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Review article

Curcumin as a therapeutic agent against cancer

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Background: Curcumin is an important chemopreventive agent against cancer**.** Recently, it has been reported that curcumin has also anti-angiogenic effects in tumor.
Objective: This article reviews the effect of curcumin against tumor angiogenesis from view-points of cancer microcirculation and biomarkers.
Results and conclusion. We demonstrated an anti-angiogenic effect of curcumin in tumors using nude mice. We studied the inhibitory effects of curcumin on tumor-induced neocapillaries and proangiogenic factors, based on our intravital fluorescent observation. Finally, we hypothesized a possible mechanism for curcumin effect in tumor.

Keywords: Anti-angiogenic effect, biomarker, cancer, curcumin, microcirculation, neocapillaries, nude mice.

Cancer is the third major cause of mortality, accounting for more than seven million deaths per year worldwide. Interestingly, the age-adjusted cancer mortality rate per 100,000 for Americans is 206.0 for men and 138.6 for women, where as in Sri Lanka, the age-adjusted cancer mortality rate per 100,000 is 29.3 for men and 26.1 for women. This observation has engendered much research activity aimed at the identification of anti-cancer agent, especially substances derived from the diet [1]. Turmeric, the dried ground rhizome of the perennial herb *Curcuma longa* L., is one example of such an agent. Turmeric contains curcuminoids and essential oils. Recently, curcumin has been considered a potentially important chemopreventive agent against several types of cancers [2]. The possible explanation for this finding is that curcumin can inhibit tumor angiogenesis which is the essential process for every tumor.

This article reviews the chemical and pharmacological properties of curcumin in brief. Special emphasis is put on its potential cancer therapeutic activity, in particular, from the view-points of cancer microcirculation and biomarkers. For abbreviations, see the last section in the text.

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Curcumin

Curcumin (diferuloylmethane) is a major yellow pigment that has been isolated from the ground rhizome of the *Curcuma* species, Zingiberaceae (ginger) family (**Fig. 1)**. There are seven major species of *Curcuma* including *Curcuma longa* L., *C. xanthorrhiza* Roxb., C. *wenyujin, C. sichuanensis*; *C. kwangsiensis*; *C. aeruginosa* Roxb., and *C. elata* Roxb. [3]. *Curcuma longa* L. or turmeric is a tropical plant native to southern and southeastern tropical Asia. It has been widely used as a spice and colouring agent in several foods, such as curry, mustard, bean cake, and cassava paste, as well as in cosmetics and drugs. Actually, the substances that are responsible for the yellow color of turmeric is called “curcuminoids”. Three major compositions of curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin (**Fig. 2**). Curcuminoids were first described by Vogel and Pellatier, and was shown to be diferuloylmethane ($C_{21}H_{20}O_6$) in 1910 [4, 5].

Curcuma longa has the highest concentration of curcumin as compared to the other species. Among the three components, curcumin was referred as the most active component in turmeric since its concentration is the highest one. It may represent up to 2-5 % of the total spice in turmeric.

Fig. 1 Turmeric, the dried ground rhizome of the perennial herb *Curcuma longa L.*

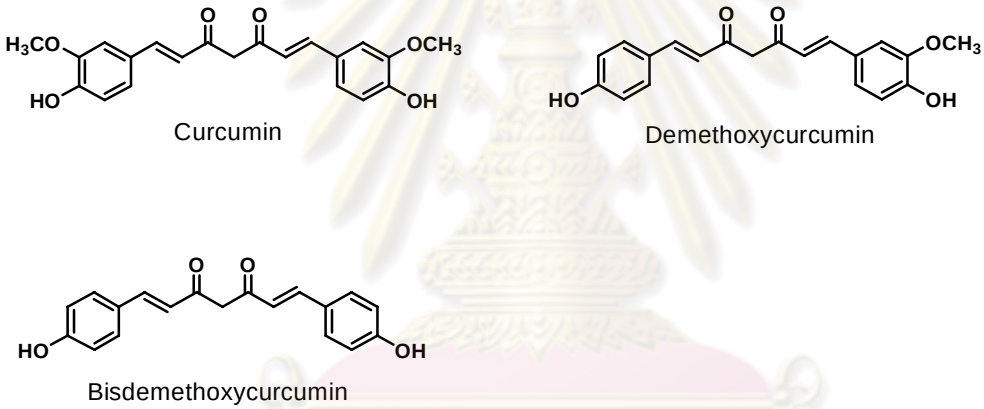

Fig. 2 Three major compositions of curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

Curcumin has been demonstrated to have several pharmacological properties as shown in **Table 1.** Recent phase I clinical trials indicate that people can tolerate a dose as high as 8 g/day [6]. In the U.S., curcumin is used as a coloring agent in cheese, spices, mustard, cereals, pickles, potato flakes, soups, ice creams, and yogurts (*www.kalsec.com*).

Curcumin and its potent antioxidant activity

From the reviews, curcumin contains more potent superoxide anion scavenging activity than the other components; demethoxycurcumin, and bisdemethoxycurcumin. Moreover, curcumin which is a fat-soluble phenolic-compound has been shown to be eight times more powerful than vitamin E in preventing lipid peroxidation [37]. As shown in **Fig. 3**, Para-hydroxyl groups [OH] are believed to be responsible for curcumin antioxidant property.

