Role of Curcumin on Tumor Angiogenesis in Hepatocellular Carcinoma

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Summary

Hepatocellular carcinoma (HCC) is a malignant tumor characterized by active neovascularization. Vascular endothelial growth factor (VEGF) is the most important angiogenic factor that regulates the HCC development. Overexpression of VEGF enhances the HCC tumor growth associated with increase of angiogenesis in the tumor, whereas suppression of VEGF attenuates the tumor growth. Similarly, the cyclooxygenase-2 (COX-2) expression stepwisely increases during hepatocarcinogenesis. Curcurmin has been shown to inhibit several angiogenic biomarkers including, VEGF and COX-2 expression. Therefore, curcumin could be used as a candidate for the combined drug treatment for HCC in the future.

Keywords: Curcumin; Angiogenesis; Hepatocellular carcinoma; VEGF; COX-2

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the five most common cancers worldwide, with a particularly high prevalence in Asian countries due to endemic hepatitis B virus infection (Parkin et al., 2001). In Thailand, it is the most common cause of death in men (Vatanasapt et al., 2002). Surgery, including transplantation, remains the only potentially curative modality for HCC, yet the recurrence rate for this particular cancer is high and long-term survival rate is rather poor. Experimental and clinical data indicate that human hepatocellular carcinoma tumor progression is associated with angiogenesis and that an increase in microvascular density is associated with a poor prognosis. Angiogenesis plays a significant role in the aggressiveness of HCC (Pang & Poon, 2006; Semela & Dufour, 2004). A better understanding of the mechanisms underlying HCC angiogenesis may provide a basis for a rational approach to develop an anti-angiogenic therapy in patients with HCC.

Anti-angiogenic agents may have the following theoretical advantages over cytotoxic chemotherapy: 1) the microvascular endothelial cells are genetically stable with a low mutation rate 2) as anti-angiogenic therapy targets the specific immature characteristics of tumor vasculature, which differs from normal quiescent vasculature, has been demonstrated low toxicity in pre-clinical studies; 3) endothelial cells are directly exposed to blood borne agents, circumventing the problem of drug delivery to tumor cells; this is a major obstacle to conventional anticancer therapy (Pang & Poon, 2006).

In this regard, discovery of non-toxic anti-angiogenic phytochemicals could have greater practical significance than non-selective cytotoxic therapies to control the tumor growth and metastasis by targeting angiogenesis. Since, many dietary and non-dietary phytochemicals do not affect survival of normal cells and also possess anti-angiogenic as well as anti-tumorigenic activities, it could be a rationale approach to examine their inhibitory effect on tumor angiogenesis. Therefore, selective targeting of tumor vasculature by non-toxic phytochemicals could be a valuable strategy for cancer control with reduced or no harmful side effects.

Many phytochemicals could have a tremendous potential as anti-angiogenic agent including green tea (catechins) (Tang et al., 2006), grape (resvevatrol) (Lee et al., 2006), soy (isoflanones) (Zhou et al., 1999), soy (genistein) (Shao et al., 1998) and turmeric (curcumin) (Gururaj et al., 2002; Singh et al., 1996; Yoysungnoen et al., 2006). These agents, which show anti-angiogenic effects on tumors, act via different mechanisms to inhibit the angiogenic process by disrupt in various components of tumor angiogenesis signaling pathway, which starts from the tumor cells secreting angiogenic factors and endings in the formation of blood capillaries by endothelial cells. Among these candidates, curcumin is the most important anti-angiogenic agent which has shown to inhibit most step of tumor angiogenic process.

Curcumin (diferuloylmethane) which is a major yellow pigment found in ground rhizome of *Curcuma longa*. It has possessed wide range of pharmacological activities including anti-inflammation (Guo et al., 2008; Jacob et al., 2007), anti-oxidant (Sandur et al., 2007; Suryanarayana et al., 2007) and anti-cancer (Kunnumakkara et al., 2008; Lin et al., 2007; Shankar et al., 2007; Yoysungnoen et al., 2008). Recently, it has been shown that the

anti-cancer property of curcumin is mediated in part by its anti-angiogenic activity (Gururaj et al., 2002; Singh et al., 1996; Yoysungnoen et al., 2006). Therefore, the purpose of this review is to discuss the possible role of curcumin, on tumor angiogenesis, especially in hepatocellular carcinoma.

ANGIOGENESIS IN HEPATOCELLULAR CARCINOMA

Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths. Tumor angiogenesis actually starts with cancerous tumor cells releasing molecules that send signals to surrounding normal host tissue. This signaling activates certain genes in the host tissue that, in turn, make proteins to encourage growth of new blood vessels. Tumor growth and metastasis are angiogenic dependent. The development of tumor angiogenesis provides two essential functions for the growth and metastasis of cancer. First, the vessels provide a route for supply of nutrient and oxygen to sustain tumor growth, and excretion of metabolic waste. Second, the neovessels provide access for tumor cells to enter the circulation. Much of interest in angiogenesis comes from the notion that for tumors to grow beyond a critical size, they must recruit endothelial cells from the surrounding stroma to form their own endogenous microcirculation. This process is driven by the metabolic requirements of the rapidly growing tumor itself. Thus, during tumor progression, two phases can be recognized: a prevascular phase and a vascular phase.

The transition from the prevascular to the vascular phase is referred to as the "*angiogenic switch*". This angiogenic switch has been observed in different types of cancers. The angiogenic switch depends on a net balance of positive and negative angiogenic factors in the tumor (Hanahan & Folkman, 1996). It is now widely accepted that the angiogenic switch is "off" when the effect of angiogenic molecules is balanced by that of anti-angiogenic molecules, and is "on" when the net balance is tipped in favour of angiogenesis (Hanahan & Weinberg, 2000). In tumors, the switch to an angiogenic phenotype is known to be critical for disease progression. Unless a tumor can stimulate the formation of new blood vessels, it remains restricted to a microscopic size.

