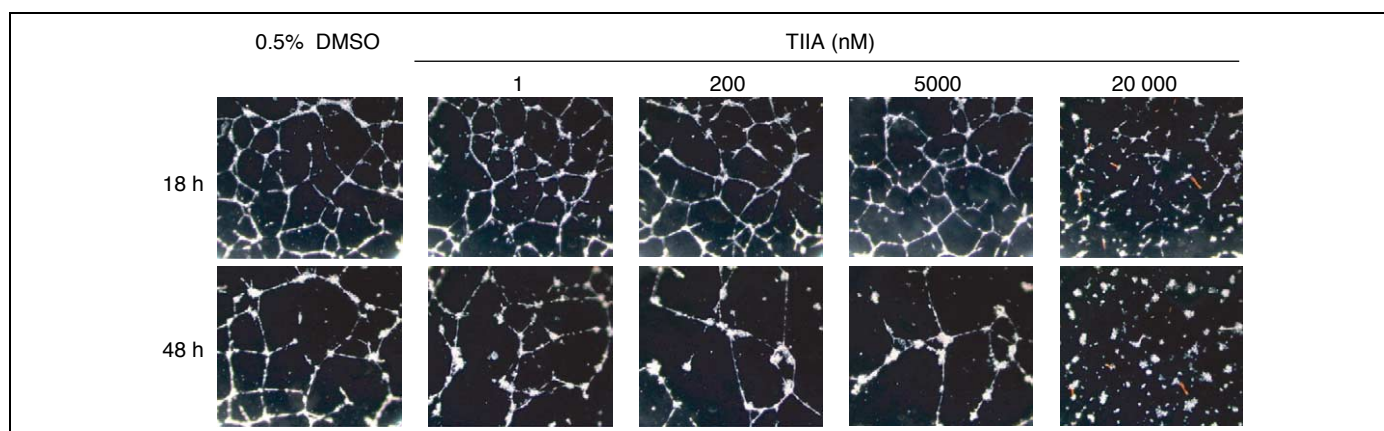
*Salvia miltiorrhiza* (*danshen*)

*Salvia miltiorrhiza* has been used widely in Asia for several hundred years to treat angiogenesis- and haemostasis-related diseases such as atherosclerosis, thromboembolism

and angina. Nearly 40 diterpene quinones comprise the pharmacologically active constituents of danshen, including tanshinone IIA (TIIA) (Figure 3) and cryptotanshinone (CT) (Figure 3). Several phenolic acids such as danshensu, salvianolic acid B (Figure 3) and  $\beta$ -sitosterol are also active. Salvianolic acid B stimulates the proliferation and tube formation of a murine simian virus resistance endothelial cell line [50] through the sequential upregulation of the genes encoding *MMP-2*, *VEGF*, *VEGF* receptor 2 and *Tie-1*.

By contrast, we observed that CT and TIIA inhibit the proliferation of HUVECs in a concentration-dependent manner at an  $IC_{50}$  of 1.65  $\mu$ M and 1.48  $\mu$ M, respectively





**Figure 4.** Antiangiogenic effects of TIIA *in vitro*. HUVECs were seeded onto matrigel in 96-well plates. After six hours of incubation, TIIA (1–20 000 nM) was added to the wells. At 18 h or 48 h, tube formation was recorded photographically. Compared with the solvent control [0.5% dimethyl sulfoxide (DMSO)], TIIA produced a concentration- and time-dependent inhibition of tube formation.

[51]. In a modified matrigel assay, both CT and TIIA inhibited HUVEC tube formation at  $2 \times 10^{-5}$  M (Figure 4). Using bovine aortic ECs, Hur *et al.* [52] reported that CT, but not TIIA, inhibits FGF-2-induced tube formation at 10- $\mu$ M ranges *in vitro* without causing cytotoxicity. Because TIIA is structurally similar to CT except at the C-15 position of the dihydrofuran ring, these results indicate that the double bond at this position has a crucial role in antiangiogenic activity.

#### Sinomenium acutum

*Qing-luo-yin* (QLY) is a TCM *fufang* that is used to treat rheumatoid arthritis. It contains the extracts of four herbs: *Sinomenium acutum*, *Sophora flavescens*, *Phellodendron amurense* and *Dioscorea hypoglauca*. The antiangiogenic effect of QLY was investigated in a rat model of collagen-induced arthritis (CIA). Oral administration of QLY (0.3 g/kg) for 27 days inhibited angiogenesis in the pannus and reduced cartilage damage [53]. In addition, the overexpression of MMP-3 mRNA and the level of the tissue inhibitor of MMP (TIMP)-1 in the synovium of CIA rats were significantly reduced by QLY. These results indicate that QLY exerts a suppressive effect on the angiogenesis of CIA rats, and the therapeutic effect of QLY could be brought about by restoring the balance of MMP-3 and TIMP-1 in rheumatoid synovium.