Curcumin and its anti-inflammatory activity

Another major biological property of turmeric and curcuminoids is their anti-inflammatory property which is comparable in strength to steroidal drugs and nonsteroidal anti-inflammatory (NSAIDs) drugs such as indomethacin and phenylbutazone [38, 39]. Curcuminoids inhibit enzymes which participate in the synthesis of inflammatory substances in the body derived from arachidonic acid. Arachidonic acid is a compound metabolized in the body to yield important hormone-like substances which play major roles in the process of inflammation. Arachidonic acid can be converted by the action of the enzyme cyclooxygenase to prostaglandins (PG) and thromboxanes (TX), and by action of the enzyme lipoxygenase to hydroxyeicosatetraenoic acids (HETE) and leukotrienes (LT). Some of the prostaglandins like PGE_2 and PGI_2 dilate blood vessels,

Table 1. Therapeutic potential of Curcumin.

Potent antioxidant	Kunchandy E, Rao MNA (1990) [7]; Subramanian M *et al.* (1994) [8], Sreejayan Rao MN (1994) [9], Rushworth S (2006) [10]
Anti-inflammation	Huang M-T *et al.* (1988, 1991, 1997) [11-13], Shih and Lin (1993) [14]
Carcinogen-DNA adduct inhibitor	Conney *et al.* (1991) [15]
Tumorigenesis	Huang MT *et al.* (1992, 1994, 1995) [16-18], Rao CV *et al.* (1995) [19]
Cardiovascular disease	Ghoneim AH *et al.* (2002) [20], Sreejayan Rao MNA (1994) [9], Soni KB *et al.* (1992) [21], Shoba G *et al.* (1998) [22]
Alzheimer disease	Frautschy SA *et al.* (2001) [23], Lim GP *et al.* (2001) [24]
Diabetes	Sajithlal GB *et al.* (1998) [25], Babu PS *et al.* (1995) [26], Patumraj S *et al.* (2006) [27]
Nephrotoxicity	Venkatesan N *et al.* (1999) [28]; Ali BH *et al.* (2005) [29]
Cataract	Awasthi S *et al.* (1998) [30]
Would healing	*www.freepatentsonline.com*
HIV	Vajragupta O *et al.* (2005) [31] La Colla P (1998) [32]
Anti-angiogenesis	Adamd BK *et al* (2004) [33], Patumraj S *et al.* (2005) [34] Yoysungnoen P *et al.* (2005, 2006) [35, 36]

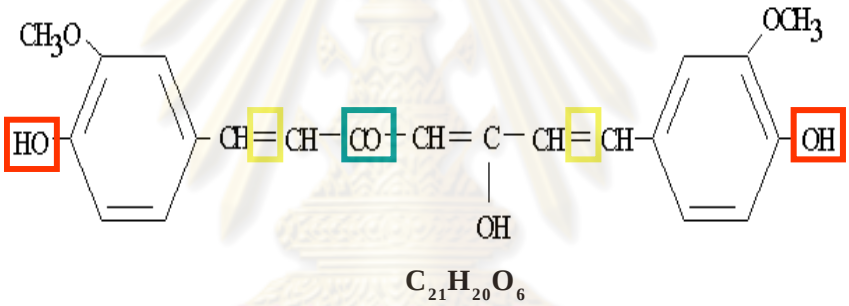

Fig. 3 Curcumin, polyphenolic compound, has two molecules of ferric acids and carbonyl groups which are called "diferuloylmethane" ($C_{21}H_{20}O_6$). This figure demonstrates the locations of hydroxyl groups, keto group, and double bonds.

while certain leukotrienes; LTB_4, LTC_4 and LTD_4, increase vessel permeability resulting in tissue swelling, which characterizes inflammation. Increased levels of some prostaglandins like PGE_2 produce redness, swelling and pain at the inflamed part of the body.

There are two isoforms of cyclooxygenase **(**COX) enzymes, namely COX-1 and COX-2. COX-1 is constitutively expressed in many tissues and is considered to be involved in various physiological functions, whereas COX-2 is induced by pathological stimuli, such as inflammation, various growth factors and cytokines produced by tumor cells [40, 41].

As shown in **Fig. 3**, keto groups and double bonds are believed to have the responsibility for curcumin anti-inflammatory properties. By using the chronic inflammation rat-model, Banerjee et al. (2003) showed that curcumin could reduce the increased levels of tumor necrosis factor-alpha and interleukin-1β [42].

Recently, by using adjuvant arthritis model mice, Tohda and his co-workers studied the inhibitory effects of *Curcuma* extracts on *in vitro* enzymatic activities measured against COX-2 and COX-1 [43]. The methanol extract of *Curcuma phaeocaulis* (CP, 500 μg/ml) significantly inhibited COX-2 activity (inhibition rate: 24.4 %). However, *Curcuma longa L.* (CL, 500 μg/ml) extract demonstrated no significant inhibition. For COX-1, both CP and CL extracts showed inhibitory activity, and the inhibition rate with the CP extract was the most remarkable (inhibition rate: 38.4 %). Indomethacin, a COX inhibitor, inhibited COX-2 (inhibition rate: 45.5 % inhibition at 100 μM) and COX-1 (inhibition rate: 37.2 % at 100 μM) activities dose dependently. The inhibitory efficacy of COX-2 with the methanol extract of CP was weaker than that of 100 μM indomethacin [43].