The switch to the angiogenic phenotype involves a change in the local equilibrium between activators and inhibitors of the growth of microvessels. Secretion by HCC cells, tumor-infiltrating inflammatory cells and hepatic stellate cells of factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factors (bFGF), angiopoietins, platelet derived growth factor (PDGF), placental growth factor (PLGF), transforming growth factor (TGF)-β and others promotes the sprouting of new vessels from nearby existing vessels (Hanahan & Folkman, 1996; Jung et al., 2003). Additionally, hypoxia in the center of the growing tumors leads to intracellular stabilization of hypoxia-inducible factor (HIF)-1α, the key transcription factor in hypoxic tissues, and induces the expression of several hypoxia response genes, such as *VEGF* (Harris, 2002). On the other hand, hypoxia decreases anti-angiogenic factors like thrombospondin-1 (Fox et al., 2001). Moreover, genetic alterations in tumor suppressor genes (loss of function) and oncogenes (gain of function) like *p53*, *ras*, *myc*, *c-jun* and others can upregulate proangiogenic factors (Longo et al., 2002). Hepatitis B virus X protein has also been shown to increase the transcriptional activity and protein level of HIF-1 and therefore promoting angiogenesis during hepatocarcinogenesis (Moon et al., 2004).

Angiogenesis in HCC and other solid tumors is based on the same fundamental principles of activation, proliferation and migration of endothelial cells: secreted angiogenic factors activate resting endothelial cells in adjacent blood vessels. Activated endothelial cells loosen interendothelial cell contacts and break down the surrounding basement membrane and extracellular matrix by secreting proteases. Matrix proteins contain and sequester different angiogenic factors such as VEGF, which are liberated after degradation of the matrix and further stimulate endothelial cells. These, then proliferate and migrate, involve different integrins during migration, and finally assemble to a tubular structure. Subsequent formation of a lumen then leads to the formation of a new blood vessel (Semela & Dufour, 2004). Pericytes are involved in the stabilization and maturation of the newly formed vessel. Tumor vessels differ from normal vessels in many aspects due to dysregulation of signaling pathways involved in angiogenesis and, therefore altered gene expression of angiogenic factors. In comparison to normal blood vessels, such newly formed tumor vessels are structurally and functionally abnormal.

By intravital fluorescent microscopy, our study demonstrated that there was a significant increase in the numbers of neocapillary density with the heterogeneous network in hepatocellular carcinoma (HepG2)-implanted nude mice in comparison to the controls (Yoysungnoen et al., 2006). Particularly, we have noticed the changes of host arterioles, and neocapillary vessels as that they appeared to be tortuosity dilatation, and hyperpermeability, respectively (Figure 1). These changes of host microvessels might be a result of angiogenic growth factors (in particular, VEGF) binding to its receptor on endothelial cells. These

specific characteristics of tumor vessel have an impact on diagnostic and therapeutic strategies in tumors. For example, leakiness of tumor vessels leads to increased interstitial fluid pressure which compromises the delivery of drugs into tumor tissue (Tong et al., 2004) or the diffusion of contrast agents for tumor imaging.

Tumor cells regulate angiogenesis process of endothelial cells via paracrine effect. This paracrine effect also imparts survival value to the endothelial cells (Carmeliet, 2005). Therefore, it can be inferred that paracrine regulation of endothelial cells by tumor cells is the initial event in breaking dormancy of initiated tumor cells. The important angiogenic factors secreted from tumor cells are VEGF, bFGF and insulin like growth factor (IGF-1), which stimulates tumor neo-angiogenesis and vascular permeability. Interleukin-8 (IL-8), Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are among the inflammatory angiogenic molecules secreted by tumor cells, which influence the growth and development of tumor vasculature and metastasis. Among these VEGF appears to be the most critical angiogenic factor regulating angiogenesis in HCC.

ROLE OF VEGF IN HCC-ASSOCIATED ANGIOGENESIS

VEGF is one of the most important angiogenic activator. It has a specific mitogenic effect on endothelial cells, and it also increases vascular permeability (hence also known as vascular permeability factor) and promotes extravasation of proteins from tumor vessels, leading to the formation of a fibrin matrix that supports the growth of endothelial cells and allows invasion of stromal cells into the developing tumor (Dvorak et al., 1992). The effects of VEGF are mediated via its receptors, VEGF-1 (Flt-1) and VEGF-2 (KDR/Flk-1), by endothelial cells (Veikkola et al., 2000). In normal hepatic parenchyma Flt-1 is expressed on endothelial cells within portal tracts and on macrophages. KDR/Flk-1 is expressed on sinusoidal endothelial cells (Yamaguchi et al., 2000). Both receptors, especially KDR/Flk-1, are expressed on endothelial cells of HCC vessels. The mRNA of KDR/Flk-1 is significantly more abundant in the HCC in comparison to the non-tumoral parenchyma (Yamaguchi et al., 2000). VEGF is thought to be a specific angiogenic factor, recent evidence suggested that some tumor cells may also express VEGF receptors, and VEGF may act as an autocrine growth factor in stimulating the proliferation of such cancer cells (Masood et al., 2001). The direct correlation between overexpression of VEGF in tumor cells and tumor angiogenesis in HCC has been demonstrated. Mise et al. (1996) first reported that the level of VEGF mRNA was found to be significantly correlated with the intensity of angiographic tumor staining. A subsequent study also demonstrated a strong association between VEGF immunostaining and angiographic vascularity, which suggested an important role of VEGF in the development of neovascularization in HCC (Torimura et al., 1998).

The degree of VEGF expression during development of HCC correlates with microvascular density, unpaired arteries (i.e., arteries not accompanied by bile ducts, indicative of angiogenesis) and with CD34 staining as a marker of sinusoidal capillarization (Park et al., 2000). In addition, tumor expression of VEGF (mRNA and protein expression) significantly correlates with serum VEGF level in patients with HCC providing the basis for using circulating VEGF as a prognostic marker (Poon et al., 2003). Concentration of circulating VEGF increases with advancing HCC stage, the highest levels being in patients with metastasis (Jinno et al., 1998). Furthermore, an essential role for VEGF in tumor angiogenesis has been demonstrated in animal models by the findings that neutralizing VEGF antibodies and dominant-negative VEGF receptors inhibit both angiogenesis and the progression of the disease (Kim et al., 1993). In agreement with these studies, our results also showed that serum VEGF increased significantly in hepatocellular carcinoma cell (HepG2)-implanted nude mice as compared to control (Yoysungnoen et al., 2006). These data suggest that VEGF is an important angiogenic factor in HCC.

