The Plumbing System in Our Body - Angiogenesis and Cancer

Wong Weng Yew*; Kanga Rani Selvaduray* and Kalanithi Nesaretnam*

INTRODUCTION

Our vascular system which consists of mainly blood vessels is something comparable to a plumbing system within our body through which blood circulates, transferring various supplies that are essential for the growth of our body as well as ridding it of wastes. Proteins, vitamins, minerals, ions or even waste are transported from one part of the body to the other through this complicated vascular network. Being an important structure, blood vessels are generated whenever necessary. The process that is responsible for generating blood vessels in the body is known as angiogenesis. It is a physiological process involving the formation of new blood vessels from the existing or established vessels found in the body. Angiogenesis is a normal process in growth and development, as well as in wound healing. However, it is also a fundamental step in the transition of tumours from a dormant state to a malignant state as angiogenesis also provides the blood vessels to tumours, feeding them with nutrients for further growth.

THE PLUMBING PROCESS – ANGIOGENESIS

Studies reveal that angiogenesis employs several steps that involve secretion, lysis, proliferation, migration, adhesion and remodelling of blood vessels that are tightly regulated by a series of 'on' and 'off' switches which are regulatory proteins. The 'on' switches are the angiogenesis-stimulating factors such as the vascular endothelial growth factor (VEGF), the basic fibroblast growth factor (bFGF) and the transforming growth factor-β (TGF- β)], interleukin-8 (IL-8) and angiopoietin-1 (Claffey *et al*., 1996; Bussolino *et al.*, 1997; Goldman *et al.*, 1998). On the other hand, the 'off' switches represent a series of angiogenesis inhibitors such as angiostatin, endostatin and interleukin-12 (IL-12) (Hayes *et al.*, 1999).

As mentioned earlier, blood vessels are formed when the body senses the need to create new blood vessels in a certain region. One of the conditions is hypoxia (lack of oxygen). During the development of organs or when blood vessels are clogged, supply of nutrients and oxygen diminishes. This triggers the body to secrete angiogenic factors to generate new blood vessels to prevent the cells from being deprived of nutrients and oxygen.

ANGIOGENSIS: PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

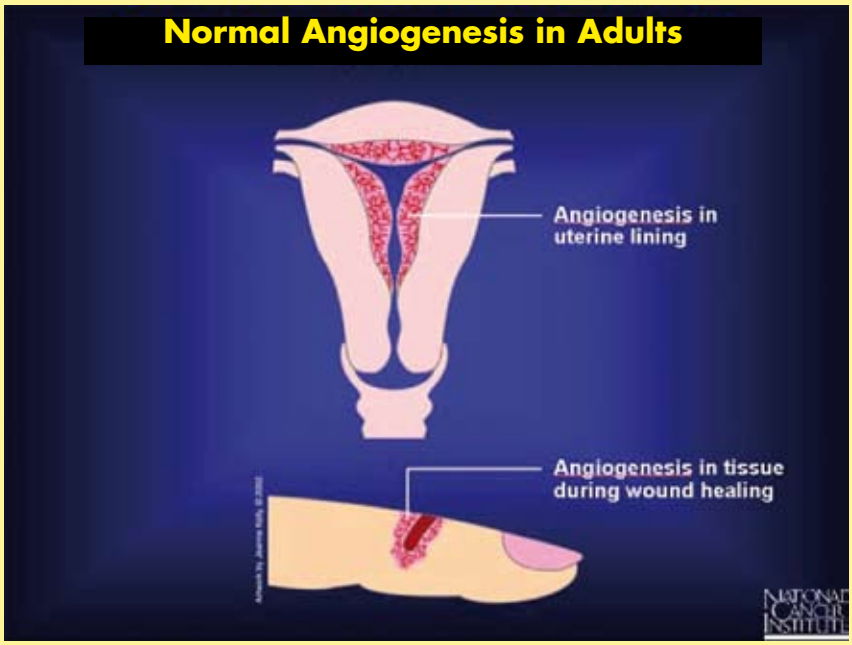
Angiogenesis takes place under both physiological and pathological conditions (Hsieh *et al*., 2006). It is an essential process in normal physiology that occurs during growth, wound healing, embryonic development, menstrual cycle, *etc*. (*Figure 1*) (Sunilla and Kuttan, 2006). However, in an abnormal physiological condition (*i.e*. in a diseased state), the body loses control of angiogenesis. This causes the pathogenesis of various maladies such as solid tumour growth and metastasis, diabetic retinopathy, rheumatoid arthritis, psoriasis, and many more (Folkman, 1971; 1995; Hanathan and Folkman, 1996; Sunilla and Kuttan, 2006).