Curcumin and its cancer preventing activity

Turmeric extract and curcumin were also found to be cancer preventing compounds in different tumor models. Recently, curcumin has been considered a potentially important chemopreventive agent against cancer [42 44]. Animal studies have demonstrated that curcumin inhibits carcinogenesis in various sites, including skin [12], colorecum [17, 18], mouth [41], forestomach [17, 44] and breasts [45, 46]. The genetic changes in carcinogenesis in these organs involve different genes, but curcumin is effective in preventing carcinogenesis in several organs. Since angiogenesis (blood vessel formation) is essential for tumor growth and metastasis, it has been suggested that curcumin can inhibit several cancers through its potential role as anti-angiogenic agent.

There are different active sites within the chemical structure of curcumin. This could be a good reason why curcumin has been used for multiple purposes. Moreover, through its unique chemical structure, these multi-purposed actions of curcumin can also act simultaneously or/and sequentially. At this point, it may be worth to note that the actions of curcumin in anti-tumor, anti-oxidant and anti-inflammation might have "cross-talk" through their signalling pathways as shown in **Fig. 4**.

Anti-angiogenic effects of curcumin

Normally, the process of angiogenesis occurs as an orderly series of events: *(http://www.angio.org/understanding/understanding.html)*

1. Diseased or injured tissues produce and release angiogenic growth factors (proteins) that diffuse into the nearby tissues
2. The angiogenic growth factors bind to specific receptors located on endothelial cells (EC) of nearby preexisting blood vessels
3. Once growth factors bind to their receptors, the endothelial cells become activated. Signals are sent from the cell's surface to the nucleus. The endothelial cell's machinery begins to produce new molecules including enzymes
4. Enzymes dissolve tiny holes in the sheath-like covering (basement membrane) surrounding all existing blood vessels
5. The endothelial cells begin to divide (proliferate),

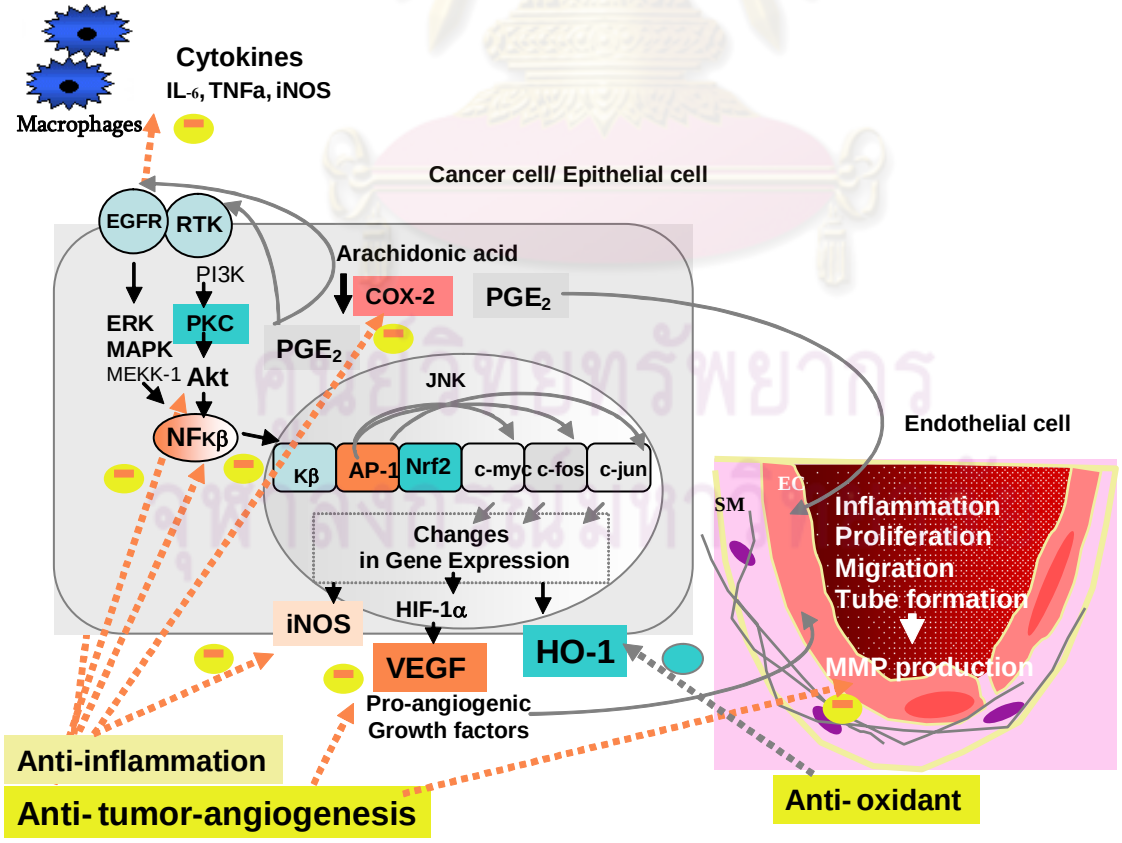

Fig. 4 Cell signalling pathways proposed for curcumin actions, including anti-tumor (phosphatidylinositol 3-kinase-Akt pathway [47], anti-oxidant (PKC-Nrf2-HO-1) [10], and anti-inflammation (3-kinase-Akt-NF-kβ-AP-1) [48].

and they migrate out through the dissolved holes of the existing vessel towards the diseased tissue (tumor)
6. Specialized molecules called adhesion molecules, or integrins (avb3, avb5) serve as grappling hooks to help pull the sprouting new blood vessel forward
7. Additional enzymes (matrix metalloproteinases or MMP) are produced to dissolve the tissue in front of the sprouting vessel tip in order to accommodate it. As the vessel extends, the tissue is remolded around the vessel
8. Sprouting endothelial cells roll up to form a blood vessel tube
9. Individual blood vessel tubes connect to form blood vessel loops that can circulate blood
10. Finally, newly formed blood vessel tubes are stabilized by specialized muscle cells (smooth muscle cells, pericytes) that provide structural support. Blood flow then begins.