Sinomenine (Figure 3) is an alkaloid from *S. acutum* that has a wide range of pharmacological actions, including anti-inflammatory and antirheumatic effects. Because angiogenesis has a crucial role in the development of rheumatoid arthritis, we tested whether sinomenine has antiangiogenic properties [54]. Sinomenine inhibited the basic (b)FGF-induced proliferation of HUVECs and arrested the cell cycle in G1 phase. It also disrupted tube formation in HUVECs on matrigel and suppressed the chemotaxis of HUVECs. Importantly, sinomenine reduced neovascularization in a matrigel plug assay, in addition to microvascular outgrowth in a rat aortic-ring sprouting assay. These results indicate that sinomenine inhibits bFGF-induced angiogenesis *in vitro* and *in vivo*. In addition, sinomenine reduced the transmigration of granulocytic differentiated HL60 cells across an IL-1 $\beta$ -activated HUVEC monolayer. Therefore,

the inhibition of leucocyte migration across blood vessel walls and the antiangiogenic effect of sinomenine might contribute to its therapeutic mechanisms in alleviating the pathogenesis of rheumatoid arthritis. Thus, we have identified a single antiangiogenic compound from a TCM *fufang*. Further work could reveal other active compounds for drug development.

#### Tripterygium wilfordii

*Tripterygium wilfordii* has also been used to treat rheumatoid arthritis and other autoimmune inflammatory diseases. Triptolide (Figure 3), the main diterpenoid from this herb, is an immunosuppressive agent with anti-inflammatory and antitumour properties [55]. Li *et al.* [56] reported that the extract of *T. wilfordii* inhibits cyclooxygenase (COX)-1, COX-2 and 5-lipoxygenase at IC<sub>50</sub> values of 27, 125 and 22  $\mu$ g/ml, respectively. Recent studies have shown that triptolide inhibits VEGF expression and secretion in ECs treated dose dependently with 12-*O*-tetradecanoylphorbol 13-acetate [57,58], and angiogenesis in CIA rats [53]. This effect could be one of the mechanisms underlying the therapeutic effects of triptolide on rheumatoid arthritis.

#### Future prospects of Chinese medicine in angiotherapy

##### Applying genomics and proteomics to TCM

There are ~30 000 genes in the human genome but there are only 600–1500 potential drug targets [59]. It is interesting that the ~6000 currently marketed drugs interact with fewer than 120 molecular targets and that the 100 bestselling drugs interact with 43 targets. Of particular relevance to this article is the fact that 61% of the 877 drugs introduced between 1981 and 2002 can be traced to, or are inspired by, natural products. A quick survey reveals that 74% of anticancer drugs in current use are derived from nature.

It is possible to use bioactivity-directed fractionation to identify angiomodulators from TCM herbs that are efficacious in the treatment of angiogenic diseases. With the advent of genomics, proteomics, glycomics and metabolomics and with major advances in techniques for analysis (e.g. surface-enhanced laser desorption ionization time-of-flight mass spectrometry), chemical

synthesis and high-throughput screening of active compounds, there is an unprecedented opportunity to evaluate and explore the huge medicine chest that is embedded in TCM. As a starting point, cDNA microarray technology has been applied to elucidate the mechanisms of action of Rg1. The differential gene expression profile of HUVECs following treatment with Rg1 revealed the expression of genes related to cell adhesion, migration and the cytoskeleton, including those encoding RhoA, RhoB, IQ-motif-containing GTPase-activating protein 1 (IQGAP1), calmodulin (CALM2), Vav2 and laminin- $\alpha$ 4 (LAMA4). Our results indicate that Rg1 promotes angiogenesis by modulating genes that are involved in cytoskeletal dynamics, cell-cell adhesion and migration [60]. More recently, proteomic analysis was applied to study the effects of Rg1 on HUVECs stimulated by tumour necrosis factor- $\alpha$  [61].

**Chemical genomics: a new platform for drug discovery**  
Chemical genomics is another powerful way to discover better and safer drugs. Instead of finding drugs for targets in the current pharma orthodoxy (i.e. 'omics  $\rightarrow$  target  $\rightarrow$  compound  $\rightarrow$  effect), this strategy finds targets for drugs (i.e. effect  $\rightarrow$  compound  $\rightarrow$  target  $\rightarrow$  better drug) [6]. Here, a chemical tool called 'perturbogen' is used to perturb

a biological system and, thus, unravel the biochemistry underlying a chemotype (i.e. a chemically induced phenotype). A classic example of chemical genomics strategy is the discovery by John Vane in 1971 of prostaglandin synthesis as the target pathway for the effects of aspirin. This work led to the isolation and cloning of COX-1 and COX-2, resulting in the invention of more-potent and safer COX-2 selective nonsteroidal anti-inflammatory drugs.

