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Review

Molecular Mechanisms of Anti-metastatic Activity of Curcumin

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Abstract. *Cancer is the leading cause of death worldwide. Although cancer occurs as a localized disease, its morbidity and mortality rates remain high due to the ability of cancer cells to break-off from the primary tumor and spread to distant organs. Currently, chemotherapy is the main treatment for cancer; however, the increase in proportion of drug-resistant cancer cells and unpleasant side-effects of chemotherapy are still the major challenges in cancer therapy. Curcumin is a natural polyphenol compound and the main bioactive constituent of Indian spice turmeric, widely used in Indian and Chinese medicines. Curcumin has well-known therapeutic actions, including anti-inflammatory, anti-microbial, anti-oxidant and anti-cancer properties. Curcumin induces cancer cell apoptosis through regulating various signaling pathways and arresting tumor cell cycle. Curcumin's therapeutic/ preventative actions on metastatic cancers have not been yet fully understood and studied. The present review explores the potential anti-metastatic mechanisms of curcumin, including inhibition of transcription factors and their signaling pathways (e.g., NF-κB, ApP-1 and STAT3), inflammatory cytokines (e.g., CXCL1, CXCL2, IL-6, IL-8), multiple proteases (e.g., uPA, MMPs), multiple protein kinases (e.g., MAPKs, FAK), regulation of miRNAs (e.g., miR21, miR181b) and heat shock proteins (HLJ1). In addition, possible synergistic actions of combination therapy of curcumin with current chemotherapies are discussed in this review.*

Despite all recent advances in oncology, cancer is still one of the deadliest diseases around the world (1). Cancer occurs as a localized disease but can spread to distant organs through migration, invasion and metastasis (2). Metastatic cancer is one of the major causes of death in cancer patients. Metastasis is a complex process involving multiple steps: (i) local migration through degrading basement membrane and extracellular matrix (ECM), (ii) intravasation into blood and/or lymphatic vessels, (iii) circulating to the target organ site, (iv) extravasation into target organ tissue and, finally, (v) multiplication in the target organ (2, 3). These steps are mediated by various factors, including growth factors, proteolysis degradation of extracellular matrix, cell-cell adhesion, cytoskeleton remodeling and changes of genes' expressions (2, 3). Metastasis is a non-random process that means each metastatic cancer type has its own preferred site of metastasis. For example, breast cancer cells preferentially metastasize to regional lymph nodes, liver, lungs and bone (4). Nowadays, there are different therapeutic approaches available for patients with metastatic cancers, including surgery, radiotherapy and chemotherapy. Chemotherapy remains the main treatment modality for cancer patients because of its ability of preventing invasion and metastasis. However, the morbidity and mortality rates in patients with metastatic cancer still remain high since current chemotherapy agents fail to selectively and effectively kill cancer cells without destroying normal cells at the sites of metastasis (5). Therefore, finding a safe and effective preventative approach to inhibit invasion of cancer cells from primary tumor and, thereby, prevent metastatic process in the first place is a critical step to cure the disease or increase life expectancy of cancer patients.

Curcumin (diferuloylmethane), the main biologically active compound of Indian spice turmeric, is a polyphenol compound (Figure 1) derived from the roots of *Curcuma longa*. Turmeric commonly used as a dietary spice and coloring agent in different countries and has been widely used as a therapeutic and preventive agent for various illnesses and medical conditions. Curcumin has well-known biological activities that include anti-inflammatory, anti-oxidant, anti-microbial, wound healing and anti-cancer properties (6-10). Studies have shown that curcumin is able

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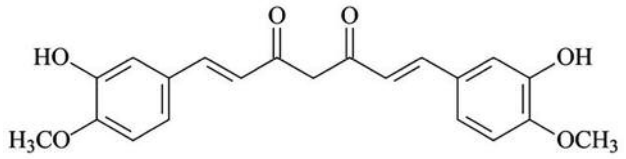

Figure 1. *Chemical structure of curcumin.*

to interfere with tumor cell cycle, thereby suppressing the growth of cancer cells and preventing invasion and metastasis (8, 10-13). Curcumin also has the ability to inhibit invasion and metastasis of cancer cells through regulating the expression of inflammatory cytokines, growth factors, growth factors' receptors, enzymes, adhesion molecules, apoptosis-related proteins and cell cycle proteins (10, 13-16). Curcumin modulates the activity of oncogenes, tumor suppressor genes, several transcription factors and their signaling pathways (10, 13, 15, 17).

