CURCUMIN AS AN INHIBITOR OF ANGIOGENESIS

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Abstract: Angiogenesis, the formation of new blood vessels from host vasculature, is critical for tumor growth and metastases. Curcumin, a novel small-molecular-weight compound, has been shown to inhibit carcinogenesis in different organs and the common link between these actions is its antiangiogenic effect. Curcumin is a direct inhibitor of angiogenesis and also downregulates various proangiogenic proteins like vascular endothelial growth factor and basic fibroblast growth factor. Curcumin's antiangiogenic effect is also in part due to its inhibitory effect on signal transduction pathways, including those involving protein kinase C and the transcription factors NF-κB and AP-1. Curcumin has an inhibitory effect on two groups of proteinases involved in angiogenesis that are the members of the matrix metalloproteinase family and the urokinase plasminogen activator family. Cell adhesion molecules are upregulated in active angiogenesis and curcumin can block this effect, adding further dimensions to curcumin's antiangiogenic effect. Curcumin shows a dose-dependent inhibition on tumor necrosis factor, a versatile cytokine, which has its effect on angiogenesis through the signal transduction pathways, expression of proangiogenic factors, and cell adhesion molecules. Curcumin's effect on the overall process of angiogenesis compounds its enormous potential as an antiangiogenic drug.

1. INTRODUCTION

Angiogenesis, the growth of new capillary blood vessels, is crucial for tumor growth and expansion.[1,2] Tumors require a constant supply of oxygen and nutrients, and diffusion from nearby capillaries can supply adequate nutrition for tumors less than 2 mm^2, but for continued growth, tumors must develop their own blood supply.[3] Tumor masses acquire the ability to produce proangiogenic factors that stimulate the growth of host blood vessels.[4] The acquisition of the proangiogenic factors is mediated by a switch to an angiogenic phenotype that induces angiogenesis and allows rapid expansion of tumor growth.[5,6] Angiogenic tumors also produce positive regulators of angiogenesis and mobilize angiogenic promoters from the extracellular matrix. Antiangiogenic therapy can therefore interfere with any or all of these mechanisms and prevent tumor cells from developing a viable blood supply.[3]

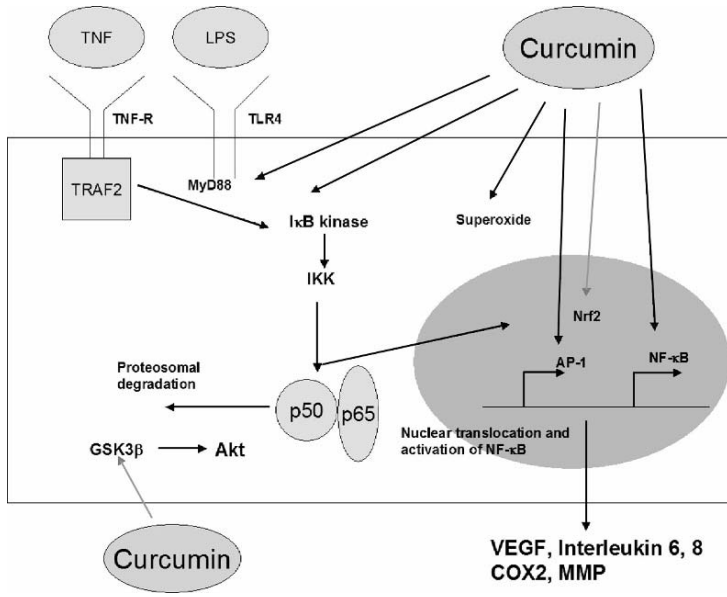

Figure 1. Suppression of angiogenesis pathway by curcumin.

Angiogenesis inhibitors can be divided into two classes. The first class, or direct angiogenesis inhibitors, are relatively specific for endothelial cells and have little effect on tumor cells. Indirect inhibitors might not have direct effects on endothelial cells, but they downregulate the production of angiogenesis stimulators. Curcumin has been shown by Arbiser et al.[7] to be a direct inhibitor of angiogenesis and also plays an important role in the downregulation of proangiogenic proteins (Figure 1). Curcumin's antiangiogenic effect is also in part due to its inhibitory effect on the production of cytokines relevant to tumor growth such as tumor necrosis factor and its antiapoptotic effect: its inhibitory effect on endothelial cell attachment, motility and proliferation.