The factors that regulate VEGF expression in cancer have been elucidated. Mitogenic cell survival and inflammatory signals play an essential role in the synthesis and secretion of angiogenic factors from tumor cells. This process occurs via the activation of receptor tyrosine kinases, such as EGFR, IGF-1R and PDGFR-β, leading to the phosphorylation of important signaling molecules, including ERK1/2 and PI3K, causing expression of VEGF in tumor cells (Bancroft et al., 2002).

Hypoxia is another factor correlated with the poor survival and increased tumor vasculature and metastasis (Harris, 2002). The expression of VEGF in hypoxic area of HCC was regulated through the HIF-1α pathway (Wu et al., 2007). An et al. (2000) demonstrated that seven times more endothelial cells were positive for VEGF antibody in carcinoma areas than in non-carcinoma areas in HCC, suggesting that VEGF is an important angiogenic factor for HCC. Hypoxia-induced transcription of VEGF mRNA is apparently mediated, at least in part, by the binding of HIF-1α to an HIF-1α binding site located in the VEGF

promoter, and by the activation of a stress inducible PI3K/Akt pathway. In fact, progressive growth of tumor creates ongoing hypoxia, which up-regulates several pro-angiogenic compounds including VEGF, bFGF, IL-8, TNF-α, TGF-b etc. These compounds, via several mechanisms such as increase of vessel hyperpermeability, release of plasma proteins, induction of proteases, fibrin formation, EC proliferation, migration etc., promote angiogenesis and fibrinolysis resulting in continued tumor growth and dysfunctional vasculature, which further positively feedback to create continuing hypoxia inside the tumors (Gupta & Qin, 2003).

Recent studies have demonstrated that Hepatitis B virus X protein, which is an important oncogenic protein of hepatitis B virus, activates VEGF through the HIF-1α pathway and plays a significant role in inducing angiogenesis in hepatitis B virus related hepatocarcinogenesis (Yoo et al., 2003). Moreover, Hepatitis B virus X protein enhanced transcriptional activity of HIF-1α in the reporter gene encoding hypoxia response element or VEGF promoter, and the expression of HIF-1α and VEGF was increased in the liver of HBx-transgenic mice (Yoo et al., 2003). Later Moon et al. (2004) have also demonstrated that HCC is detected in the liver of the HBx-transgenic mice at the age of 11-18 months. They investigated whether the inductions of HIF-1α and VEGF are involved in HBx-induced angiogenesis using the 12-month-old HBx-transgenic mice. In immunohistochemical analysis, HBx was more strongly detected in the dysplastic lesion than in the non-neoplastic region of the HBx transgenic liver. HIF-1α and VEGF were also strongly detected in the dysplastic lesion of the HBx-transgenic liver. In contrast, HIF-1α and VEGF were rarely detected in the liver tissues of non-transgenic mice. The capillary-like microvessels stained with PECAM-1 antibody were more frequently and strongly detected in the dysplastic lesion than in the non-neoplastic region of the HBx-transgenic liver. Moreover, they have shown that HBx interacts and stabilizes HIF-1α through inhibition of the interaction between von Hippel-Lindau protein (pVHL) and HIF-1α and the ubiquitin-dependent degradation. These findings suggest that HBx may promote the development of HCC by the overexpression of HIF-1α and the induction of angiogenesis at the early stage of hepatocarcinogenesis. Figure 2 summarized the major pathway which regulated VEGF production in hepatocellular carcinoma-associated angiogenes is (Gupta & Qin, 2003).

In addition, COX-2 is involved in the regulation of VEGF-induced angiogenesis in HCC (Cheng et al., 2004). Cheng et al. (2004) reported that Up-regulation of COX-2 correlates with VEGF expression and tumor angiogenesis in HBV-associated hepatocellular carcinoma. They have also demonstrated that COX-2 up-regulates VEGF expression in a HCC cell line, possibly via PGs production. In a study from our group, both the overexpression of COX-2 and elevation of serum VEGF were observed in hepatocellular carcinoma-implanted nude mice. Taken together, these results indicated that COX-2 and VEGF were closely related with each and both of them appear to play an important role in the angiogenesis in HCC. Further studies on the molecular mechanisms will be required to clarify the critical role of VEGF and COX-2 in regulating angiogenesis in HCC. The next review section I will propose the role of COX-2 in hepatocellular carcinoma-associated angiogenesis.

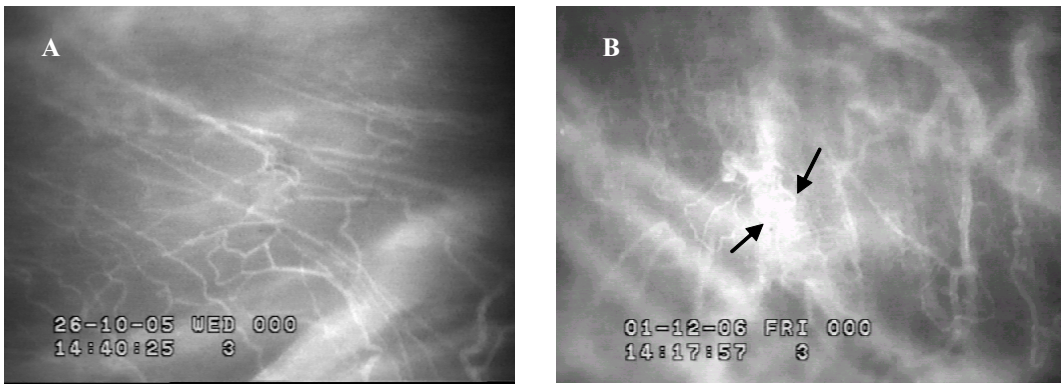

Figure 1. A: Fluorescence videoimage of the microvasculature for control; B: Fluorescence videoimage of the microvasculature for 21 days after HCC cell (HepG2) inoculation onto the upper layer of the dorsal skin in nude mice.