Several possible mechanisms of the observed anti-angiogenic effects of curcumin have been examined. Arbiser and his co-workers [49] tested the molecule for its ability to inhibit the proliferation of primary endothelial cells with or without the presence of bFGF, a potent angiogenic factor. They assayed the ability of curcumin to inhibit proliferation of an immortalized endothelial cell line, and to inhibit phorbol ester-stimulated VEGF mRNA production. The capacity of curcumin to inhibit bFGF-induced corneal neovascularization in the mouse cornea was investigated. It was found that curcumin inhibited bFGF-induced proliferation of endothelial cells in vitro and angiogenesis in vivo. Thus, the ability of curcumin in inhibiting carcinogenesis in several organs may be mediated in part through angiogenesis inhibition. Although the exact mechanisms of action of curcumin remain to be elucidated, the inhibitory effects of curcumin on tumor angiogenesis may be mediated through two major biomarkers COX-2 and VEGF.

Cyclooxygenase-2: COX-2

One of the mechanisms by which COX-2 supports tumor growth is by inducing the angiogenesis necessary to supply oxygen and nutrients to tumors >2 mm in diameter [50, 51]. Current evidence indicates that COX-2 promotes tumor-specific angiogenesis [52-54], inhibits apoptosis [55, 56], and induces proangiogenic agents such as VEGF [57, 58], inducible nitric oxide synthase (iNOS) promoter [59], IL-6 [60], IL-8 [61], and Tie-2 [62]. Williams et al. (2000) [63] demonstrated that mice lacking the COX-2 gene had deficient production of VEGF by fibroblasts and treatment of wild-type fibroblasts with selective COX-2 inhibitor suppressed VEGF production. Over expression of COX-2 in colon cancer cells induced expression of VEGF and other angiogenic factors and this effect was inhibited by the COX-2-specific antagonist NS-398 [64]. Consistent with these findings, prostaglandins enhanced VEGF production in many different cells [65]. COX-2 is also involved in the regulation of VEGF-induced vascular permeability and endothelial cell proliferation [66, 67].

The molecular mechanism responsible for PGE_2-induced colorectal cancer cell migration and invasion is known to involve an epidermal growth factor receptor (EGFR)–phosphatidylinositol 3-kinase–Akt pathway [48]. However, the mechanisms by which PGE_2 modulates apoptosis are still largely unknown. One potential mechanism with regard to its regulation of programmed cell death is that PGE_2 reduces the basal apoptotic rate by increasing the level of antiapoptotic proteins such as BCL-2 [68] or other members of the BCL gene family, such as MCL-1. In addition, COX-independent effects of NSAID-induced apoptosis have also been reported [69]. In general, COX-2-derived PGE_2 suppresses immunosurveillance by down-regulating T and B cell proliferation, cytotoxic activity of natural killer cells, and cytokines such as IL-12 and tumor necrosis factor [69].

Vascular endothelial growth factor (VEGF)

VEGF has received attention as a target for therapeutic angiogenesis [70]. There is a correlation between tumors with higher densities of blood vessels and metastasis and poorer clinical outcome. Expressions of VEGF, as well as bFGF and PDGF, are associated with tumor growth, angiogenesis, and metastasis [70, 71]. The expression of VEGF correlates both temporally and spatially with the onset of neovascularization [70]. Elevations in VEGF levels have been detected in the serum of some cancer patients [72], and a correlation has been observed between VEGF expression and microvascular density in primary breast cancer sections [73]. A postoperative survey indicated that the relapse-free survival rate of patients with VEGF-poor tumors, suggesting that VEGF expression is associated with stimulation of angiogenesis and with early relapse in primary breast cancer. Moreover, there is compelling evidence that circulating VEGF levels are of prognostic significance

in a variety of tumor types [74-80]. 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 [81, 82]. These results are consistent with the hypothesis that angiogenesis is necessary for tumor growth, and that VEGF is a potent stimulator of the angiogenic response.

Taken together, these results indicated that both COX-2 and VEGF are crucial biomarkers for tumor angiogenesis.

How curcumin produces its anti-angiogenic effects

How curcumin produces its anti-angiogenesis is not fully understood, but it is believed that curcumin probably mediated in part through its effects on vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2).

By using intravital fluorescence video microscopy, the angiogenic process of inoculated human hepatocellular carcinoma cell lines (HepG2; obtained from the American Type Culture Collection (ATCC)) was studied [34-36]. HepG2 cell caused the changes of microvascular network of the skin-fold of nude mice as follows: 1) Three days after implantation, the characteristics of inflammatory processes, including arteriolar dilatation, tortuosity and hyperpermeability with extravasations of fluorescence tracer from the host existing microvessels, were observed (**Fig. 5**). 2) Percentage changes of tissue perfusion on day 7 and day 14 in HepG2 groups were significantly increased (43.2±9.5 % and 53.9 5.6 %, respectively). 3) On day 7, at the early stage of tumor angiogenesis onset, tumor neocapillaries were observed, in which the mother-host vessels underwent endothelial cell sprouting. Some of the proliferating neovessels appeared ready to migrate out, approaching to the tumor area (**Fig. 6**). 4) In HepG2 groups, the capillary density increased from 51.7±4.3 % (on day 7) to 69.2 ± 2.4 % (on day 14).

