CHAPTER V

DISCUSSION

In the present study, the experiments were conducted to investigate the effects of curcumin on tumor angiogenesis and levels of angiogenic biomarkers, COX-2 and VEGF using HepG2-implanted nude mice model.

The observations for tumor angiogenesis

- ***Dilatation, Tortuosity, Hyperpermeabitity***

By using intravital fluorescent microscope, within the tumor area, the changes of microvasculature and the induction of neocapillary could be observed. Intravital fluorescence videomicroscope has been reported for its potential to quantitative assessment of the dynamic process of angiogenesis in neoplastic tissue by several studies as well (Lehr *et al.*, 1993; Niimi *et al.*, 2003;Nageswari *et al.*, 2002). In our study, we have found that on day 3 after the inoculation of tumor cells, there was no significant increase in host-capillary diameters. However, we have noticed for the changes of host arterioles, and capillaries vessels as that they appeared to be tortuosity and dilatation, hyperpermeability to macromolecule, rhodamin-dextran (70,000 MW), respectively. These early changes of host microvessels might be a result of angiogenic factors growth factors (in particular, VEGF) binding to its receptor on endothelial cells of host blood vessels. In fact, it has been reported by Hanahan and Folkman (1996) that microvascular dilatation, tortuosity, hypermeability, edema appeared as earliest histopathological features. It is also possible that VEGF elaborated from the tumor cells increases the permeability of local microvessels so that the tumor microcolony is bathed in nutrients even before neovascularization begins. Further, plasma and fibrin leakage from the microvessels could facilitate chemotaxis and alignment of tumor cells (Senger *et al.*, 1994), as well as subsequent migration of endothelial cells (Kadish *et al.*, 1979). VEGF also up-regulates synthesis of nitric oxide in vascular endothelial (Ziche *et al.*, 1997; Papapetropoulos *et al.*, 1997; Garcis-Cardena and Folkman, 1998). The NO was derived from tumor

vascular endothelia and/or tumors cells, which might be a correspondence for these events.

Moreover, the result showned that the early tumor induction of COX-2 expression on 3 days after HepG2 inoculation might be another factor to cause microvascular dilatation, tortuosity, hypermeability, and edema. Since the up-regulation of COX-2 in endothelial cells has been shown for its strongly influence on cellular COX-2 activity (Caughey *et al.*, 2001), and resulted in an increase in prostanoid synthesis including thromboxane A2 (TXA_2), PGI_2, and PGE_2. COX-1 was linked to TXA2 production, whereas the induction of COX-2 shifted prostanoid synthesis to favor PGE_2 and PGI_2 production (Caughey *et al.*, 2001). Therefore, the increased COX-2 could lead to prostanoids production in endothelial cells and then resulting in vasodilatation and hyperpermeability.

At this point, we might briefly summarize that the tumor-derived growth factor caused the early observed event such as vasodilatation, tortuosity, and hypermeability of host microvasculature. Vasodilation and vessel tortuosity permit endothelial elongation and thus may be a prerequisite for endothelial cells to undergo mitosis and migration in response to angiogenesis.

- ***Neocapillary characteristics***

It has been demonstrated in Figure 4.4 that the tumor angiogenesis could be observed on day 7 after the inoculation of HepG2. It appears that the tumor angiogenesis was observed with endothelial cell sprouting in the mother vessel. This phenomenon is a characteristic process of tumor angiogenesis. In the present observation, some of these proliferating neovessels mostly migrated out approaching to the tumor area.

The mean capillaries diameters were significantly increased in 7 and 14 days of HepG2 groups (Table 4.4 and Figure 4.10). Other investigators also reported an increase of vessel diameter in tumor with time (Liotta *et al.*, 1974; Leunig *et al.*, 1992). In addition, the host vessels appeared to be tortuous and leaky (Figure 4.6). The increases of capillaries diameters in these groups might be due to the increment of

angiogenic factors. Accordingly, the serum VEGF was markedly increased in 7 and 14 days of HepG2 groups (Table 4.7 and Figure 4.12). Moreover, COX-2 proteins were overexpressed on these periods. The expressions of the COX enzyme induced capillary diameter changes might be due to its activity to produce a number of eicosanoid products including prostaglandins and prostacyclin.