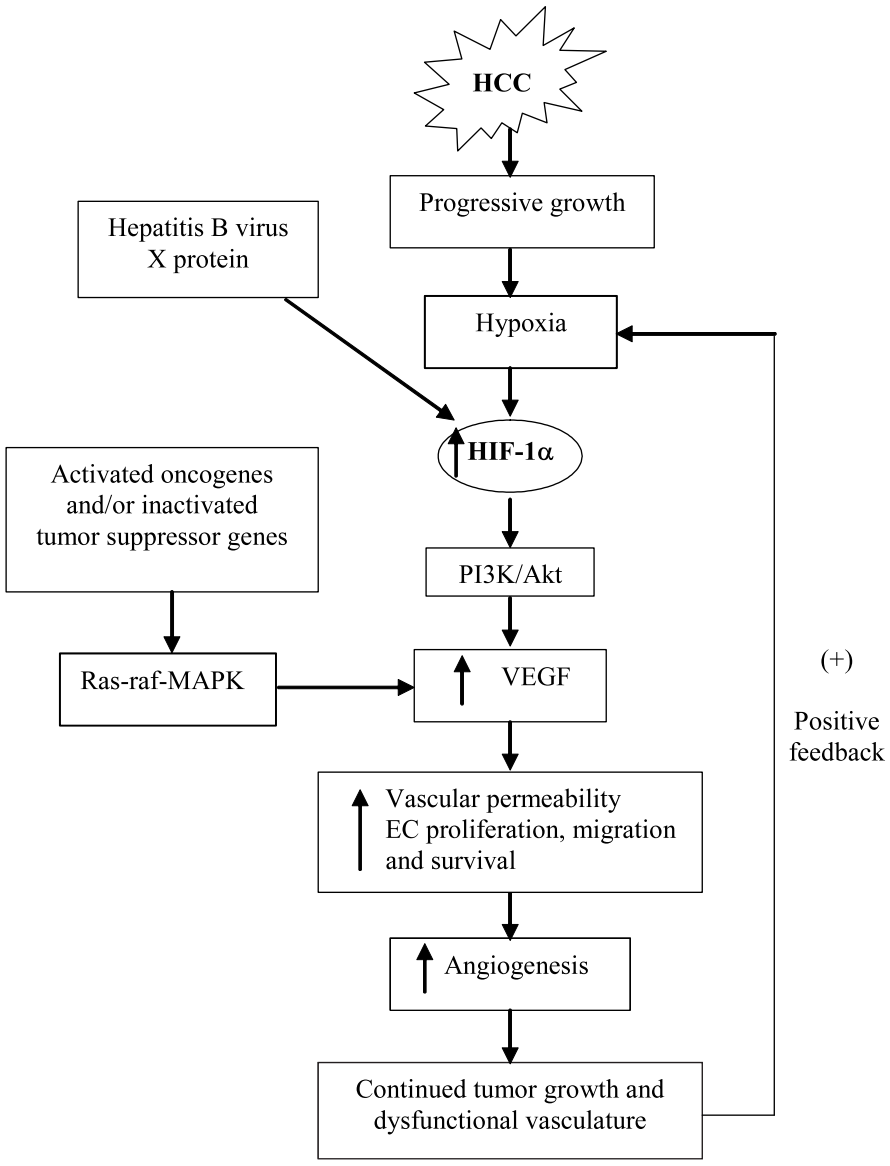

Figure 2. The major pathway which trigger for VEGF production in hepatocellular carcinoma-associated angiogenesis (modified from Gupta & Qin, 2003).

ROLE OF COX-2 IN HEPATOCELLULAR CARCINOMA-ASSOCIATED ANGIOGENESIS

Recent studies have highlighted the potential role of COX-2 in angiogenesis. Overexpression of COX-2 has been demonstrated in HCC and HCC cell lines (Bae et al., 2001; Cervello & Montalto, 2006; Zhao et al., 2007). Moreover, a correlation between COX-2 expression and tumor angiogenesis in HCC has been identified in previous studies (Cheng et al., 2004; Rahman et al., 2001; Tang et al., 2005). These results indicated that targeting COX-2 to prevent HCC development might be a fascinating therapeutic strategy. However, there are little known about the involvement of the COX-2 pathway in HCC dependent angiogenic responses.

Cyclooxygenase is a membrane bound enzyme that is expressed in at least two different isoforms, and it is responsible for the oxidation of arachidonic acid to prostaglandin G 2 and its subsequent reduction to prostaglandin H 2 (Hla et al., 1999). COX-1, a constitutively expressed isoform, is expressed in many normal tissues and is involved in a number of homeostatic body functions, such as hemostasis, vasodilatation in renal vessels, cytoprotection of the gastric mucosa, and platelet aggregation (Morita, 2002). COX-2 is an inducible form of cyclooxygenase and is also known as prostaglandin (PG) H synthase. The expression of COX-2 can be induced by various stimuli, such as growth factors and cytokines. Current study showed that COX-2 can stimulate angiogenesis

and is associated with tumor growth, invasion, and metastasis (Costa et al., 2002). COX-2 is up-regulated not only in tumor cells but also in stromal components, such as endothelial cells, macrophages, chondrocytes, and fibroblasts. Even though COX-2 is also known to have an anti-apoptotic effect on tumor cells (Bae et al., 2001), its angiogenic property is believed to play a major role in its relationship with cancer growth and progression (Fosslien, 2001).