In our study, the Western blot and Enzyme Immunoassay (EIA) for COX-2 and Serum VEGF data demonstrated that COX-2 and VEGF strongly expressed on day 3 and 7, respectively, in HepG2 group and maintained throughout the experimental period (**Figs. 7.a, 7.b**). Moreover, the linear regression line of Y=22850x+23699 also implied that the expression of COX-2 was significantly correlated with tumor neogenesis. The more COX-2 expression, the more neocapillary density was obtained (r^2=0.9607, $p<0.01$) [36]. This finding, therefore, suggests that the over expression of COX-2 is functionally significant for tumor angiogenesis. In particular, during the initiation state of the angiogenic switch (day 3 in our findings), we thought that COX-2-derived PGE_2 was responsible for what we have observed as shown in **Fig. 5** including arteriolar dilatation, tortuosity and hyperpermeability with extravasations of fluorescence tracer from the host existing microvessels.

Interestingly, 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 post- inoculation significantly (**Figs. 8A-D**). Importantly, this inhibition effect of curcumin on tumor angiogenesis has been well correlated by its effect on COX-2 and VEGF inhibition (**Figs. 7a, 7b**). Even though the relationship between these inhibition effect. 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 ($p<0.001$).

In this study, anti-angiogenic activity of curcumin showed that curcumin inhibited COX-2 expression and VEGF production. It is likely that COX-2 is an upstream important mediator of the angiogenic pathway. COX-2 and VEGF may co-modulate angiogenesis in different pathways. The activity of curcumin in inhibiting angiogenesis may be mediated in part through reduction of angiogenic stimulators or biomarker production according to the mechanism proposed above (**Fig. 4**).

The diagram shown in **Fig. 9** is the proposed mechanism that we hypothesize [83]. The idea is that once a tumor grows to a certain size, the cells in the center are too far removed from existing blood vessels to receive the necessary nutrients for cell survival. Then the condition of “hypoxia”, lack of oxygen, occurs. 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 promoter region via a direct phosphorylation of hypoxia-inducible factor-1α (HIF-1α). Hypoxia induces VEGF expression via HIF-1/AP-1 pathway represent the “noninflammatory process”. In addition, hypoxia could influence many other transcriptional pathways, such as those mediated

by *fos* and *jun* [84] and NF-kB [85] 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 [86, 87]. However, COX-2 expressed via the inflammatory cells in the tumor microenvironment is also involved in the regulation of the angiogenic process. Inflammatory cells, particularly macrophage are important sources of pro-angiogenic factors like VEGF and COX-2. These tumor-associated macrophages in turn produce increased levels of VEGF [48, 88]. COX-2-derived PGE_2 could suppress immunosurveillance by down-regulating T and B cell proliferation and it also enhanced expression of VEGF. The up-regulation of both VEGF and COX-2 expression in HepG2-implanted nude mice may be responsible for switching and maintaining tumor angiogenesis. In other words, both COX-2 and VEGF may co-modulate tumor angiogenesis.

As shown in **Fig. 4**, the significance of curcumin multi-molecular targets mediated through those cell signalling pathways including: anti-tumor (phosphatidylinositol 3-kinase-Akt pathway, anti-oxidant (PKC-Nrf2-HO-1), and anti-inflammation (3-kinase-Akt-NF-Kβ-AP-1) might explain our findings for curcumin. However, further study is required to confirm that the anti-angiogenic effect of curcumin is mediated through these cell signaling pathways.

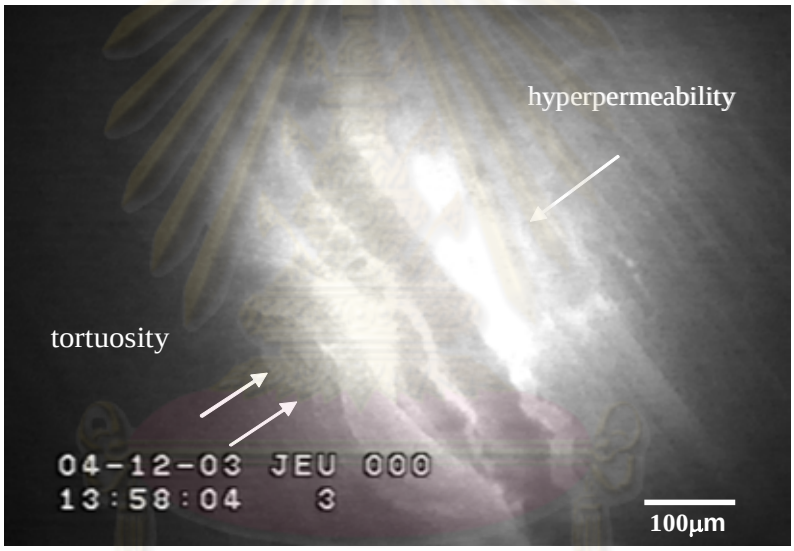

Fig. 5 Video image of 3-day HepG2 demonstrated tortuosity and hyperpermeability [34].