2. CURCUMIN: INHIBITIOR OF PROANGIOGENIC PROTEINS

The angiogenic switch, which is essential for angiogenesis, is mediated by angiogenic oncogenes,[8] which upregulate the expression of proangiogenic proteins such as VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) and reduce the expression of angiogenesis inhibitors.[2,9] Among the proangiogenic proteins, VEGF and bFGF are crucial factors in pathological angiogenesis.[7,10] VEGF-A is considered the most important of the five isoforms. VEGF mediates angiogenic signals through its VEGFR-1, -2, and -3 receptors, which initiate signaling events that begins with dimerization and transautophosphorylation of TK residues in the receptors, which, in turn, activate phospholipase C-γ, phospho-inositide 3 (PI3) kinase (PI3-K), GTPase activating

protein (GAP), mitogen-activated protein kinase (MAPK), and others.[10] Cui et al. showed that VEGF secretion by U937 and Raji cells is increased by tumor necrosis factor-α (TNF-α) treatment and suppressed by curcumin treatment.[11] TNF-α augments the expression of VEGF165 and VEGF121 mRNA and curcumin reduces the expression. Angiogenesis was tested by network formation of endothelial cells on Matrigel and no networks or cords formed in control and curcumin groups and there was tube formation on matrigel in the supernatants of the Raji culture group and the supernatants groups treated by the VEGF group and TNF-α in Raji cells. The study concluded that expressions of VEGF mRNA in U937 and Raji cells were increased by TNF-α and suppressed by curcumin. VEGF and TNF-α can induce angiogenesis, and curcumin can inhibit angiogenesis in ECV304 cells and can, therefore, inhibit potential mechanisms controlling tumor neovascularization.

Overexpression of VEGF and cyclooxygenase-2 (COX-2) has been demonstrated in the HepG2 cell line (hepatocellular carcinoma cell line).[12,13] COX-2 supports tumor angiogenesis both directly and indirectly, as shown by Millianta et al.[14] and directly stimulates the production of angiogenic factors from tumor cells. Antiangiogenic activity of curcumin is further compounded by its ability to reduce the tumor-induced overexpression of VEGF and COX-2 as, shown by Yoysungnoen et al.[15] HepG2 cells were inoculated onto the upper layer of the skin-fold chamber and curcumin solutions were orally fed to the HepG2 cell-implanted nude mice. The tumor neocapillary density (NCD) was evaluated using a digital image analysis and demonstrated the NCD of HepG2-groups were significantly increased on day 7 and 14, compared to the aged-matched controls and this was attenuated by daily treatment of curcumin solution (3000 mg/kg BW). Curcumin treatment thereby inhibits tumor angiogenesis by reduction of angiogenic biomarkers such as VEGF and COX-2 and this inhibition also occurs with liposomal curcumin as shown by Li et al.[16]through attenuation of the NF-κB mechanism.

Basic fibroblast growth factor (bFGF) is another potent angiogenic factor and stimulates both endothelial proliferation and migration. The activity of bFGF on endothelial cells might be in part through stimulation of protein kinase C.[7,17] Curcumin and its analogues results in potent inhibition of bFGF-induced corneal neovascularization assessed by measuring vessel length and density in the normally avascular cornea. Intraperitoneal administration of curcumin at doses up to 300 mg/kg BW did not inhibit corneal neovascularization, which might be due to the well-known rapid metabolism of curcumin. Curcumin inhibits the proliferation of primary endothelial cells in the presence and absence of bFGF and also inhibits proliferation of an immortalized endothelial cell line, as shown by Arbiser et al.[7]

3. EFFECT ON ENDOTHELIAL CELL MIGRATION AND INVASION

The role of endothelial cell migration is a crucial step in angiogenesis. The effect of curcumin on endothelial cell migration, attachment, and tube formation was studied on Matrigel by Aggarwal and Natarajan.[18] Curcumin had no effect on

endothelial cell migration or attachment to either plastic of Matrigel, but caused a dose-dependent inhibition of tube formation when the cells were treated before plating or at the time of plating on Matrigel. Curcumin treatment inhibited angiogenesis in a subcutaneous Matrigel plug model in mice and caused the preformed tubes to break down.