In spite of their efficacy as anti-angiogenic agent, the precise mechanism(s) for the effect of COX-2 inhibitors remains unclear. Tsujii et al. (1998) firstly demonstrated the ***direct action of COX-2*** on angiogenic process. They hypothesized that stimulation of tumor angiogenesis could be done by the products of COX-2 activity, i.e. PGs, TXA_2. Since, they found that activated human microvascular endothelial cells produce a number of eicosanoid products including thromboxane A2 (TXA_2). Selective COX-2 antagonists have been shown to inhibit TXA_2 production and endothelial migration as well as corneal angiogenesis, an effect that is reversed with the use of a TXA_2 agonist U46619 under COX-2-inhibited conditions. TXA_2 may represent an important intermediary of the angiogenic process. Cianchi et al. (2001) have also found a significant relationship between prostaglandinE2 (PGE_2), main product of COX-2 and tumor stage. Higher PGE_2 levels were found in tumor specimens with distant or lymph node metastases than those without any metastases. Experimental studies have shown that PGE_2 production or the addition of PGE_2 to cell cultures can mediate important carcinogenic mechanisms. Among these are the inhibitions of apoptosis by the increasing of Bcl-2 levels (Sheng et al., 1998), the stimulation of angiogenesis (Tsujii et al., 1998), the inhibition of the immune response against cancer (Kambayashi et al., 1995), and the invasiveness of neoplastic cells by increasing matrix metalloprote inase-2-activation (Tsujii et al., 1997). These effects can be reversed by selective COX-2 inhibitors. In addition, Dormond et al. (2001) investigated the potential links between $\alpha V\beta 3$ integrin, an adhesion receptor critically involved in mediated tumor angiogenesis and COX-2. They demonstrated that inhibition of endothelial-cell COX-2 by NSAIDs suppressed $\alpha V\beta 3$-dependent activation of the small GTPases, Cdc-42 and Rac, resulting in inhibition of endothelial-cell spreading and migration *in vitro* and suppression of FGF-2-induced angiogenesis *in vivo*. These results provide a functional link between the direct effects of COX-2 on angiogenic process.

The ***indirect action of COX-2*** on tumor angiogenesis might be mediated by an up-regulation of the expression of angiogenic factors like VEGF. Tsujii et al. (1998) used an endothelial cell/colon carcinoma coculture model system to explore the role of COX-2 in tumor related angiogenesis. They demonstrated that COX-2 overexpressing Caco-2 and HCA-7 cells stimulated endothelial motility and tube formation by the increased production of proangiogenic factors, such as VEGF, basic FGF, transforming growth factor beta, and platelet-derived growth factor. These effects can be blocked by NS-398, a selective inhibitor of COX-2. The current evident demonstrated a significant correlation between tumor cytosolic COX-2 and VEGF levels in HCC (Tang et al., 2005). It has been shown that COX-2-derived prostaglandins facilitate angiogenesis by the up-regulation of VEGF expression, and the increased levels of VEGF can be reversed by using COX-2 inhibitor, suggesting that COX-2 regulates VEGF expression and angiogenesis in HCC. These data suggest that COX-2 was related to tumor angiogenesis in up-regulating VEGF expression in hepatocellular carcinoma cells, possibly via PGs production. Therefore, the possible mechanism(s) of COX-2 on modulating tumor angiogenesis may be reside on both direct and/or indirect pathway as described above. Figure 3 summarized the mechanisms by which COX-2 derived prostaglandins are involved in the carcinogenesis (Konturek et al., 2005). Altogether, these results indicated that COX-2 is crucial for tumor angiogenesis in HCC.

ANTI-ANGIOGENIC EFFECT OF CURCUMIN

Curcumin is the principal curcuminoid in turmeric. Three major curcuminoids namely curcumin, demethoxycurcumin and bisdemethoxycurcumin occur naturally in these *Curcuma* species. It seems that *C. longa* (turmeric) contains the highest concentration of curcumin compared to other species. Commercial curcuminoids isolated from the rhizomes of *C. longa* consist of three major curcuminoids approximately 77% of curcumin, 17% of demethoxycurcumin and 3% of bisdemethoxycurcumin (Huang et al., 1995).

Curcumin exhibits a variety of pharmacological effects, and has been reported to have anti-inflammation (Guo et al., 2008; Jacob et al., 2007), anti-oxidant (Sandur et al., 2007; Suryanarayana et al., 2007) and anti-cancer activities (Kunnumakkara et al., 2008; Lin et al., 2007; Yoysungnoen et al., 2008). Anti-cancer activities of curcumin could exert both *direct* and *indirect* actions by inhibiting tumor cell proliferation and by inhibiting tumor angiogenesis, respectively. ***Direct action of curcumin*** for inhibiting carcinogenesis has been shown to inhibit cell proliferation and induce apoptosis in hepatic cancer cells (Cao et al., 2007; Lin et al., 1998; Yoysungnoen et al., 2008). Curcumin has also exhibited significant anti-invasion activity in human

HCCSK-Hep-1 cells, an effect that is associated with curcumin inhibited action on matrix metalloproteinase-9 (MMP-9) secretion (Aggarwal et al., 2007).

Furthermore, curcumin can inhibit proliferation and induces apoptosis in wide types of cancer cell *in vitro* including cancers of the bladder, breast, lung, pancreas, prostate, cervix, head and neck, ovary, kidney, brain, bone marrow, and skin (Aggarwal et al., 2003). It has also been shown to potentiate the effect of chemotherapeutic agents (Aggarwal et al., 2005; Kamat et al., 2007; Kunnumakkara et al., 2007) and of γ-radiation (Chendil et al., 2004). *In vivo* curcumin has been used both to prevent and to treat various cancers (Kunnumakkara et al., 2008).