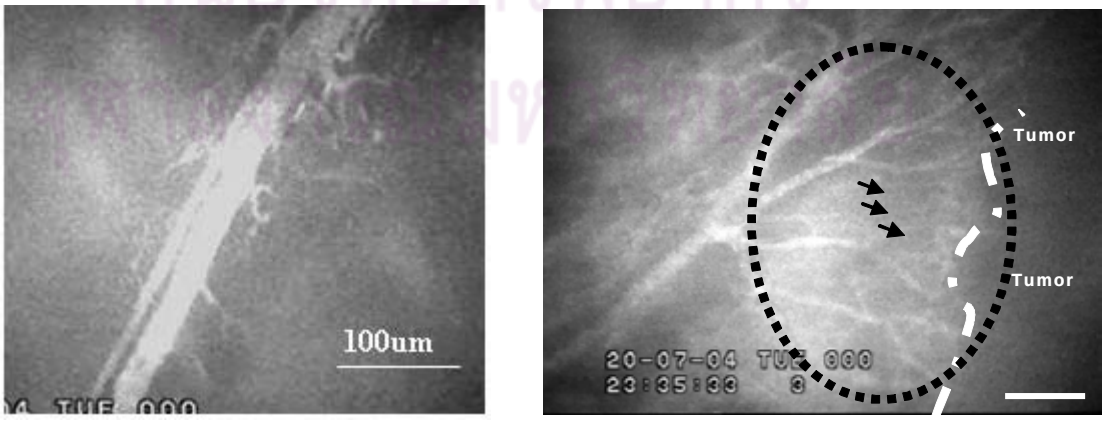

Fig. 6 The mother-host vessels underwent endothelial cell sprouting (**a**). Some of the proliferating neovessels appeared already to migrate out, approaching to the tumor area (**b**). Bar, 100 μm [34].

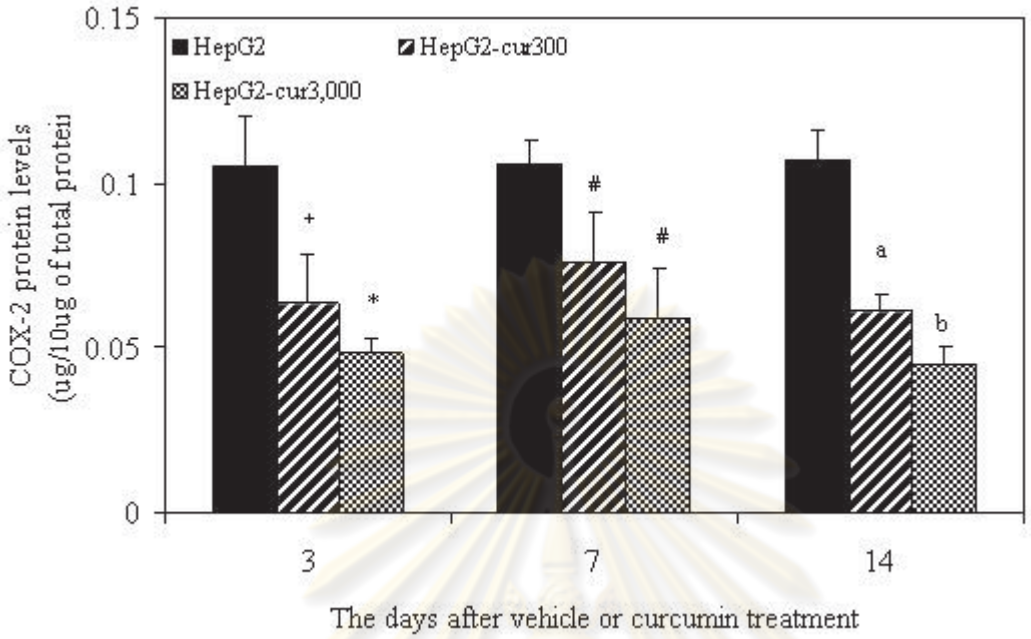

Fig. 7a Comparisons of COX-2 protein levels by using image analysis (Global Lab II software) measurement in 3, 7, and 14 days HepG2 group with and without curcumin treatment (means±SEM). $^{+}P<0.05$ significantly different compared to 3 days HepG2. $^{*}P<0.001$ significantly different compared to 3 days HepG2. $^{\#}P<0.005$ significantly different compared to 7 days HepG2. $^{a}P<0.01$ significantly different compared to 14 days HepG2; $^{b}P<0.001$ significantly different compared to 14 days HepG2 [36].