- ***Neocapillary density (NCD) and Tissue perfusion***

Since Neocapillary density (NCD) is an important parameter for quantitative assessment of the development of angiogenesis in tissues, various methods have been developed to measure it at different period after the inoculation of tumor cells. In the present study, by using Global LabII software we expressed the neocapillary density (NCD) as the corresponding ratio of total number of pixels within capillaries and total number of pixel within studied area (equation 1). Several studies have used and represented the NCD in terms of number of capillary per area (number/mm^2) as well (Niimi *et al.*, 2000; Komi *et al., in press*). The technique of NCD determination using the application of image analysis will allow for convenient and precise evaluation of the tumor vasculature progression. This could facilitate validation of mechanisms of action of putative antiangiogenic compounds.

By intravital fluorescent microscopy, our study demonstrated that there was a significant increase in the numbers of NCD with the heterogeneous network in HepG2 groups as compared to controls (Table 4.3 and Figure 4.9). In particular this increment of neocapillary density was characterized as a time-dependent manner (NCD of 14 days > NCD of 7days). In our study, it is actually confirmed the fact that angiogenesis has already occurred on day 7 after HepG2 inoculation. A large number of neocapillaries was observed and corresponded with the increase in tumor-area tissue perfusion. Within HepG2 groups, the adaptation microvasculature network reflecting the increase in metabolic rate and oxygen consumption in the tumor-bearing tissue was directly observed (Folkman, 1990).

Tumor angiogenesis and its biomarkers : COX-2 and VEGF levels

COX-2:

In our study, the Western blot data demonstrated that COX-2 strongly expressed on day 3 in HepG2 group and maintained throughout the experimental period. Moreover, the linear regression line of Y= 22850x - 23699 (Figure 4.17) also implied that the expression of COX-2 was significantly correlated with tumor neogenesis. The more COX-2 expression, more neocapillary density was obtained (r^2 = 0.9607, $p<0.01$). This finding, therefore, brings us the idea that the overexpression of COX-2 is functionally significant for tumor angiogenesis. As which COX-2 could be referred as biomarker for tumor angiogenesis. In 1999, the study of Koki *et al.* (1999) found that COX-2 was also detected in non-cancerous cell immediately adjacent to tumor cells and in the angiogenic vasculature within tumors and in pre-existing blood vessels adjacent to tumors, as well as the epithelial cells and some inflammatory cell. Tomozawa *et al.* (2000) demonstrated a significant correlation between COX-2 expression and tumor recurrence, especially hematogenous metastasis. Their findings had support to the hypothesis that the overexpression of COX-2 may enhance the development of hematogenous metastasis through the promotion of tumor angiogenesis.

Several epidemiological studies have demonstrated that the 40-50% decrease in the relative risk of colorectal cancer could occurr by the use of non-steroidal anti-inflammatory drugs (NSAIDs) (Rosenberg *et al.*, 1991; Thun *et al.*, 1991; Dubois *et al.*, 1996). Cyclooxgenase-2, an inducible isoenzyme of COX, has been considered to be the major target molecule in cancer chemoprevention induced by NSAIDs. Furthermore, using rodent studies of colon carcinogenesis, one had reported the preventive and/or therapeutic efficacy by COX-2 inhibitors (Oshima *et al.*, 1996; Reddy *et al.*, 2000). Thus, cancer chemoprevention with COX-2 inhibitors is regarded as the potent anticancer strategy. Although, the roles of COX-2 in tumor development and progression have been described, *the mechanisms by which COX-2 deficiency decreases tumorigenesis are still unclear.*