If the diseased cells produce large amounts of angiogenesis-stimulating factors, the body generates more blood vessels to transfer supplies essential for the growth of these diseased cells. This is classified as excessive angiogenesis. On the other hand, when the body does not produce adequate angiogenic factors, or produces excessive angiogenesis inhibitors, insufficient angiogenesis occurs. This causes complications such as coronary artery and ischemic diseases (Risau, 1998).

ANGIOGENESIS IN CANCER

As previously mentioned, the body forms blood vessels not only in normal physiology but also in abnormal physiology, *i.e*. when angiogenesis is under the control of the diseased cells. One of the most studied abnormal angiogenesis is

* Malaysian Palm Oil Board,
P. O. Box 10620,
50720 Kuala Lumpur,
Malaysia.
E-mail: wengyew@mpob.gov.my



Source: US National Cancer Institute.

Figure 1. Examples of angiogenesis in our body.

tumour-induced angiogenesis, or the formation of blood vessels by cancer. This was brought up back in 1971 by Dr Judah Folkman. He stated that cancer actually stimulates blood vessel growth by producing its own angiogenic factors similar to that of normal physiology (Folkman, 1971).

Cancer cells, like any other ordinary cells, need blood vessels for their survival. Cancer initially begins as a small lump, forming within clusters of normal cells. Cancer cells in the initial stage compete with normal cells to get food supplies. In order to grow and to develop into different stages, cancer cells require nutrients. Perhaps, due to the case of 'survival of the fittest', this small lump produces its own angiogenic factors to stimulate blood vessel formation so that it can tap into the body's blood supply and draw on the oxygen and nutrients to survive.

Thus, the 'on' switch is turned on by cancer cells. With that, endothelial (blood vessel) cells begin to proliferate and migrate towards the lump, forming a vascular network surrounding the lump. Following this, the lump begins to grow steadily and develop into a malignant cancer. Cancer does not stay in one place for too long. The cancer cells will take advantage on these new blood vessels by invading the vessels and migrating to other parts of the body. This is known as metastasis, the spreading of tumour cells to different parts of the body from its original location. Therefore, blood vessels are not only important for cancer to survive but also for it to metastasize (Skobe *et al.*, 1997).

STARVING THE CANCER: ANTI-ANGIOGENIC THERAPY IN CANCER

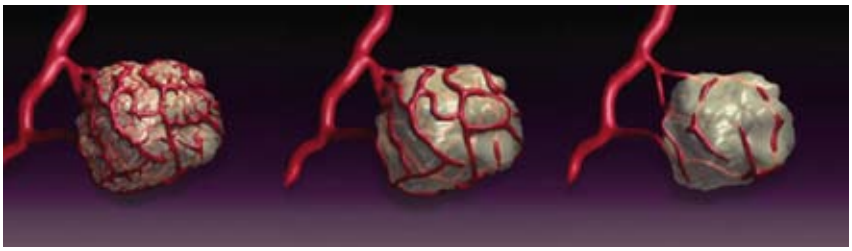
As angiogenesis plays an essential role in the tumour progression, angiogenic therapy is considered a promising approach in the treatment of cancer and other pro-angiogenic diseases. Modern medicine came up with the idea of starving the cancer which is called the anti-angiogenic therapy for cancer patients. It is hoped that by cutting off the blood vessels surrounding the tumour, blood supplies will be disrupted, cutting off the nutrients and other essential needs to the tumour. Eventually, the tumour will shrink, regress in size and, hopefully, die off (*Figure 2*).

Anti-angiogenic therapy has many advantages and is currently a promising approach for cancer treatment. This treatment targets the endothelial cells specifically. Endothelial cells do not transform and are thus unlikely to undergo mutations resulting in drug resistance, which usually occurs in the case of using drugs targeting cancer cells. Secondly, anti-angiogenic treatment is directed to the blood vessels, thus it is applicable in all kinds of cancers, regardless of the tumour origin (Hayes *et al*., 1999).

In the past few years, anti-angiogenic therapy has been successfully employed in pre-clinical as well as clinical trials (Liekens *et al.*, 2001; Malafa *et al.*, 2002; Ziche *et al.*, 2004). In 2003, *bevacizumab* (Avastin®) became the first anti-angiogenic drug tested in a large-scale clinical trial to inhibit the growth of tumour blood vessels and prolong survival in patients with metastatic colorectal cancer (Kabbinavar *et al*., 2003) and other malignancies.