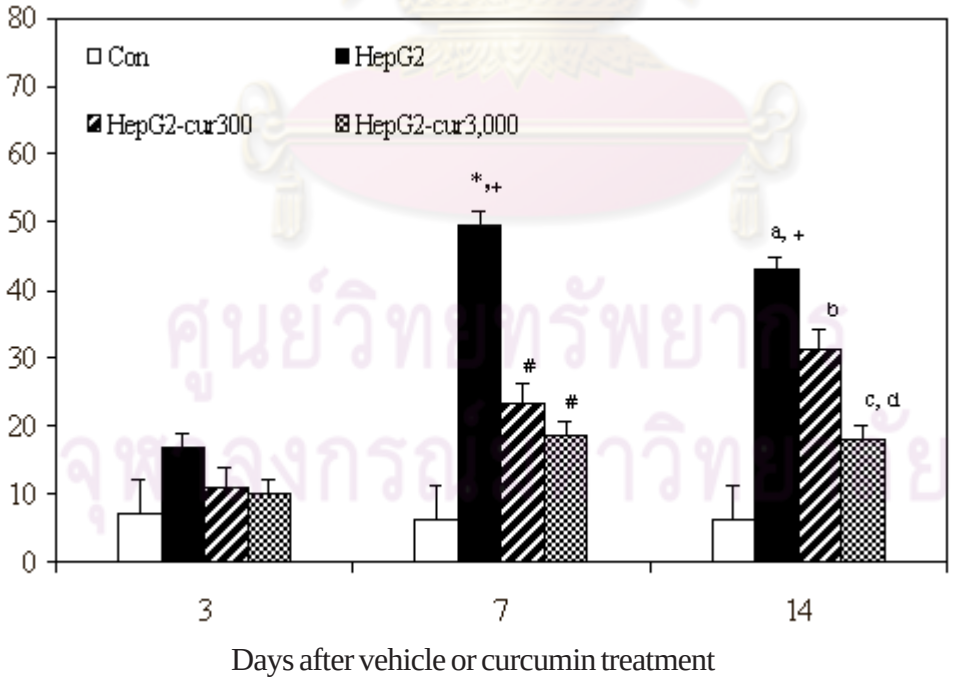

Fig. 7b Serum VEGF (ng/ml) of control and HepG2 groups after 3, 7 and 14 days with and without curcumin treatment (means ± SEM, all n=5). $^{*}P< 0.001$ significantly different compared to 7 days Con. $^{+}P<0.001$ significantly different compared to 3 days HepG2. $^{\#}P<0.001$ significantly different compared to 7 days HepG2. $^{a}P< 0.001$ significantly different compared to 14 days Con. $^{b}P< 0.01$ significantly different compared to 14 days HepG2. $^{c}P<0.001$ significantly different compared to 14 days HepG2. $^{d}P< 0.01$ significantly different compared to 14 days HepG2-cur300 [36].

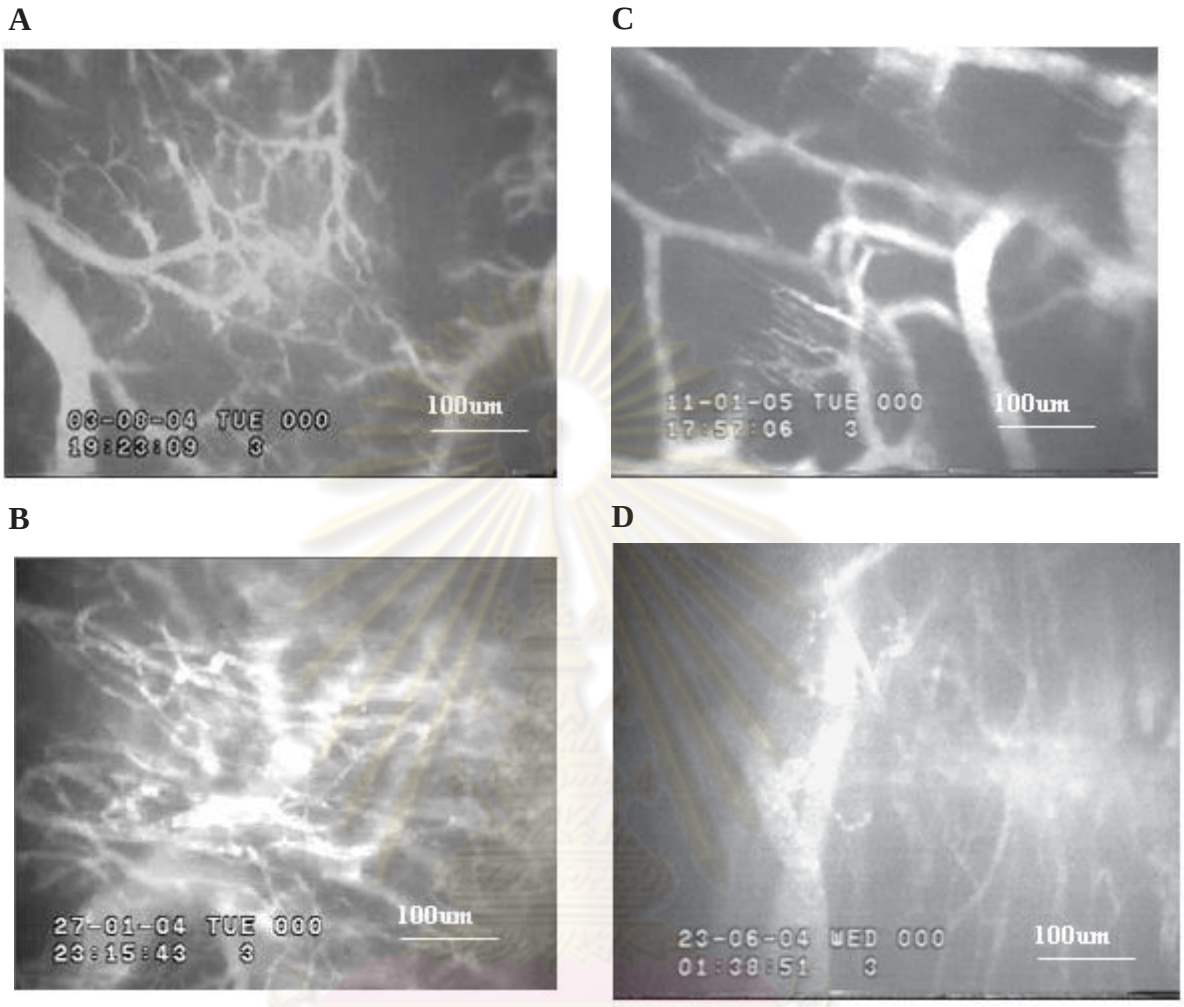

Fig. 8 (A-D) Fluorescence video images of neocapillaries on 7 (**A,C**) and 14 days (**B,D**) after the implantation of tumor cells without and with treatment of curcumin (3,000 mg/kgBW) for HepG2 (**A, B**) and HepG2-cur 3,000 groups (**C, D**). Bar, 100 μm [35].