The direct effect of COX-2 on angiogenic process had been demonstrated by Daniel *et al.*(1999). They hypothesized that the stimulation of tumor angiogenesis was 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. Besides, the other main product of COX-2, namely prostaglandinE2 (PGE_2) was also found in high levels in tumor cells (Schrey *et al.*, 1995; Uefuji *et al.*, 2000). Cianchi *et al.* (2001) have also found a significant relationship between PGE_2 levels and tumor stage. However, PGE_2 levels were not significantly related to either COX-2 or VEGF expression, even though PGE_2 levels tended to increase with increasing COX-2 and VEGF staining grades. Nevertheless, *other cell types such as endothelial cells, tumor-infiltrating macrophages, and lymphocytes, can express COX-2 and may be responsible for PG production in tumors.* Higher PGE_2 levels were found in tumor specimens with distant or lymph node metastases than in tumor specimens 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 metalloproteinase-2-activation (Tsujii *et al.*, 1997). These effects can be reversed by selective COX-2 inhibitors. A recent study by 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 COX-2, integrin $\alpha V\beta 3$ and Cdc-42/Rac-dependent endothelial-cell migration.

The indirect pathway 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 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.

Thus, COX-2 appears to be related to induction of angiogenesis in a variety of tumors. The possible mechanism(s) of COX-2 on modulating tumor angiogenesis may be resided on both direct and/or indirect pathways as described above.

VEGF:

To investigate whether COX-2 may involved in the up-regulating of angiogenic factor, especially, VEGF, or not, we therefore, evaluated the production of VEGF by using EIA-kit (Chemicon International, Inc., CA). The result has demonstrated that serum VEGF increased significantly in HepG2 groups on 7 and 14 days as compared to their age-matched controls, *but not for 3 days after the HepG2 inoculation.* Our finding agrees with several studies such that the preoperative level of VEGF in the serum had been defined for its use to predict the stage of cancer (Kumar et al., 1998; Adams *et al.*, 2000; Bian *et al.*, 2000; Loncaster *et al.*, 2000; Mineta *et al.*, 2000; Stockhammer *et al.*, 2000; Yoshikawa *et al.*, 2000; Broll *et al.*, 2001; Hirai *et al.*, 2001). In addition, Warren *et al.* (1995) found that serum VEGF was significantly raised in mice after inoculation with tumor cells. The animals treated with neutralizing antibodies against VEGF show an inhibition of tumor and metastasis growth compared with untreated animals. Moreover, it has been shown that VEGF plays an important role in determining the biological aggressiveness of neoplastic cells and thus, their metastatic potential (Mandriota *et al.*, 1995; Nakata *et al.*, 1998).

Several studies have shown a strong correlation among VEGF, angiogenesis, and tumor staging (Takahashi *et al.*, 1995; Kang *et al.*, 1997; Ishigami *et al.*, 1998).

Our study has gained more confounding that it is not a simply linear correlation between the elevation of serum VEGF and the tumor neovascularization. Since the result showed in Figure 4.15 demonstrated that VEGF level and NCD could not be linearly fit ($r^2 = 0.7681$, $p<0.32$).

Although the molecular mechanism of the "angiogenic switch" by which quiescent endothelium becomes activated is unknown, ***VEGF seems to be the main inducer of tumor angiogenesis.*** Tumor hypoxia and oncogenes up-regulate VEGF levels in the neoplastic cells. The hypoxia, in combination with the locally increased VEGF concentrations, could up-regulate VEGFR-1 and VEGFR-2 on tumor endothelial cells (Kremer *et al.*, 1997;Plate *et al.*, 1992;Shweiki *et al.*, 1992). VEGF derived from hypoxic areas of tumors is likely to mediate increased permeability. From the literature reviews, *VEGF contributes to tumor angiogenesis by both direct and indirect mechanisms :* on one hand, VEGF stimulates endothelial cell proliferation and migration; on the other, it renders vessels hyperpermeable, leading to formation of a matrix or matrix reorganization for supports blood vessel growth. VEGF not only increases vascular permeability, it is also chemotactic for macrophages. These tumor-associated macrophages in turn cause more VEGF production. Indeed, there were several documents shown that other mediators of neovascularization including interleukins, and some growth factors may produce their effects by altering the expression of VEGF. Therefore, it implied that *VEGF might be the final common pathway for all pathological in vivo angiogenesis (Ferrara, 1996).*

***COX-2 and VEGF*:**

Moreover, our results also demonstrated that there was not a direct correlation between overexpressions of COX-2 and VEGF. As shown in Figure 4.19, the plot between these two parameters could not be significantly fit by linear correlation ($r^2 = 0.5829$, $p<0.447$).