FUNCTIONAL FOOD COMPOUNDS WITH ANTI-ANGIOGENIC PROPERTY

Today, apart from commercial drugs, there are many natural compounds and micronutrients which are believed to have anti-angiogenic property. Numerous studies on the screening of potential anti-angiogenic compounds found naturally have been carried out to confirm their efficacies. A number of natu-



Source: Genentech.

Figure 2. Inhibiting angiogenesis may eventually cut off supplies to the tumour and the tumour eventually dies off.

ral compounds derived from dietary and traditional medicinal plants have been found to possess such property which was not known in the past. As angiogenesis can now be overcome by a non-toxic low dose of angiogenesis inhibitors over a long period of time, this offers other options for the treatment and prevention of angiogenic-related disease using natural products.

Vitamin E: Tocotrienols

Among all the nutrients studied, vitamin E is one that has been shown to have anti-angiogenic effect on cancer. Appearing in nature as a mixture of two classes of compounds, tocopherols and tocotrienols, vitamin E is proven in our laboratory to be capable of reducing tumour size. Of the two classes of vitamin E, tocotrienols are shown to be more potent as an anti-cancer agent than tocopherols. Malaysians are indeed fortunate as tocotrienols are found abundantly in palm oil, the main commodity of our country, and also the most consumed oil in every household. Other tocotrienol sources include cereal grains like barley, wheat and rice. Tocotrienols have been shown to have functional properties as an antioxidant, anti-inflammation, reduction of plasma cholesterol and anticancer (Hood, 1996; Jiang *et al*., 2000). Apart from their function as antioxidants, recent studies suggest anti-angiogenic activity of vitamin E in combating tumour growth (Shklar and Schwartz., 1996; Malafa *et al*., 2002; Miyazawa *et al*., 2004; Nesaretnam *et al*., 2007).

Studies suggest that tocotrienols inhibit both the proliferation and tube formation of bovine aortic endothelial cells (Miyazawa *et al*., 2003; 2004; Nakagawa *et al*., 2004). A study in our laboratory demonstrated that chick embryo (chorioallantoic membrane assay, a model for angiogenesis studies) treated with different concentrations of tocotrienol-rich fractions (TRF) showed some inhibition in blood vessel growth within the egg at a high concentration (*Figures 3* and *4*). At the physiological level, approximately 1 μM tocotrienol is detected in human plasma (O'Byrne *et al*., 2000). The concentration used in our experiment was 200 μg ml^{-1}, equivalent to approximately 500 μM, and the effect was localized**.** Tocotrienols at 1 μM showed no anti-angiogenic effect (Miyazawa *et al*., 2004). Therefore, we suggest that tocotrienol may be considered a potential therapeutic agent in cancer treatment targeting the blood vessels surrounding the tumour without harming the normal angiogenesis in our body. Our *in vivo* study also revealed that tumour size and serum VEGF level were significantly reduced in mice treated with TRF compared with the untreated mice (unpublished data).

Red Wine, Grape: Resveratrol

Resveratrol was first isolated in 1940 from the roots of hellebore. In 1963, it was then isolated from the roots of *Polygonum cuspidatum*, a traditional Chinese and Japanese medicinal plant. Resveratrol did not attract interest until 1992. Resveratrol is a natural compound found in grapes, peanuts, mulberries, pines and their related products. Of all the products, red wine is very likely the most consumed drink that is rich in resveratrol.

Resveratrol showed anti-cancer activity in several tumour models regardless of cell type. A study showed that resveratrol suppresses the growth of new blood vessels in

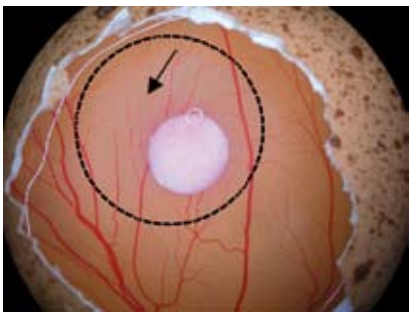


Figure 3. Chick embryo treated with 200 μg ml^{-1} of TRF at 0 hr. Blood vessels formed where the arrow points.