In recent years, anti-cancer properties of curcumin have attracted attention in medicine. There exist many studies that have shown curcumin is able to suppress the growth of cancer cells by various molecular mechanisms, but mainly through apoptosis. As metastasis is one of the major factors contributing to poor cancer survival rate, there are many studies that looked into the anti-invasive and anti-metastatic effects of curcumin in various cancers. This review will look into the effects of curcumin on different signaling pathways and explore the possible underlying anti-invasion and anti-metastasis mechanisms of curcumin on cancer cells. Table I summarizes various molecular mechanisms and signaling pathways through which curcumin inhibits cancer cell migration, invasion and metastasis.

Potential Anti-migration, Anti-invasion and Anti-colony Formation Mechanisms of Curcumin on Cancer Cells

Curcumin inhibits nuclear factor-kappa B (NF-κB) activation. NF-κB is the one of the primary transcription factors to regulate genes that control cell proliferation and survival (18). Although the activation of NF-κB signaling pathway is important for immune system to fight against infections, the constitutive activation of NF-κB (Figure 2) will mediate carcinogenesis by suppressing apoptosis and leading to progression of cancers. NF-κB-regulated gene products that control tumor invasion include matrix metalloprotinases (MMP), urokinase-type plasminogen activator (uPA), interlukin-8 (IL-8) and other chemokines (18, 19). Once NF-κB is activated, for example by bacterial or viral infections, it activates IκB kinase (IKK) enzymes, that are responsible to phosphorylate and degrade IκBα (inhibitor of κB, alpha). IκBα is one of the inhibitors of κB (IκB) protein family, which inhibits the NF-κB activation by keeping them sequestered in an inactive state in the cytoplasm and by blocking their ability to bind to DNA (20, 21). Increasing IKK activation, promotes phosphorylation and, thereby, degradation of IκBα, leading to a constitutive activation of NF-κB. However, NF-κB activation also turns on the expression of genes that result in synthesizing of new IκBα, which then re-inhibits NF-κB and forms an auto-feedback loop to stop further NF-κB activation (22). Therefore, the inhibition of NF-κB transcription factor is one of the main targets for preventing cancer progression. Studies have also reported that NF-κB activation is one of the mechanism for chemotherapy resistance in cancer cells (23) and that the inhibition of NF-κB pathway can increase the efficacy of cancer therapy.

Cancer tumor promoter molecules, such as phorbol ester (12-O-tetradecanoylphorbol-13-acetate is the most common one), tumor necrosis factor (TNF) and hydrogen peroxide induce the activation of NF-κB (17, 24) and several works have shown curcumin can inhibit this activation. Bachmeier *et al.* (25) and Killian *et al.* (26) reported that curcumin prevents NF-κB activation by inhibiting phosphorylation and degradation of IκBα. Curcumin is also shown to inhibit the activation of p65 (one of the NF-κB transcription factor family) in estrogen receptor (ER)-negative breast cancer cells (25) and in prostate cancer cells (26). Singh *et al.* showed that curcumin inhibits NF-κB activation pathway in the signal transduction cascade of NF-κB activation before the IκBα phosphorylation but after the point at which signals transduced by various stimuli converge (27). Zong *et al.* showed that curcumin is an inhibitor of NF-κB in a dose-dependent manner in a breast cancer cell line (11). Inhibiting or down-regulating NF-κB activation by curcumin leads to down-regulation of the expression of various proliferative genes and induction of apoptosis, therefore, preventing tumor cells' invasion and metastasis (Figure 3).