Cianchi *et al.* (2001) found that COX-2 and VEGF were expressed in tumor specimens of colorectal cancer patients as compared with normal tissues. The immunohistological expressions of both COX-2 and VEGF were significantly correlated with microvessel density. Moreover, Cheng *et al.* (2004) found a

significantly correlation between COX-2 and VEGF expression which examined by immunohistochemistry in hepatocellular carcinoma patient. Treatment of the COX-2 overexpressing cells with a COX-2-selective inhibitor, NS-398 (10 microM), decreased PGE_2 level and attenuated VEGF expression. They suggested that COX-2 was related to tumor angiogenesis in up-regulating VEGF expression in hepatocellular carcinoma cells, possibly via PGs production. Therefore, it is likely that VEGF has been mostly referred as one of the most important mediator of the COX-2 induced angiogenic pathway.

At this point, we could be simply summarized our findings that both overexpression of COX-2 and VEGF were significantly needed for tumor angiogenesis as that found in HepG2 groups. The overexpression of COX-2 occurred significantly prior before the increase in VEGF (shown by the result of 3 day experimental period). In other word, COX-2 may be a primary mechanical process responding to angiogenic pathway. VEGF may be acted as a secondary key process mediated **indirectly** by COX-2, or it may co-modulate via different pathways.

The effects of curcumin on HepG2 angiogenic biomarkers, COX-2 and VEGF levels

Over centuries, native medicines of Asian countries employed natural products to treat a variety of diseases. Besides, human has consumed many medicinally important natural products as our diet. For instance, in Ayrveda, an alternative medicine, turmeric, is an important food ingredient known for its anti-tumorigenic action (Rao *et al.*, 1995) as well as anti-inflammatory properties (Huang *et al.*, 1991). Curcumin, which is our important agent trial in this study, is a small molecular-weight component of tumeric.

In this study, we have demonstrated that curcumin could inhibit angiogenesis which occurred in dorsal skin-fold chamber using HepG2-implanted nude mice model. Especially, the high dose of curcumin, 3000mg/kgBW, which has not been reported for any toxicity (Krishnaswamy and Raghuramulu, 1998; Luo *et al.*, 1998), could give the significant inhibiting effect on tumor angiogenesis. Interestingly, our

study has also indicated that the inhibition of tumor angiogenesis by curcumin is a dose-dependent manner as well.

During the early stage of 3 day after HepG2 inoculation, the results of curcumin treatments (both 300 and 3000 mg/kgBW) have shown that pathological features including host-microvascular dilatation, tortuosity, and hypermeability, induced by tumor could be attenuated.

Moreover, our findings have indicated that the high dose of curcumin (3,000 mg/kgBW) could inhibit tumor neovascularization observed on day 7 and day 14 pos-inoculations significantly (Table 4.3 & Figure 4.9 and 4.10). Importantly, this inhibition effect of curcumin on tumor angiogenesis has been well correlated by its effect on COX-2 inhibition as shown in Figure 4.17. Even though the relationship between this inhibition effects of curcumin on tumor angiogenesis with VEGF-inhibition could not be represented by the simply linear correlation, but curcumin was still able to suppress the expression of VEGF significantly with dose-dependent manner ($p<0.001$).

Moreover, the histological examination using hematoxylin-eosin as shown in Figure 4.8 has demonstrated for the inhibition effects of curcumin on tumor growth as a result of its anti-angiogenesis property as well.

Although the underlining mechanism of curcumin on tumor anti-angiogenesis has not been well clarified yet. From our findings, at this moment we therefore would like to propose that its mechanism(s) should be corresponded through its inhibitory effects on tumor biomarkers expression, both COX-2 and VEGF. Previous studies had demonstrated that curcumin could inhibit both of cyclooxygenase and lipoxygenase enzymes in mouse epidermis (Huang *et al.*, 1991, Zhang *et al.*, 1999).