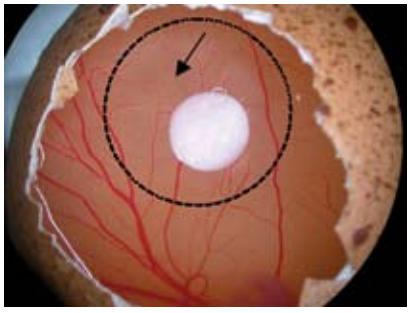


Figure 4. Chick embryo treated with 200 μg ml^{-1} of TRF at 24 hr. Blood vessels diminished where the arrow points.

animals (Brakenhielm *et al*., 2001). Resveratrol inhibits angiogenesis by suppressing the phosphorylation of MAP kinase, a pathway that is common for two key angiogenic growth factors, FGF and VEGF (Brakenhielm *et al.*, 2001). In addition, it has attracted considerable attention as one of the most promising cancer-chemopreventive agent in recent years (Cao *et al.*, 2002).

Soya Product: Genistein

Epidemiological studies indicate that the incidence of breast and prostate cancers is lower in Asian countries such as China and Japan compared with USA and European countries (Fan *et al.*, 2006). One possible reason is dietary habit. Japanese and Chinese consume diets high in soya products, and soya products are high in genistein, a predominant isoflavone found in soyabean (Zhou *et al*., 1999). Several studies *in vivo* and *in vitro* have shown that genistein is a promising agent for cancer-chemoprevention and treatment. Increased consumption of soyabean products has been hypothesized to contribute to reduced prostate cancer risk (Messina *et al*., 1994; Hebert *et al.*, 1998).

Genistein is reported to inhibit cancer in animal models, inhibiting endothelial cell proliferation and *in vitro* angiogenesis (Fotsis *et al*., 1993). Histological examination of tumour tissues showed that consumption of soyabean products significantly reduced tumour cell proliferation and micro vessel density (Zhou *et al.*, 1999). Therefore, dietary soya products may inhibit experimental tumour growth through a combination of direct effects on the tumour cells and indirect effects on tumour blood vessel growth.

Green Tea: Epigallocatechin-3-gallate (EGCG)

Epigallocatechin-3-gallate (EGCG) is a catechin found in green tea. It is reported to show positive cancer-preventive effects in pancreatic, colon and rectal cancers (Ji *et al*., 1997) in studies on Asians who drink predominantly green tea (Bushman, 1998). There are also reports linking the consumption of green tea with the improved prognosis in breast cancer (Nagata *et al*., 1998; Nakachi *et al.*, 1998; Fujiki *et al.*, 1999).

Studies reported that green tea and EGCG significantly prevented angiogenesis (Cao and Cao, 1999), inhibited breast cancer and endothelial cell proliferation (Sartippour *et al.*, 2002). Drinking green tea could be beneficial in preventing and treating angiogenesis-related disease such as cancer and diabetic retinopathy. This is because the level of EGCG present in an *in vivo* experiment which showed anti-angiogenic effects is similar to the level of EGCG in humans after drinking two or three cups of green tea (Fan *et al.*, 2006). A report of a Phase I clinical trial on green tea has shown encouraging results. In that study, green tea extract dispensed orally showed protective effects against tumours, and that it can be safely taken for at least six months at the recommended dose during a clinical study. The maximum-tolerated dose was 4.2 g m^{-2} once daily or 1.0 g m^{-2} three times daily, equivalent to seven to eight Japanese cups (120 ml) of green tea three times daily (Pisters *et al*., 2001).

CONCLUSION

Angiogenesis involves a complex process that depends on the coordination and regulation of several factors and cells. In a normal physiological state, it plays a vital role in the development and maintenance of the human body, with many factors interacting with one another in keeping the balance on the angiogenesis process. However, in a pathological state, the mechanism of angiogenesis behaves differently, which most of the time is harmful to our body. In recent years, it has become increasingly evident that excessive, insufficient or abnormal angiogenesis contributes to the pathogenesis of many disorders. In the case of cancer, it helps the tumour to secure a food supply and to metastasize.

Ongoing studies in understanding the mechanism involved in angiogenesis at the biochemical and molecular levels may provide further diagnostic therapeutic benefits for a variety of angiogenesis-related diseases. Further pre-clinical and clinical trials are required to test various treatment strategies and to determine their toxicity profiles. Nevertheless, anti-angiogenic therapy is a promising approach, especially in cancer treatment.

REFERENCES

BRAKENHIELM, E; CAO, R and CAO, Y (2001). Suppression of angiogenesis, tumour growth and wound healing by resveratrol, a natural compound in red wine and grapes. *FASEB J., 15*: 1798-1800.

BUSHMAN, J L (1998). Green tea and cancer in humans: a review of the literature. *Nutr. Cancer, 31*: 15-159.