Curcumin inhibits activator protein 1 (AP-1) activation. AP-1 is another important transcription factor that regulates the expression of different genes affecting cellular processes, such as proliferation and apoptosis. Activation of AP-1 complex in different cancer cells is regulated, at least in part, by activation of stress-activated kinase C-Jun N-terminal kinase (JNK) that stimulates the transactivating potency of AP-1 activation, thereby increasing the expression of AP-1 target genes (24, 28). It has been shown that curcumin inhibits the activation of AP-1 by interacting with the AP-1 DNA-binding motif and inhibits JNK activation (24). Curcumin inhibits the activation of AP-1 in colorectal (29) and breast cancer cells (14). Wang *et al.* reported that neurotensin increased AP-1 DNA-binding activity and curcumin inhibited the neurotensin-mediated AP-1 activation in a colon cancer cell line (30).

Table I. *Summary of potential underlying molecular mechanisms of curcumin to inhibit cancer migration, invasion and metastasis.*

Mechanism of actions	*In vitro*/*In vivo*	Outcomes/effects	Ref.
Nuclear factor-kappa B (NF-κB) activation	*In vitro*	Curcumin inhibits NF-κB activation in a dose-dependent manner	(11, 17, 24-26)
Activator protein 1 (AP-1) activation	*In vitro*	Curcumin inhibits AP-1 activation	(14, 30)
Signal transducer and activator of transcription 3 (STAT3)	*In vitro*	Curcumin inhibits STAT3 phosphorylation	(16, 24)
Inflammatory cytokines' expression	*In vitro* *In vivo* (mice)	Curcumin suppresses CXCL-1, -2 expression and protein secretion	(25, 26)
Interleukin expression	*In vitro*	Curcumin inhibits neurotensin-induced *IL-8* gene expression and protein secretion in a dose-dependent manner	(30)
Urokinase plasminogen activator (uPA) expression	*In vitro*	Curcumin suppresses uPA expression in a dose-dependent manner	(11)
Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases' (TIMPs) expression	*In vitro* *In vivo* (mice)	Curcumin suppresses MMP-2, -9 expression and enhances TIMP-1, -2, -4 expressions	(12-14, 42, 44-47)
Focal adhesion kinase (FAK) activity	*In vitro* *In vivo* (mice)	Curcumin suppresses FAK activity	(49, 50)
MicroRNAs (miRNAs) expression	*In vitro* *In vivo* (chicken and mice)	Curcumin suppresses miR21 expression and enhances miR181b expression	(51, 53)
DnaJ-like heat shock protein 40 (HLJ1) expression	*In vitro* *In vivo* (mice)	Curcumin up-regulates HLJ1	(54)

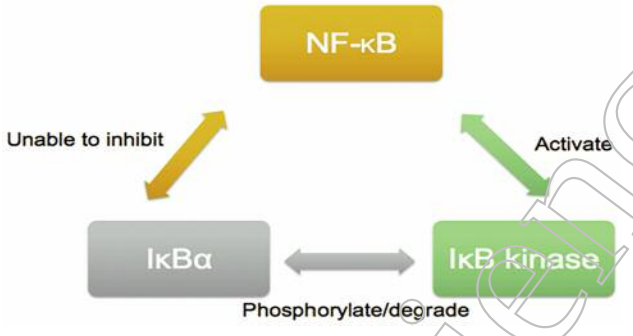

Figure 2. *Constitutive activation of NF-κB signaling pathway. The activation of NF-κB (e.g., induced by viral infection) is initiated by degradation of IκB (inhibitor of κB) proteins: NF-κB activates IκB kinase (IKK) enzymes that phosphorylate and degrade IκBα. Phosphorylated IκBα is unable to inhibit the activation of NF-κB, which leads to a constitutive activation of NF-κB.*