During angiogenesis, extracellular proteolysis has been implicated in different steps such as provisional matrix remodeling, basement membrane degradation, cell migration, and invasion.[19–21] The group of proteinases involved in extracellular matrix (ECM) remodeling comprises four different families based on the nature of the chemical group responsible for catalytic activity: the serine, cysteine, aspartic, and metalloproteinases.[22] Curcumin's antiangiogenic property is due in part by its inhibitory action on metalloproteinases and the serine proteinase family, the urokinase plasminogen activator system (uPA). uPA interacts with a specific receptor (uPAR) via the epidermal growth factor (EGF)-like domain in the urokinase amino-terminal fragment (ATF).[23] Its angiogenic effect is due to its effect on the migration of endothelial cells and through the activation and/or release of several angiogenic factors such bFGF, transforming growth factor (TGF), TNF, hepatocyte growth factor (HGF), and VEGF.

In mouse keratinocytes, uPA expression/secretion is increased by TGF-β1. Curcumin decreases the uPA levels induced by TGF-β1 in transformed keratinocytes; inhibits the TGF-β-induced synthesis of fibronectin, an early response gene to the growth factor; and reduces TGF-β-stimulated cell migration and invasiveness.[24,25] Curcumin also inhibits EGF-stimulated urokinase production, although not statistically significant. Curcumin modulates the EGF-stimulated uPA production, which involves the activation of the extracellular signal-regulated kinases 1/2 and JNK signaling pathways and also inhibits the phosphorylation of the EGF receptor.[26] In another study by Parra et al.,[27] uPA induced by N-methyl-N-nitro-N-nitrosoguanidine (MNNG) was inhibited by curcumin. Curcumin acted at the (1) AP-1 binding to the uPA enhancer element, (2) uPA transcriptional activity, and (3) uPA mRNA expression to abrogate the uPA secretion. The multifunctional properties render the uPA–uPAR system an attractive target for curcumin as antiangiogenic therapy.

The other family of proteinases involved in ECM remodeling is the metalloproteinases. Endothelial cell attachment to the extracellular matrix, detachment, and migration/invasion are functions of matrix metalloproteinases (MMPs), which have been clearly implicated in angiogenesis by Collins et al.[28] and Stetler-Stevenson et al.[29]Gelatinase A (MMP-2) and gelatinase B (MMP-9) are metalloproteinases that cause the formation of new capillaries by activating growth factors and it was shown that curcumin inhibits the gelatinolytic activities of secreted 53- and 72-kDa MMP and suppresses the expression and transcription of the 72-kDa MMP, indicating its inhibitory effect at both the transcriptional and posttranscriptional level. Gelatinase-B expression is induced by the transcription factor AP-1, which, in turn, is regulated by FGF-2 and this expression is inhibited by curcuminoids. Using corneal implantation pellets, it has been shown that the FGF-2 pellet was inhibited by coimplantation of curcuminoid pellet and this

correlates with the inhibition of endogenous gelatinase-B expression. These results provide evidence that curcuminoids inhibit expression of gelatinase-B and target the FGF-2 angiogenic signaling pathway and that curcumin acts as an angiogenesis inhibitor by modulating MMPs.[24]

4. EFFECT ON ADHESION MOLECULES

Cell adhesion molecules play a determining role in tumor metastasis and curcumin can downregulate their expression. Adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1, also called CD54), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1, also called E-selectin) are induced by TNF.[30–32] ICAM-1, VCAM-1, and ELAM-1 are 95-kDa, 110-kDa, and 115-kDa proteins, respectively, and are expressed in endothelial cells. Treatment of endothelial cells with TNF augmented the adhesion of monocytes to endothelial cells and this adhesion was due to increased expression of ICAM-1, VCAM-1, and ELAM-1 in the endothelial cell. Curcumin completely blocked the adhesion of monocytes[32] with endothelial cells as well as the cell surface expression of ICAM-1, VCAM-1, and ELAM-1. TNF-induced expression of adhesion molecules on human umbilical vein endothelial cells has been reported by others.[31] Although curcumin inhibited adhesion even when administered 1 h after TNF treatment, maximum inhibition occurred when added either 1 h before or at the same time as TNF. The downregulation of adhesion molecules by curcumin might contribute to its anticancer properties.