BUSSOLINO, F; MANTOVANI, A and PERCISCO, G (1997). Molecular mechanism of blood vessel formation. *Trends Biochem. Sci., 22*: 251-256.

CAO, Y and CAO, R (1999). Angiogenesis inhibited by drinking tea. *Nature, 398*: 381.

CAO, Y; CAO, R and BRAKENHIELM, E (2002). Antiangiogenic mechanism of diet-derived polyphenols. *J. Nutr. Biochem., 13*: 380-383.

CLAFFEY, K P; BROWN, L F; AGUILA, L F; TOGNAZZI, K; YEO, K T; MANSEAU, E J and DVORAK, H F (1996). Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumour growth, angiogenesis, and experimental metastasis. *Cancer Res., 56*: 172-181.

FAN, T P; YEH, J C; LEUNG, K W; YUE, P Y K and WONG, R N S (2006). Angiogenesis: from plants to blood vessels. *TRENDS in Pharmacol. Sci*., *27(6)*: 297-309.

FOLKMAN. J (1971). Tumour angiogenesis; therapeutic implication. *N. Engl. J. Med., 285*: 1182-1186.

FOLKMAN, J (1995). Angiogenesis in cancer, vascular. Rheumatoid and other disease. *Nat. Med., 1*: 37-31.

FOTSIS, T; PEPPER, M; ADLERCREUTZ, H; FLEISCHMANN, G; HASE, T; MONTESANO, R and SCHWEIGERER, L (1993). Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc. Natl. Acad. Sci. USA, 90*: 2690-2694.

FUJIKI, H; SUGANUMA, M; OKABE, S; SUEOKA, E; SUGA, K; IMAI, K; NAKACHI, K and KIMURA, S (1999). Mechanistic findings of green tea as cancer preventive for human. *Proc. Soc. Exp. Biol. Med., 220*: 225-228.

GENENTECH (2006). http://www.gene.com/gene/products/information/oncology/avastin/vegf-angiogenesis-cancer.jsp 14 August 2006.

GOLDMAN, C K; KENDALL, R L; CABRERA, G; SOROCEANU, L; HEIKE, Y; GILLESPIE, G Y; SIEGAL, G P; MAO, X; BETT, A J; HUCKLE, W R; THOMAS, K A and CURIEL, D T (1998). Paracrine expression of a native soluble vascular endothelial growth factor receptor inhibits tumor growth, metastasis and mortality rate. *Proc. Natl. Acad. Sci. USA, 95*: 8795-8800.

HANATHAN, D and FOLKMAN, J (1996). Patterns and imaging mechanism of the angiogenic switch during tumorigenesis. *Cell, 186*: 353-364.

HAYES, A J; LI, L Y and LIPPMAN, M E (1999). Antivascular therapy: a new approach to cancer treatment. *BMJ, 318*: 853-856.

HEBERT, J R; HURLEY, T G; OLENDZKI, B C; TEAS, J; MA, Y and HAMPL, J S (1998). Nutritional and socioeconomic factors in relation to prostate cancer mortality: a cross-national study. *J. Natl. Cancer Inst., 90*: 1637-1647.

HOOD, R l (1996). Tocotrienol and cholesterol metabolism. *Nutrition, Lipids, Health and Disease* (Ong, A S H; Niki, E M and Packer, L eds.). Champaign, IL: AOCA Press. p. 96-103.

HSIEH, M Y; CHEN, W Y; JIANG, M J; CHENG, B C; HUANG, T Y and CHANG, M S (2006). Interleukin-20 promotes angiogenesis in a direct and indirect manner. *Genes and Immunity, 7*: 234–242.

JI, B T; CHOW, W H; HSING, A W; McLAUGHLIN, J K; DAI Q; GAO, Y T; BLOT, W J and FRAUMENI, J F Jr (1997). Green tea consumption and the risk of pancreatic and colorectal cancers. *Int. J. Cancer, 70:* 255–258.

JIANG, Q; ELSON-SCWHWAB, I; COURTEMANCHE, C and AMES, B N (2000). γ-Tocopherol and its major metabolite, in contrast to α-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. *PNAS, 97(21):* 1494-11499.

KABBINAVAR, F; HURWITZ, H I; FEHRENBACHER, L; MEROPOL, N J; NOVOTNY, W F; LIEBERMAN, G; GRIFFING, S and BERGSLAND, E (2003). Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with colourectal cancer. *J. Clin. Oncol, 21*: 60-65.