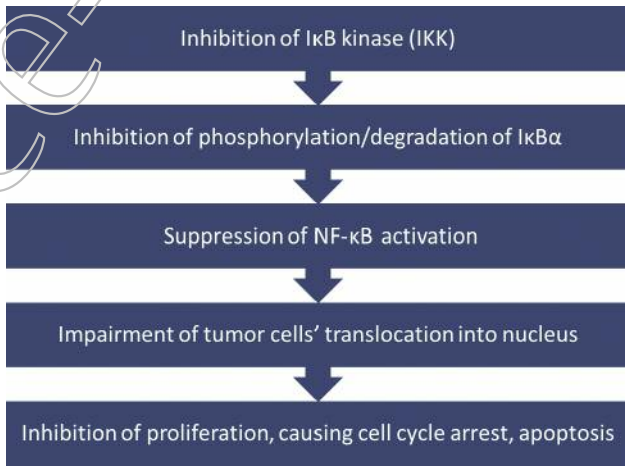

Figure 3. *Curcumin suppresses NF-κB activation.*

Curcumin inhibits signal transducer and activator of transcription 3 (STAT3). STAT3 is constitutively activated in cancer cells and STAT3-regulated gene products are involved in cellular cycle, survival, angiogenesis and metastasis (18, 31). Particularly, it has been shown that the level of activated STAT 3 is associated with metastasis in different types of cancer tumors. Curcumin acts as a suppressor of interleukin-6 (IL-6) cytokine signaling pathway and binds to the Janus-activated kinase (JAK) activation loop, thereby blocking subsequent signaling that requires the phosphorylation and activation of STAT 3 (16). Yang *et al*. reported that curcumin inhibited IL-6-inducible STAT3 phosphorylation in a time- and dose-dependent manner in small-cell lung carcinoma (SCLC) cells (16). Their results also indicated that phosphorylation of STAT3 depended on the activation of JAKs, especially JAK-2, and curcumin inhibited JAK phosphorylation in SCLC cells. STAT3-regulated gene products, including vascular endothelial growth factor (VEGF) and MMP-2 and -9, were

all down-regulated by curcumin. As STAT3 and its regulated gene products (VEGF, MMP-2 and -9) were significantly suppressed by curcumin, curcumin treatment reduced the metastasis rate in SCLC cells. Kunnumakkara *et al*. also demonstrated that curcumin inhibited IL-6-induced STAT3 phosphorylation in myeloma cells (24).

Curcumin down-regulates inflammatory cytokines' expression. Chronic inflammation is the key risk factor for cancer development and progression (32, 33). Inflammatory cytokines contribute to this process as they have the ability to attract mononuclear cells to cancer sites and provide growth factors to enhance cancer development. Inflammatory cytokines CXCL1 and CXCL2 are shown to promote angiogenesis *in vivo*, which is an important process for tumor cells' growth (34). It has been shown that CXCL1 is able to promote migration of breast cancer cells *in vitro* (35). Bachmeier *et al*. have identified CXCL1 and -2 expression as the major targets of anti-cancer activity of curcumin in a breast cancer cell line (25). Their research demonstrated that curcumin down-regulates mRNA expression and protein secretion of CXCL1 and -2 in a metastatic breast cancer cell line (MDA-MB-231). Helbig *et al*. showed that NF-κB directly induces the expression of chemokine receptor CXCR4 to promote breast cancer cell migration and metastasis (4). As curcumin inhibits the activation of NF-κB, curcumin can, thereby, reduce breast cancer metastasis through reducing NF-κB-mediated expression of two inflammatory cytokines, CXCL1 and -2, and reducing expression of the chemotactic receptor CXCR4.

Killian *et al*. showed that CXCL1 and -2 share an almost identical proximal promoter region that contains a perfect NF-κB binging site (26). This result shows activation of NF-κB increases the expression of CXCL1 and -2. Their results demonstrated that curcumin down-regulates CXCL-1 and -2 by targeting NF-κB signaling pathway in metastatic prostate cancer cell line. The group has also conducted an *in vivo* study in an orthotopic mouse model of hematogenous metastasis. Their results indicated that curcumin treatment significantly inhibited the formation of prostate cancer lung metastasis. Metastasis of prostate cancer was induced by maintaining a positive pro-inflammatory and pre-metastatic feedback loop between NF-κB and CXCL1 and -2 signaling pathways. Curcumin interrupted this feedback loop by inhibiting NF-κB activation and down-regulating CXCL1, -2, which led to reduced metastasis of prostate cancer in the lungs.