However, the mechanisms on inhibition of COX-2 expression by curcumin were not well defined. It has been demonstrated that curcumin could inhibit both cyclooxygenase and peroxidase activities (Zhang *et al.*, 1999). Therefore, curcumin may be superior to commonly used NSAIDs, which have anti-inflammatory and

chemopreventive effects as well. The inhibition of curcumin on expression of COX-2 in cancer cells gastrointestinal cancer (SK-GT-4, SCC450, IEC-18 and HCA-7) was studied by Zhang *et al.* (1999). The overexpression of COX-2 in cancer cells was induced by chenodeoxycholate (CD)- or phorbol ester (PMA). They found that treatment with curcumin suppressed CD- and PMA-mediated induction of COX-2 mRNA as well as protein level and synthesis of prostaglandinE2 in these cell lines. Nuclear run-offs revealed increased rates of COX-2 transcription after treatment with CD or PMA, and these effects were inhibited by curcumin treatment (1-20 μM) in dose dependent manner. Treatment with CD or PMA increased the binding of AP-1 to DNA. This effect was also inhibited by curcumin. Furthermore, the activity of COX-2 was found to be directly inhibited by curcumin *in vitro*. Additionally, the study of Goel *et al.* (2001) also found that curcumin (5-75 μM) markedly inhibited the mRNA and protein expression of COX-2 but not COX-1. These findings suggest that curcumin has a specific inhibition on COX-2 expression.

Our results also showed that in mice bearing HepG2 cells, curcumin could inhibit the increase in serum VEGF, thereby, it inhibited the formation of new blood vessels. Conversely, the recent report on anti-angiogenic activity of curcumin showed that curcumin inhibited FGF-induced neovascularization, but did not have any effect on TPA-induced VEGF mRNA expression (Arbiser *et al.*, 1998).

The previous study reported that elements for transcription factor AP-1 are essential for VEGF-gene expression (Illi *et al.*, 2000). Since curcumin is a known inhibitor of binding of AP-1 factor to its DNA domain (Huang *et al.*, 1991), therefore, ***it is possible that curcumin exerts its influence on VEGF gene expression via inhibition of AP-1.*** Moreover, curcumin had been reported for its properties on inhibiting the expression of angiogenic ligands, VEGF and angiopoietin-1 (Ang1) in NIH3T3 cells (Gururaj *et al.*, 2002). As such anti-angiogenic effect of curcumin, one has reported that it is not secondary to the pro-apoptotic property of curcumin (Gururaj *et al.*, 2002). Therefore, it may say that tumors did not respond to curcumin in terms of apoptosis but still respond in terms of angiogenesis.

Curcumin not only interfered with the gene expression of angiogenic ligands but also inhibited the receptor, KDR gene expression in HUVECs. Curcumin blocks NF-κB translocation to the nucleus by inhibiting IκB kinase enzyme and consequently inhibiting dissociation of IκB from NF-κB. Since KDR promoter is up-regulated by NF-κB (Hideyasu *et al.*, 1999), the mode of down regulation of KDR gene expression by curcumin could be via blocking of NF-κB activity by curcumin. However, the further studies are required to elucidate the exact mechanism of down-regulated of VEGF and KDR gene expression by curcumin.

Typically, angiogenic inhibitors may be divided into two classes. The first one, direct angiogenic inhibitors, refers to those agents which are relatively specific for endothelial cells and have little effect on tumor cells (Arbiser, 1997). Indirect inhibitors may not have direct effects on endothelial cells but may down-regulate the production of an angiogenic factors, such as VEGF (Arbiser *et al.*, 1997; Gess *et al.*, 1996). Previous studies have indicated that curcumin effectively inhibited endothelial cell proliferation in a dose-dependent manner (Arbiser *et al.*, 1998). Curcumin demonstrated significant inhibition of bFGF-mediated corneal neovascularization in the mouse. Curcumin had no effect on phorbol ester-stimulated VEGF production. They suggested that curcumin has direct anti-angiogenic activity both *in vitro* and *in vivo*. However, the study by Gururaj *et al.* (2002) demonstrated that the angiogenic inhibitory effect of curcumin *in vivo* was colloborated by the results on down-regulation of the expression of pro-angiogenic genes, EAT, NIH3T3, in endothelial cells.