LIEKENS, S; DE CLERCQ, E and NEYTS, J (2001) Angiogenesis: regulators and clinical applications. *Biochem. Pharmacol., 61*: 253–70.

MALAFA, M P; FOKUM, F D; SMITH, L and LOUIS, A (2002). Inhibition of angiogenesis and promotion of melanoma dormancy by vitamin E succinate. *Ann. Surgical Oncology, 9(10)*: 1023-1032.

SARTIPPOUR, M R; SHAO, Z M; HEBER, D; BEATTY, P; ZHANG, L; LIU, C; ELLIS, L; LIU, W; LIANG, V L and BROOKS, M N (2002). Green tea vascular endothelial growth factor (VEGF) induction in human breast cancer cells. *J. Nutr., 132*: 2307-2311.

MESSINA, M; PERSKY, V; SETCHELL, K D and BARNES, S (1994). Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutr. Cancer, 21*: 113-131.

MIYAZAWA, T; INOKUCHI, H; TSUZUKI, T; NAKAGAWA, K and IGARASHI, M (2003). Anti-angiogenic potential of tocotrienol *in vitro. Biochemistry, 69(1)*: 67-69.

MIYAZAWA, T; TSUZUKI, T; NAKAGAWA, K and IGARASHI, M (2004). Anti-angiogenic potency of vitamin E. *Ann. N. Y. Acad. Sci., 1031*: 401-404.

NAGATA, C; KABUTO, M and SHIMIZU, H (1998). Association of coffee, green tea and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in pre menopausal Japanese women. *Nutr. Cancer, 30*: 21-24.

NAKACHI, K; SUEMASU, K; SUGA, K ; TAKEO, T; IMAI, K and HIGASHI, Y. (1998). Influence of drinking green tea on breast cancer malignancy among Japanese patients. *Jpn. J. Cancer Res., 89*: 254-261.

NAKAGAWA, K; EITSUKA, T; INOKUCHI, H and MIYAZAWA, T (2004). DNA chip analysis of comprehensive food function: Inhibition of angiogenesis and telomerase activity with unsaturated vitamin E, tocotrienol. *BioFactors,* 21: 5-10.

NESARETNAM, K; WENG-YEW, W and BASRI, M W (2007). Tocotrienols and cancer: beyond antioxidant activity. *Eur. J. Lipid. Sci. Technol., 109*: 445-452.

O' BYRNE, D; GRUNDY, S; PACKER, L; DEVERAJ, S; BALDENIUS, K; HOPPE, P P; KRAEMER, K; JIALAL, I and TRABER, M G (2000). Studies of LDL oxidation following alpha-, gamma- or delta- tocotrienyl acetate supplement of hypercholesterolemic humans. *Free Radical Biology and Medicine, 29 (9)*: 834-845.

PISTERS, K M W; NEWMAN, R A; COLDMAN, B; SHIN, D M; KHURI, F R; HONG, W K; GLISSON, B S and LEE, J S (2001). Phase I trial of oral green tea extract in adult patients with solid tumours. *J. Cli. Oncol., 19*: 1830-1838.

RISAU, W (1998). Angiogenesis is coming of age. *Circ. Res., 82*: 926-928.

SHKLAR, G and SCHWARTZ, J L (1996). Vitamin E inhibits experimental carcinogenesis and tumor angiogenesis. *Eur. J. Cancer B. Oral Oncol., 32B*: 114-119.

SKOBE, M; ROCKWELL, P; GOLDSTEIN, N; VOSSELER, S and FUSENIG, N E (1997). Halting angiogenesis suppresses carcinoma cell invasion. *Nat. Med., 3*: 1222-1227.

SUNILLA, E S and KUTTAN, G (2006). *Piper longum* inhibits VEGF and proinflamatory cytokines and tumour-induced angiogenesis in C57BL/6 mice. *International Immunopharmacology, 6*: 733-741.

US NATIONAL CANCER INSTITUTE (2006). http://www.cancer.gov/cancertopics/ understanding-cancer/ angiogenesis. 20 August 2006.

ZHOU, J R; GUGGER, E T; TANAKA, T ; GUO, Y; BLACKBURN, G L and CLINTON, S K (1999). Soybean pytochemicals inhibit growth of transplantable human prostate carcinoma and tumour angiogenesis in mice. *J. Nutr., 129*: 1628-1635.

ZICHE, M; DONNINI, S and MORBIDELLE, L (2004). Development of new drugs in angiogenesis. *Curr. Drug Targets, 5*: 485-93.