These properties of curcumin were further studied by Aggarwal et al.[33] and showed that curcumin also acts through the TNF-induced NF-κB-dependent pathway. VEGF, MMP-9, and ICAM-1 are regulated by NF-κB, and Western blot analysis revealed that curcumin blocked TNF-induced VEGF, ICAM-1, and MMP-9 protein expression in a time-dependent manner. These results suggest that curcumin plays a role in suppressing angiogenesis and metastasis through various different pathways.

5. EFFECT ON APOPTOSIS OF MELANOMA CELLS

The NF-κB transcription factor plays a central role in the pathogenesis of melanoma.[34] NF-κB activity is inhibited in part under conditions in which melanoma cells undergo apoptosis and previous reports have described NF-κB inhibition with exposure to higher concentrations of curcumin (60 μM) for shorter periods (6 h) in melanoma cell lines.[35] Therefore, the NF-κB machinery is suppressed both by short exposures to high concentrations of curcumin and by longer exposures to lower concentrations of curcumin. Liposomal curcumin was also shown by Li and Kurzrock[16] to have an inhibitory effect on NF-κB. Under apoptosis-inducing conditions, IKK, the upstream regulator of NF-κB, is inhibited strongly by curcumin. Partial inhibition of NF-κB but strong inhibition of IKK by curcumin suggests that, in melanoma cells, signaling molecules other than IKK can

regulate NF-κB activity. The ERK1/2-mediated pathway and an Akt-mediated pathway contribute to NF-κB activation independent of IKK[36,37] and play a role in melanoma cell proliferation or survival,[38–40] but under apoptosis-inducing conditions, neither the B-Raf/ERK pathway nor the Akt pathway was inhibited by curcumin. This shows that curcumin's proapoptotic acivity is associated with inhibition of the IKK/NF-κB transcriptional machinery, but not the B-Raf/ERK or Akt pathways, which implies that suppression of the viability of melanoma cells can occur despite the continued activation of the B-Raf/MEK/ERK and Akt pathways.[34] Interleukin (IL)-8, a pleiotropic chemokine that previously has been shown by Hoffmann et al.[41] to play a role in the promotion of malignant cell proliferation as well as in angiogenesis, is regulated in part by NF-κB, and although curcumin can cause NF-κB inhibition, it has surprisingly increased IL-8 levels in the high-secreting IL-8 melanoma cell lines. Therefore, it appears that IL-8 secretion is independent of curcumin-induced NF-κB inhibition and this might be due to IL-8 expression is regulated transcriptionally by AP-1 and C/EBP as well as NF-κB.[42] Curcumin also represses TNF-induced NF-κB-dependent antiapoptotic gene products. NF-κB regulates the expression of antiapoptotic proteins IAP1/2, XIAP, Bcl-2, Bcl-xL, Bfl-1/A1, and FLIP induced by TNF. Also, curcumin was shown to block the expression of these TNF-induced antiapoptotic proteins as well.[33]

6. EFFECT ON CELL MOTILITY AND PROLIFERATION

Cell motility is essential for a wide range of cellular activities, including angiogenesis.[24] In the highly invasive SK-Hep-1 cell line of human hepatocellular carcinoma (HCC), an *in vitro* assay, without or with the Matrigel matrix, was used to quantitate cellular migration and invasion. Curcumin, at 10 μM, inhibited cellular migration and invasion of SK-Hep-1. This cell line also showed a higher secretion of MMP-9, which was inhibited by curcumin in a dose-dependent fashion. Curcumin, therefore, has a significant anti-invasion activity in SK-Hep-1 cells, and this effect is associated with its inhibitory action on MMP-9 secretion.[43]