Our study curcumin could inhibit COX-2 expression, significantly on 3, 7, and 14 day post-inoculations, and reduce VEGF production, significantly on 7, and 14 day post-inoculations. With this chronological study design, it indicated that inhibitory effects of curcumin on tumor angiogenesis might be due to its direct actions on both biomarkers of COX-2 and VEGF, ***but*** by different pathways. Or this anti-angiogenesis effects of curcumin might be explained by that curcumin was firstly act on inhibiting COX-2 expression, and then result to the ***indirect*** inhibition on the VEGF expression.

Diagram shown in Figure 5.1 is the proposed mechanism that we would like to hypothesize. The idea is that once a tumor grows to a certain size, the cells in the center are too far away from existing blood vessels to receive the necessary nutrients for cell survival. Then the condition of "Hypoxia", lack of oxygen, is occurred to those tumor cells. The hypoxic tumor cells then were stimulated and produced VEGF through the recruitment of the AP-1 factor, the essential elements for VEGF gene expression, on the VEGF promotor region via a direct phosphorylation of hypoxia-inducible factor-1α (HIF-1α). In addition, hypoxia could influence many other transcriptional pathways, such as those mediated by *fos* and *jun* (Webster *et al.*, 1994), and NF-kB (Koong *et al.*, 1994) which is the essential transcription factor for COX-2 gene expression. COX-2 is an immediate early response gene that can be induced by direct hypoxia and a variety of cytokines and growth factors as well (Schmedtje *et al.*, 1997;Yang *et al.*, 2002).

The inflammatory cells present in the tumor microenvironment are also involved in the regulation of the angiogenic process. Inflammatory cells, and particularly macrophage (Harmey *et al.*, 1998), are also important sources of pro-angiogenic factors like VEGF and COX-2. VEGF is also chemotactic for macrophages. These tumor-associated macrophages in turn produce increased levels of VEGF. The up-regulation of both VEGF and COX-2 expression in the HepG2-implanted nude mice may be responsible for switching on angiogenesis. Both COX-2 and VEGF may co-modulate angiogenesis.

COX-2 correlated with angiogenesis through an up-regulation of the expression of VEGF ***indirectly***. Then, VEGF binds to receptors on endothelial cells of existing blood vessels, stimulating angiogenesis by both direct and indirect pathways. Both pathways involved several mechanisms such as increase of vessel hyperpermeability, induction of proteases, EC proliferation, migration etc., promote angiogenesis resulting in continued dysfunction vasculature and tumor growth. Dysfunction vasculature and tumor growth further positive feedback to crate continuing hypoxia inside tumors, therefore further increase angiogenesis.

In this study, anti-angiogenic activity of curcumin showed that curcumin inhibited COX-2 expression and VEGF production. . Curcumin might inhibit the binding of AP-1 factor, a responsed element of VEGF, to its DNA domain (Huang *et al.*, 1991), or blocking of NF-κB activity. Therefore, the inhibitory effects of curcumin on angiogenesis may be act as either direct actions on both biomarkers of COX-2 and VEGF angiogenesis inhibitor or firstly act on inhibiting COX-2 expression firstly, and then result to the indirect inhibition on the VEGF expression.

The findings seem to imply that COX-2 is an upstream important mediator of the angiogenic pathway. COX-2 and VEGF are necessary co-modulators for angiogenesis but through different mechanisms. Importantly, our results have provided originally an *in vivo* evidence for anti-angiogenic activity of curcumin, in particular by using hepatocellular-carcinoma inoculated skin-chamber model. The mechanisms that we have proposed for the activity of curcumin in inhibiting angiogenesis are mediated significantly through the reduction of angiogenic biomarkers, COX-2 and VEGF, productions (as shown in Figure 5.1).