Curcumin inhibits expression of interleukins (ILs). Interleukins are a group of cytokines that mediate and regulate acute inflammatory immune reactions. Freund *et al*. reported that IL-8 was overexpressed in ER-negative cancer cells but not in ER-positive cell line (36). Their results further concluded that IL-8 expression is negatively correlated to ER status and expressed preferentially in invasive cancer cells, which suggests a potential correlation between IL-8 expression and tumor progression and invasiveness. Another study also suggested that the overexpression of IL-8 associated with progression and metastasis of cancer cells in colon (37). Wang *et al*. found that neurotensin, a gut tridecapeptide that acts as a cellular mitogen in colorectal and pancreatic cancers, selectively stimulated *IL-8* gene expression in human colon cancer cells; thus, cancer cell migration is increased when treated with IL-8 (30). As mentioned above their results showed that treatment of colon cancer cells using curcumin inhibited neurotensin-induced *IL-8* gene expression and protein secretion in a dose-dependent manner, preventing migration of cancer cells (30). Curcumin also decreased IL-8 expression in human pancreatic cancer cell line (38).

Curcumin inhibits urokinase plasminogen activator (uPA) expression. uPA is a serine-specific protease that is released from tumor cells. It binds to uPA receptor (uPAR) to activate protease plasmin, which then degrades extracellular matrix (ECM) (3, 11). Extensive degradation of ECM of the primary tumor cells is a critical step for cancer invasion and subsequently metastasis. NF-κB is responsible for regulating uPA secretion. Zong *et al*. showed that curcumin significantly decreased the expression of uPA in a dose-dependent manner and NF-κB DNA binding activity in breast cancer cell line (MCF-7) (11). Based on their results, they suggested that curcumin inhibits the adhesion and invasion of breast cancer cells through down-regulating the expression of uPA *via* inhibiting NF-κB activation.

Curcumin suppresses the expression of matrix metalloproteinases (MMPs). MMPs are a group of zinc-containing endopeptidases capable of degrading extracellular matrix proteins and, thereby, play a major role in regulating ECM turnover and remodeling (3). By degrading the ECM and basement membrane, MMPs promote tumor migration of cancer cells from the original tumor and invade surrounding tissues and other organs by entering into blood stream and lymphatic system and travel to distant organ sites. During normal physiological conditions, the expression of MMPs is finely controlled and their activities are regulated by tissue inhibitors of metalloproteinases (TIMPs). However, when the balance between MMPs and TIMPs is disrupted, over-expression of MMPs will result in numerous pathogenic processes, including tumor invasion and metastasis (39, 40). Overexpression of MMPs observed in most of human cancer cells and high levels of MMPs are linked to metastasis (12). MMP-2 and -9 are the two main proteases that degrade the major component of basement membrane, type IV collagen, which, in turn, promote metastasis, especially in breast cancer (13, 14). Therefore, MMP-2 and -9 are the key therapeutic targets for anti-metastatic agents.

12-O-tetradecanoylphorbol-13-acetate (TPA) is a tumor promoter and promotes invasion of breast cancer cells (MCF-7) by inducing MMP-9 *via* protein kinase C (PKC) pathways (14, 41). In fact, TPA activates PKCs by binding to the Cl domain. Kim *et al*. demonstrated that curcumin inhibited TPA-induced MMP-9 expression and cell invasion *via* suppressing NF-κB and AP-1 activation. Curcumin also strongly inhibited the TPA-induced phosphorylation of p38 and JNK, as well as TPA-induced translocation of PKCα from the cytosol to the membrane (14).