Curcumin prevents a variety of carcinogen-induced cancers in animals including 7,12-dimethyl-benz(*a*)anthracene(DMBA)-induced lymphoma and leukemia (Huang et al., 1998), DMBA-induced mammary cancer (Singletary et al., 1996), methyl-(acetoxymethyl)-nitrosamine(MNA)-induced oral mucosal tumor (Tanaka et al., 1994), and 12-O-tetradecanoylphorbol-13-acetate(TPA)-induced skin tumor (Huang et al., 1997). Furthermore, curcumin has been used to suppress the mutagenic effects of capsaicin, tobacco, cigarette smoke condensate, 2-AAF, benzo(a)pyrene, and aflatoxin B1 (Anto et al., 2002; Deshpande et al., 1996; Singletary et al., 1996; Vanisree & Sudha, 2006)

A number of preclinical studies showed that curcumin exhibited anti-tumor effects and has been used in treatments of various cancers. In one of earlier study, Ruby et al. (1995) reported significant reduction in tumor volume due to curcuminoid treatment. In Cui et al. (2006) showed that oral administration of curcumin (50-200 mg/kg) inhibits the growth of leukemia (HL 60) and lymphoma (SGC7901) cells induced xenografts in nude mice. In a human breast cancer xenograft model study using nude mice, Aggarwal et al. (2005) observed that the administration of curcumin (2% in diet) significantly decreased the incidence of breast cancer metastasis to the lung and suppressed the expression of NF- κB, COX-2, and MMP-9. Recent study by Kunnumakkara et al. (2007) investigated the chemosensitization effect of curcumin using an orthotopic pancreatic cancer model. They found that combinations of curcumin and gemcitabine treatment could reduce tumor volume when compared to gemcitabine treatment alone, indicating curcumin had a chemosensitizing effect. In another study Li et al. (2007) evaluated the anti-tumor, chemosensitizing, and radiosensitizing effect of curcumin using a xenograft prostate cancer model. The experiment showed that xenografts treated with combinations of curcumin and gemcitabine reduced the expression of MDM2 oncogene. These results indicate a novel mechanism of action that may be essential for curcumin's chemotherapeutic effects (Li et al., 2007).

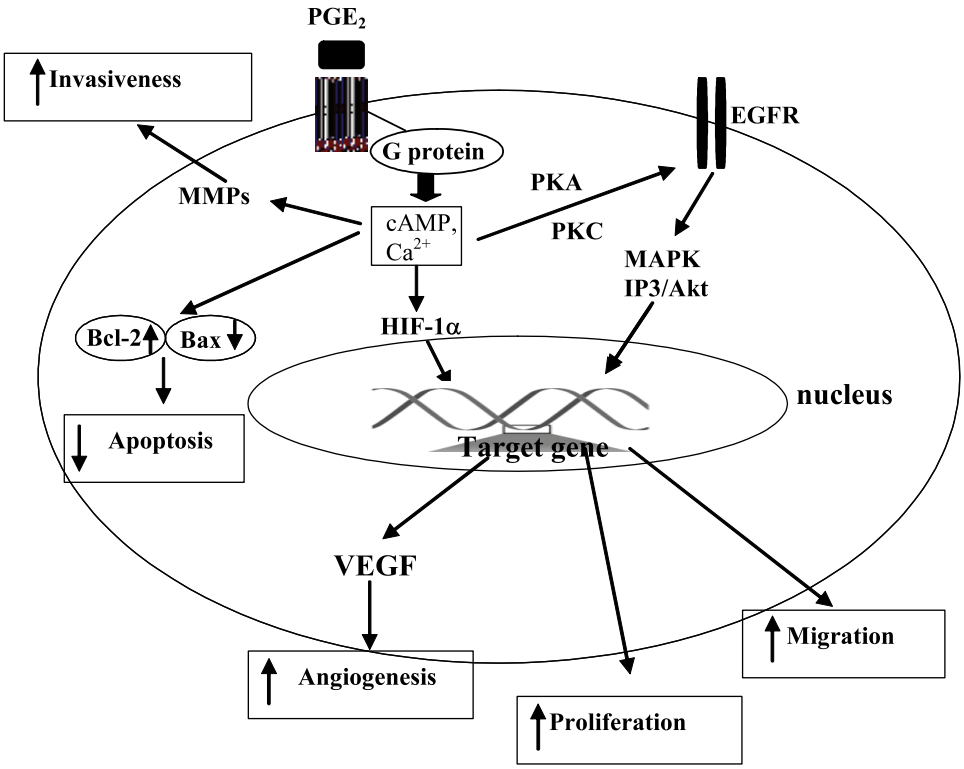

Figure 3. Role of prostaglandins, mediators of COX-2 derived in carcinogenesis (Modified from Konturek et al., 2005).

Clinical trials with curcumin have been reported in several types of cancer such as oral (Kuttan et al., 1987), breast (Kuttan et al., 1987), vulva (Kuttan et al., 1987), skin (Cheng et al., 2001), liver (Cheng et al., 2001), colorectal (Plummer et al., 2001; Sharma et al., 2001, 2004), bladder (Cheng et al., 2001), and cervical cancer (Cheng et al., 2001). In a Phase I clinical trial, a dose of 8,000 mg/kg of curcumin daily for 3 months by oral administration resulted in histologic improvement of precancerous lesions in patients having uterine cervical intraepithelial neoplasm (1/4 patients), intestinal metaplasia (1/9 patients), bladder cancer (1/2 patients) and oral cancer (2/7 patients) (Cheng et al., 2001). However, in clinical trials of oral administration of curcumin to human cancer patients, the systemic availability of curcumin was found to be negligible, especially outside the gut, due to poor absorption of the compound (Garcea et al., 2005; Sharma et al., 2004). Many analogs of curcumin have been synthesized to increase the potentials of curcumin and circumvent the low bioavailability while keeping its low toxicity. These derivatives also decreased the expression levels of oncoproteins, including β-catenin, Ki-ras, cyclin D1, and ErbB-2, at concentrations much lower than those normally used curcumin (Ohori et al., 2006).

However, recent study performed by Dhillon et al. (2008) evaluated a Phase II clinical trial of biological effects of curcumin with potent nuclear factor-kappaB (NF-kappaB) and tumor inhibitory properties against advanced pancreatic cancer. Patients received 8 g curcumin by mouth daily until disease progression, with restaging every 2 months. It was found that curcumin down-regulated expression of NF-kappaB, cyclooxygenase-2, and phosphorylated signal transducer and activator of transcription 3 in peripheral blood mononuclear cells from patients (most of whom had baseline levels considerably higher than those found in healthy volunteers). Whereas there was considerable interpatient variation in plasma curcumin levels, drug levels peaked at 22 to 41 ng/mL and remained relatively constant over the first 4 weeks. They concluded that oral curcumin is well tolerated and, despite its limited absorption, has biological activity in some patients with pancreatic cancer.