Ras has been implicated as a direct regulator of endothelial cell differentiation and microinjection of oncogenic H-Ras proteins into endothelial cells stimulates random motility.[44,45] Ras functions upstream of MAPK families, which include extracellular-signal regulated kinase (ERK) and c-Jun N-terminal kinase (JNK). JNK's role in endothelial cell motility has been proven by using a stable transfectant (DAR-ECV) of ECV304 endothelial cells expressing previously established oncogenic H-Ras. DAR-ECV cells showed a twofold increase in angiogenic potential compared to ECV-304 cells. Pretreatment with curcumin decreased the basal motility of DAR-ECV cells in a dose-dependent manner and suppressed the motility stimulated by known JNK agonists such as TNF-α and anisomycin. These results suggest that curcumin has an inhibitory effect on the

Ras-SEK-1-JNK pathway, which regulates the motility of endothelial cells during angiogenesis.[45]

7. EFFECT ON CYCLIN D1

Cyclin D1 is a proto-oncogene that is overexpressed as a result of the amplification or translocation in many cancers, including the breast, esophagus, lung, liver, head and neck, colon, and prostate.[24] Curcumin treatment of prostate cancer, breast cancer, and multiple myeloma cell lines correlates with the downregulation of cyclin D1 protein.[46,47] The suppression of cyclin D1 by curcumin led to inhibition of CDK-4-mediated phosphorylation of retinoblastoma protein. Curcumin-induced downregulation of cyclin D1 was inhibited by lactacystin, an inhibitor of 26S proteosome, suggesting that curcumin represses cyclin D1 expression by promoting proteolysis. Curcumin also downregulated mRNA expression and inhibited the activity of cyclin D1 promoter-dependent reporter gene expression. Thus, curcumin downregulates cylclin D1 expression through the activation of both transcriptional and posttranscriptional mechanisms, and this might contribute to the antiproliferative effects of curcumin.[24]

The precise molecular target of curcumin remains unknown. Curcumin and curcumin derivatives are an attractive pharmacophore because curcumin affects many targets, making resistance to curcumin less likely. A novel target of curcumin is the cop9 signalosome, which is a multiunit protein (at least eight units to date) that is involved in the proteolytic degradation of p53. Mdm2, the natural antagonist of p53, and an ubiquitin ligase, targets p53 for cop9/26S proteasomal-mediated degradation of p53. Proteasome inhibition has become an attractive strategy for tumor therapy, with velcade, and an inhibitor of the 26S proteasome, becoming first-line therapy for multiple myeloma. Curcumin has been found to inhibit the function of the cop9 signalosome. Inhibition of the cop9 signalosome has far-reaching effects on the angiogenic switch. p53 levels are upregulated, and p53 has been shown to be a negative regulator of VEGF. Inhibition of the cop9 signalosome also leads to downregulation of the angiogenic transcription factors Id1 and Id3. Mice deficient in Id1 and Id3 have a decreased ability to accept tumor xenografts because these mice have a decreased number of bone-marrow-derived endothelial stem cells.

The cop9 signalosome is regulated by several proteins, including casein kinase 2 and inositol 1,3,4-trisphosphate 5/6-kinase (5/6-kinase). Inositol 1,3,4-trisphosphate 5/6-kinase (5/6-kinase) phosphorylates many of the same substrates, such as Ikb, as does cop9, and it is possible that the 5/6 kinase provides some of the specificity of the cop9 signalosome and, like the cop9 signalosome, is inhibited by curcumin. Intriguingly, inositol 1,3,4-trisphosphate 5/6-kinase prevents apoptosis due to TNF-α, which might thus allow survival and hypertrophy of tissue under inflammatory conditions, a phenomenon that can be readily appreciated in inflammatory conditions such as psoriasis, rheumatoid arthritis, and inflammatory bowel disease.[48] Topical curcumin has demonstrated efficacy in psoriasis, a

TNF-mediated inflammatory disorder, as well as cutaneous metastases of internal tumors.

8. SUMMARY

Curcumin affects the overall process of angiogenesis by its downregulation of transcription factors such as NF-κB, proangiogenic factors such as VEGF, bFGF, and COX-2; inhibition of cell motility, cellular adhesion molecules, endothelial cell migration, invasion, and extracellular proteolysis. It also has antiproliferative and proapoptotic effects on tumor cells. All of these studies and the lack of toxicity of curcumin point toward curcumin's enormous potential as an antiangiogenic drug.

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