#### *Phytomedicine and TCM fufang*

To quote Hildebert Wagner:

The greatest challenge for phytomedicine research, however, will be the shift of paradigms, which is occurring in chemotherapy. This change can be described as a withdrawal from monosubstance therapy and a transition to treatment of patients with drug combinations consisting of two, three, or more single drugs. This multichemotherapy has been introduced, for example, in the treatment of AIDS, hypertension, and many other diseases. The second paradigm shift can be defined as a change in the strategy of medication, characterisable as a multitarget therapy. Taking tumor therapy as an

### Box 2. Novel molecular targets of angiogenesis

#### **Eph receptors and ephrins**

Eph receptors are the largest family of receptor tyrosine kinases. These receptors and ephrin ligands were originally identified as neuronal-pathfinding molecules. Intriguingly, gene-targeting experiments in mice have identified the EphB-ephrinB system as the crucial and rate-limiting determinant of arteriovenous differentiation during embryonic vascular development. Recent work has focused on the roles of the Eph-ephrin system in controlling tumour and vascular functions during tumorigenesis and tumour progression [72]. A better understanding of the Eph-ephrin system would lead to the selective targeting in antiangiogenic therapy of the molecules involved.

Antiangiogenic therapy is based on the normal genetic status of the target vasculature, and therefore is thought unlikely to lead to acquired resistance. However, recent data challenge this concept. Using a xenograft murine model of human Wilms' tumour, Huang *et al.* [63] characterized molecular changes in the vasculature during the apparent resumption of xenograft growth after initial inhibition by VEGF blockade, 'metronome' topotecan chemotherapy and combined agents. They showed that tumours that grow during antiangiogenic blockade develop as viable clusters surrounding remodelled vessels. These vessels display significant increases in diameter, are involved in the active proliferation of vascular mural cells and express PDGF-B, which enhances vascular integrity by stromal cell recruitment. In addition, remodelled vessels express ephrinB2, which is required for the proper assembly of stromal cells into vasculature. Thus, chronic antiangiogenesis could lead to enhanced vascular stability, resulting in increased perfusion and recurrent tumour growth. If this phenomenon occurs in human cancers, targeting the EphB-ephrinB system could be advantageous for treatment.

#### **Histone deacetylase**

Histone deacetylase (HDAC) is implicated in the alteration of chromatin assembly and tumorigenesis. Kim *et al.* [73] showed that HDAC is induced under hypoxia and elucidated a role for HDAC in the regulation of hypoxia-induced angiogenesis. The overexpression of wild-type HDAC1 downregulates p53 and von Hippel-Lindau tumour suppressor gene expression, thus stimulating the angiogenesis of human ECs. Trichostatin A (TSA) is a potent and specific inhibitor of mammalian HDAC that upregulates p53 and von Hippel-Lindau

expression and downregulates HIF-1 $\alpha$  and VEGF expression. It also blocks angiogenesis *in vitro* and *in vivo*, specifically hypoxia-induced angiogenesis in the Lewis lung carcinoma model. These results indicate that hypoxia enhances HDAC function and that HDAC is closely involved in angiogenesis by suppressing hypoxia-responsive tumour suppressor genes.

Using polymer-assisted solution-phase synthesis, we generated an array of HDAC inhibitors. These compounds have considerable potential as antiproliferative agents. Selected compounds inhibited human EC proliferation and tube formation in an *in vitro* model of angiogenesis [74]. Qian *et al.* [75] recently reported that the hydroxamic acid derivative LBH589 induces a wide range of effects on ECs that leads to the inhibition of angiogenesis and phosphatidylcholine-3 tumour growth *in vivo*. Thus, HDAC inhibitors should be considered as a therapeutic strategy for targeting both the tumour and the endothelial compartment; the clinical development of these agents in combination with other angiogenesis inhibitors is warranted.