Conclusion

We described possible explanations for potential chemotherapeutic activity of curcumin. By using intravital fluorescent videomicroscopic technique, the anti-angiogenic effect of curcumin was elucidated. We hypothesized the significance of curcumin on inhibiting the expression of two important proangiogenic factors, VEGF and COX-2. Importantly, the inhibition effect of curcumin on tumor-induced neovascularization was well correlated with its effect on COX-2 and VEGF inhibition. Therefore, these observations indicate that the anti-angiogenic effect of curcumin seems to be promising for future clinical utility.

List of abbreviations

AP-1= Activator protein-1,
ATCC=American Type Culture Collection,
COX=Cyclooxygenase,
EC=Endothelial cells,
EIA=Enzyme Immunoassay,
EGFR=Epidermal growth factor receptor,
HETE=Hydroxyeicosatetraenoic acids,
HIF-1α=Hypoxia-inducible factor-1α
HepG2=Human hepatocellular carcinoma cell,
HO-1=Heme oxygenase-1,
iNOS=Inducible nitric oxide synthase,
LT=Leukotrienes,
MMP=Matrix metalloproteinases,
PG=Prostaglandins,
TX=Thromboxanes,
VEGF=Vascular endothelial growth factor.

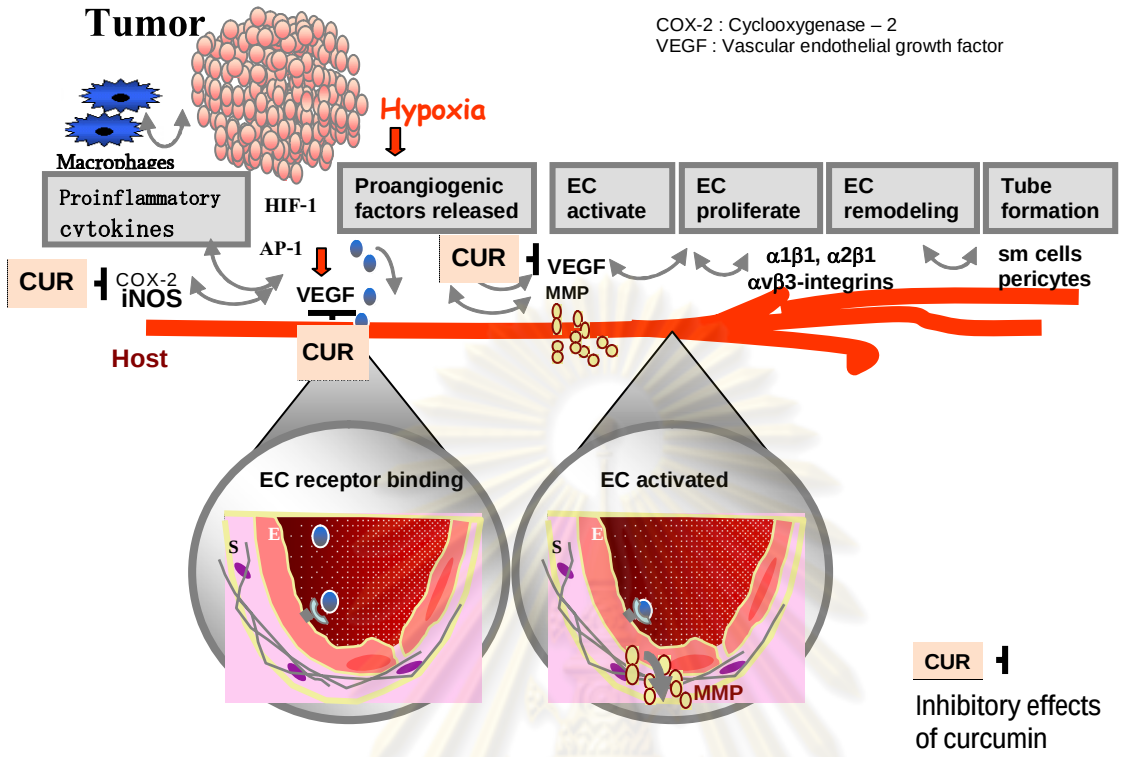

Fig. 9 The diagram demonstrates the mechanisms for curcumin on inhibiting tumor-angiogensis. Both COX-2 and VEGF behave as co-modulators for tumor angiogenesis through inflammation and non-inflammation processes. COX-2-derived PGE_2 acted as a key mediator of inflammation was suggested as a key regulator in promotion of these initiated cells, possibly by providing them with proliferating signals and by preventing apoptosis. Hypoxia induces VEGF expression via HIF-1/AP-1 pathway represent the non-inflammatory process. Consequently, this non-inflammatory factor, VEGF, in tumor cells will induce matrix metalloproteinase (MMP) expression in endothelial cells, leading to endothelial cell proliferation, tube formation and finally tumor cell invasion. Because curcumin can inhibit both major tumor-biomarkers, VEGF and COX-2, expression, therefore, it could inhibit tumor angiogenesis and tumor proliferation, respectively [83].

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The authors have no conflict of interest to declare.

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