The molecular mechanisms by which curcumin inhibits carcinogenesis have been investigated. Curcumin suppresses the activation of several transcription factors that are implicated in carcinogenesis (Aggarwal et al., 2003), including nuclear factor kappa B (NF-κB) (Aggarwal et al., 2006), activator protein 1 (AP-1) (Tomita et al., 2006), and at least two of the signal transducer and activators of transcription proteins (Stat3, Stat5), and modulates the expression of early growth response protein 1 (Erg-1), peroxisome proliferators-associated receptor gamma (PPAR-γ) (Chen & Xu, 2005). It also suppresses the expression of cyclin D1 (Mukhopadhyay et al., 2002) and induces apoptosis of tumor cells (Choudhuri et al., 2005; Sandur et al., 2007). According to the inhibitory effects of curcumin on these cell signaling pathways, curcumin could mediate its anti-proliferation by inhibiting either expression or activation of proteins required for cell survival or cell proliferation pathway.

In human hepatoma cells, curcumin has been found to inhibit Il-6 production, histone acetyltransferase (HAT) activity, and AP-1 activation (Chen et al., 2003) and induce cell death and apoptotic biochemical changes, such as the mitochondrial release cytochrome c, the cleavage of poly ADP-ribose polymerase (PARP) (Labbozzetta et al., 2006). Another proposed mechanism for curcumin's inhibition of tumor growth in HCC is through the inhibitioh of HIF-1 by degrading the aryl hydrocarbon receptor nuclear translocator (Bae et al., 2006; Choi et al., 2006). In addition, it has been shown that mitochondrial hyperpolarization is a perquisite for curcumin-induced apoptosis and that mtDNA damage is the initial event in a chain leading to apoptosis in HepG2 cells (Cao et al., 2007). *In vitro* study using hepatic cancer cells, a combination of curcumin and cisplatin had synergistic anti-tumor effects (Aggarwal et al., 2007).

Previous studies have also reported anti-carcinogenic effect of curcumin in HCC *in vivo*. In a murine hepatocarcinogenesis model, five week-old C3H/HeN mice were injected intraperitoneally with *N*-nitrosodiethylamine (DENA). The treated mice which were fed with a 0.2% curcumin mixed into the diet, had 81% less multiplicity and 62% fewer hepatocarcinomas than the non-treated mice (Chuang et al., 2000). It also prevented the induction of hepatic hyperplastic nodules and body weight loss, increased in the levels of hepatic diagnostic markers, and decreased hypo-proteinemia in DENA-initiated and phenobarbital-promoted hepatic cancer model of Wistar rats. In an orthotopic implantation model, curcumin suppressed both intraheptic metastases and the development of altered hepatic foci (AHF) in rat livers. Inhibition of tumor growth by systemic administration of 20 μg/kg curcumin for 6 consecutive days to rats bearing the highly cachectic Yoshida AH-130 ascites hepatoma was also reported (Aggarwal et al., 2003). In one of the studies, hepatocellular carcinoma cells were injected subcutaneously in mice and 3 weeks after cell injection, a tumor fragment from the injection site was implanted to liver. Curcumin (100-200 mg/kg) was administered 20 days after the implantation.

The result showed that the curcumin treatment could decrease in number of intrahepatic metastases in a dose dependent manner, although the growth of tumors at the implanted site was not affected by the curcumin treatment (Aggarwal et al., 2003). These data demonstrated direct anti-carcinogenic activities of curcumin.

On the other hand, ***indirect action of curcumin*** inhibits tumor growth via its anti-angiogenic activity. Previous study demonstrated that curcumin treatment resulted in inhibition of angiogenic differentiation of human umbilical vein endothelial cells (HUVEC) on matrigel and endothelial cell infiltration and vessel formation in matrigel plug, indicating the anti-angiogenic activity (Thaloor et al., 1998). Subsequently, it was shown to inhibit basic fibroblast growth factor (bFGF)-induced corneal neo-vascularization in the mouse cornea (Arbiser et al., 1998). This angiostatic efficacy in the cornea was also observed when curcuminoids were provided to mice in the diet (Mohan et al., 2000). Recently, it has been shown that the anti-cancer property of curcumin is mediated in part by its anti-angiogenic activity (Gururaj et al., 2002; Park et al., 2002; Singh et al., 1996; Yoysungnoen et al., 2006 & 2008). Almost all of these finding demonstrated the curcumin supplementation significantly suppressed neocapillarization under tumor progression.

Typically, angiogenic inhibitors may be divided into two classes. Firstly, ***direct angiogenic inhibitors*** refer to those agents which are relatively specific for endothelial cells (Arbiser, 1997). Endothelial cell apoptosis is necessary for repairing damaged blood vessels and for sprouting and branching of capillaries during angiogenesis. Reported by Gururaj et al. (2002), curcumin could reduce the cell number of EAT cells and HUVECs *in vitro* without having cytotoxic effect, however it did not affect the mouse fibroblast (NIH3T3) cells. This indicates the specificity of curcumin action. In another study, conducted by Shankar et al. (2007), demonstrated that curcumin could inhibit capillary tube formation and endothelial cell migration, and these effects can be enhanced by mitogen-activated protein kinase kinase (MEK) inhibitors. These results could explain in part the direct angiogenic inhibitors of curcumin.