#### **Nuclear receptors**

The structural differences in angiogenic factors and inhibitors are attributable to differences in receptor-binding preferences, pharmacology and mechanisms of action: for example, ginsenoside Rg1 and ginsenoside Rb1 – the pharmacologically active components of *Panax ginseng*. Protopanaxatriols such as Rg1 and Re have proangiogenic effects [42,43], whereas protopanaxadiols such as Rb1 and Rg3 are antiangiogenic [41,44,45,76]. Ginsenosides are triterpene saponins with subtle structural differences in the number and position of the sugar side-chain on a common steroidal backbone (see Figure 3 in main text). Studies indicate that ginsenosides exert their effect by binding to nuclear hormone receptors [77–79]. Indeed, our recent findings demonstrate that ginsenosides can bind to nuclear receptors and exert both genomic and nongenomic effects on HUVECs. We also mapped the signalling pathway of Rg1 that leads to the production of nitric oxide (K.W. Leung *et al.*, unpublished). Although ginsenosides such as Rg1 and Rb1 are similar molecules, their minor structural differences might enable them to interact differently with nuclear receptors, leading to opposing effects on angiogenesis. Further investigations could lead to the development of specific ligands for the modulation of angiogenesis.

example, this new strategy aims to destroy tumor cells not via direct interaction of the drug with the tumor cell cycle but via various other mechanisms, which do not damage healthy cells. This medication could be directed, e.g. to induce apoptosis of tumor cells, to inhibit angiogenesis, to stimulate specific and unspecific immune defense mechanisms...this new, very ambitious therapeutic strategy is still in its infancy, but it is a challenge for phytomedicine research because the attempt to treat diseases according to this strategy is actually an old phytotherapeutic concept [62].

In fact, multitarget therapy has long been exploited in TCM in the form of *fufang*. By its very nature, a TCM *fufang* works by attacking and/or modulating several targets simultaneously. This principle can be harnessed to create a new generation of 'antiangiogenic *fufang*' and 'angiogenic *fufang*'. Because of the massive database of active compounds in TCM herbs, it is conceivable to test the compounds alone and in combination in a standard set of *in vitro* and *in vivo* models of angiogenesis and novel molecular targets (Box 2). Whether an active compound interacts with a recognized or novel target protein, it can be modified by chemical genomics strategy to produce a new generation of angiomodulators.

Despite its clinical success, Avastin<sup>®</sup> monotherapy has been associated with toxicities, including hypertension, bleeding episodes and thrombotic events. To reduce such adverse effects, lower doses of Avastin<sup>®</sup> could be used in combination with other antiangiogenic compounds that have distinct mechanisms of action. The sustained ingestion of low doses (metronomic dosing) of antiangiogenic *fufang* could minimize such adverse side-effects. Antiangiogenic *fufang* might also prevent the unexpected recurrence of tumours after conventional antiangiogenic therapy [63]. By contrast, for a proper and speedy recovery from diabetic ulcers, novel angiogenic *fufang* can be used to promote angiogenesis while antibacterials control infection and bioburden. With the advent of gene chips, it would be relatively easy to perform genetic tests to evaluate a patient's natural balance of proangiogenic and antiangiogenic factors before commencement of proangiogenic treatment.

There is currently a great deal of enthusiasm for revitalizing TCM, aiming to produce evidence-based validation of the efficacy of Chinese medicine. By forging new knowledge in chemistry and biology, a common language is being created that will enable a better understanding of the principles and rationales of TCM *fufang*. Once the mechanisms of action of the active compounds are elucidated and their molecular targets identified, chemical optimization will lead to more-potent and safer analogues. These novel chemicals can then be used in mixtures that have different properties and targets as the original TCM *fufang* for the prevention, management and treatment of angiogenic ailments.

## Concluding remarks

The discoveries of Taxol<sup>®</sup>, camptothecin and combretastatin from plants are important milestones in the history of anticancer drugs. These examples of synthetic chemistry leading to more-efficacious analogues and their subsequent clinical use illustrate the power of combining medicinal chemistry and pharmacology. The elucidation of their mechanisms of action has paved the way for the identification of novel targets in the treatment of malignancy. Independently, scientists working with natural products have discovered active compounds such as ginsenosides, sinomenine and triptolide from TCM *fufang* that have been used to treat angiogenic diseases for >1000 years. These compounds have angiogenic or antiangiogenic activities, and their mechanisms of action are being investigated. Chemical genomics can be used to develop these compounds into drugs for clinical trials. In this era of genomics, proteomics, glycomics and metabolomics, there is an unprecedented array of analytical tools with which to make a great leap forward in the understanding of the philosophy and science of TCM *fufang*. Coupled with powerful techniques in drug discovery, such new knowledge has great potential in the medicine of tomorrow.

## Acknowledgements

The work in our laboratories was supported by grants from the Hong Kong Research Grants Committee, Hong Kong Baptist University and NuLiv Science Taiwan. J.-C.Y. is in receipt of a Cambridge Overseas Trust scholarship. We thank Mick Bakhle, Brian Callingham, Dan Jiang, Pieter Koolwijk, Steve Ley, Bo-Ying Ma and Rebecca Myers for their critical review of and invaluable suggestions regarding the manuscript. T.-P.F. dedicates this article to his mentors, the late Graham Lewis and John Vane.

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