Therefore, curcumin might be considered as an therapeutic agent used for treatment against cancer in the future.

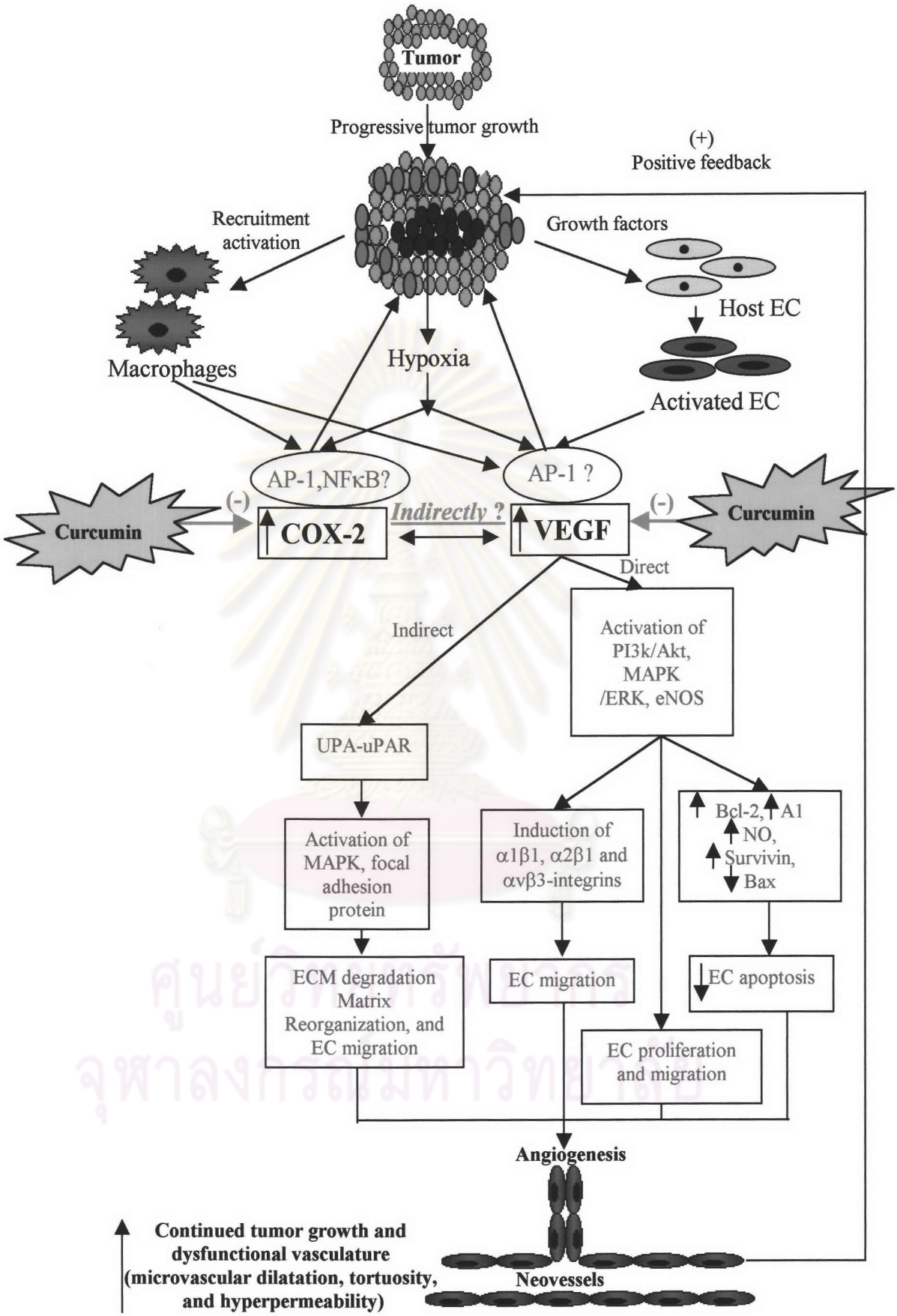

Figure 5.1. The proposed mechanism for our findings on the anti-angiogenic activity of curcumin.