Secondly, ***indirect angiogenic inhibitors*** are those which may not have direct effects on endothelial cells but may down-regulate the production of angiogenic factors, such as VEGF (Arbiser et al., 1997). Curcumin has been shown to inhibit the expression of several genes involved in angiogenesis and metastasis (*VEGF*, *COX-2*, *ICAM-1*, *CD31*, *MMP-9*) (Aggarwal et al., 2006; Bae et al., 2006; Yoysungnoen et al., 2006). Bae et al. (2006) found that curcumin significantly decreases hypoxia-induced HIF-1α protein levels in HepG2 hepatocellular carcinoma cells. Moreover, curcumin suppressed the transcriptional activity of HIF-1α under hypoxia leading to a decrease in the expression of VEGF, a major HIF-1α target angiogenic factor. Curcumin also blocked hypoxia-stimulated angiogenesis *in vitro* and down-regulated *HIF-1α* and *VEGF* expression in vascular endothelial cells. These findings suggest that curcumin may play pivotal roles in tumor suppression via inhibition of HIF-1α -mediated angiogenesis. In addition, previous study reported that elements for transcription factor *AP-1* are essential for *VEGF*-gene expression (Lee et al., 2006). Since the binding of *AP-1* factor to its DNA domain could be interfered by curcumin, it is possible that curcumin exerts its influence on *VEGF* gene expression via inhibition of *AP-1* (Grau et al., 2006). Furthermore, inhibition of *COX-2* expression by curcumin may be due to curcumin suppression of the activation of NF-kB, the essential transcription factor for *COX-2* gene expression (Lee et al., 2005). These evidence indicates inhibitory effects of curcumin on angiogenesis either by direct actions on endothelial cells or by down-regulation of the production of angiogenic factors. Because of its anti-cancer and anti-angiogenic activities, low molecular weight and lack of toxicity, curcumin could be an ideal candidate for a chemotherapeutic agent (Hahm et al., 2004). Figure 4 summarizes the proposed mechanisms by which curcumin inhibits angiogenesis in HCC.

CONCLUSIONS

Angiogenesis plays an important role in the aggressive biological behavior of HCC, one of the most vascular human cancers. From the positive correlation of VEGF and COX-2 levels in many cancers, it is reasonable to speculate that the role of VEGF and COX-2 are the most critical angiogenic factor regulating angiogenesis in HCC. However, the molecular mechanisms of association between VEGF and COX-2 in regulating angiogenesis in HCC remain to be clarified. Angiogenic inhibitors provide a target for novel therapeutic approaches to HCC. Curcumin possesses both direct and indirect anti-angiogenic activity both *in vitro* and *in vivo*. Therefore, curcumin could be a candidate for combined drug treatment strategy for HCC in the future.

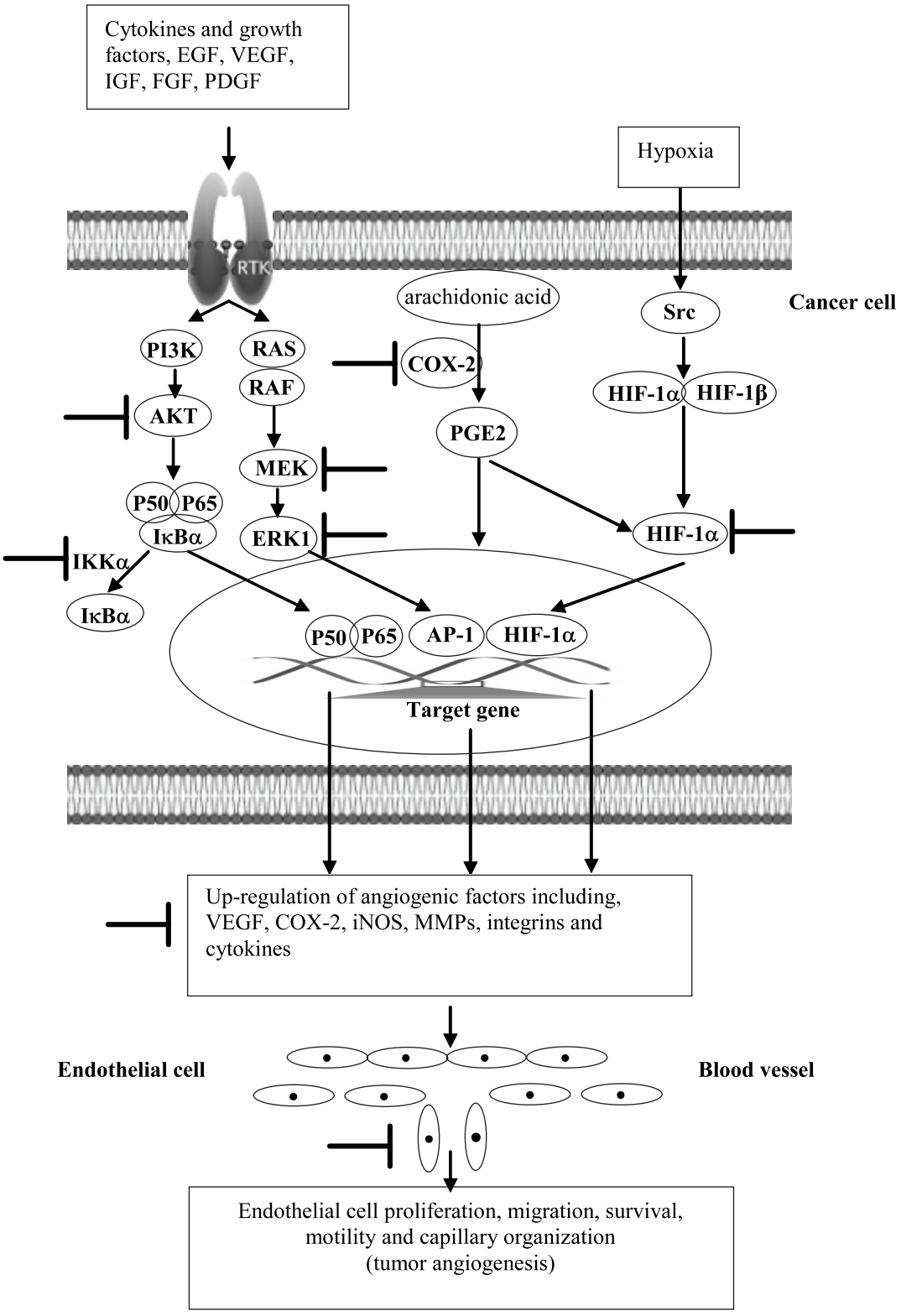

Figure 4. The proposed mechanisms by which curcumin inhibits angiogenesis in HCC.

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