In a study on curcumin's anti-invasive effects on ER-negative breast cancer cell line (MDA-MB-231), Shao *et al*. demonstrated that curcumin has a strong anti-invasive effect in a dose-dependent manner. It was suggested that curcumin down-regulates MMP-2 and up-regulates TIMP-1, -2 (42). TIMPs regulate tumor cell invasion by inhibiting MMP activity through non-covalent binding of the active zinc-binding sites of MMPs (43). Additionally, Shao *et al*. showed that curcumin is a potent inhibitor of VEGF and basic fibroblast growth factor (b-FGF) in ER-negative breast cancer cells (42). Hassan *et al*. also showed that curcumin inhibited metastasis in an ER-negative cell line by down-regulating the expression of MMP-2 and -9 and up-regulating the expression of TIMP-1 and -4 in a time- and concentration-dependent manner (12). Based on their results, they suggested that curcumin significantly decreases MMP-9 functional ability by inhibiting its production, as well as up-regulating *TIMP-1* and *TIMP-4* gene expression in breast cancer cells.

Farhangi *et al*. used dendrosomal curcumin (DNC) to test curcumin's anti-metastatic properties on a metastatic breast cancer cell line (4T1) (44). Their results indicated that curcumin inhibited 4T1 cell viability, migration and adhesion in a time- and concentration-dependent manner, which, in turn, inhibited cell metastasis. DNC suppressed NF-κB, which led to down-regulation of MMP-9, VEGF and cyclooxgenase-2 (COX-2). An *in vivo* study was also conducted and stated that DNC was safe at 80 mg/kg in mice and treated mice had higher survival rate, lower metastatic rate and smaller tumor volume than control untreated group.

Overexpression of MMPs does not only occur in breast cancer but it has also been observed in other cancers, such as in the lungs. As observed in breast cancer cells, curcumin inhibited the migration and invasion of human lung cancer cells (A549) in a time- and concentration-dependent manner by inhibiting MMP-2 and -9 and VEGF (13). Chen *et al*. also reported curcumin's anti-invasion effects *in vitro* on human lung cancer cells (801D) and *in vivo* in a mouse model (45). The results showed that curcumin inhibits tumor cell migration and invasion both *in vitro* and *in vivo* partly due to its ability of inhibiting Rac-dependent signaling pathways and down-regulating the expression of MMP-2, -9. Expression of MMPs is induced by overexpression of Ras homolog gene family member A (RhoA), which then promotes the invasion of tumor cells (46). Sun *et al*. showed the lysophosphatidic acid (LPA) activated RhoA/MMPs signaling pathways increased in breast cancer cells (MCF-7), which enhanced invasion ability of cancer cells (46). They reported that bcurcumin was able to inhibit LPA-induced invasion in breast cancer cells by attenuating RhoA/ROCK (Rho-associated protein kinase)/MMPs pathway.

Wang *et al*. demonstrated that curcumin inhibited the expression of the tumor promoter caveolin-1 (Cav-1) in mouse hepatoma Hca-F cells (15). Curcumin down-regulated the expression of cluster of differentiation (CD)147, MMP-2, -9 and inhibited the phosphorylation of epidermal growth factor receptor (EGFR) in Hca-F cells. They suggested that curcumin suppresses the migratory and invasive ability of Hca-F cells and this action is mediated through a mechanism involving inactivation of Cav-1 and EGFR signaling pathways.

Cheng *et al*. also suggested that curcumin inhibits the expression of MMP-2, -9 in prostate cancer cells (47). By comparing the expression of MMP-2 and -9, they found curcumin has more inhibitory effect on MMP-9 compared to MMP-2. Additionally, they showed that the expression of serine protease (matriptase) was suppressed by curcumin, which, in turn, inhibited the matriptase-induced invasion in prostate cancer cells.

Curcumin inhibits focal adhesion kinase (FAK) activity. FAK is a protein kinase that is highly expressed in cancer cells, particularly breast and ovarian cancers, and involved in cellular adhesion regulating tumor cells' invasion and metastasis (48). Chen *et al*. reported an *in vitro* (colon cancer cell lines) and *in vivo* (mice) study to identify the effects of curcumin in terms of inhibiting metastasis (49). They suggested that curcumin inhibits migration and invasion *in vitro* and curcumin treatment significantly reduced primary tumor growth and number of liver metastatic nodules. They suggested that curcumin inhibited CD24, one of the major cell surface glycosylated proteins that function as an adhesive molecule of tumor cells. CD24 reduces FAK activity *via* inhibition of FAK phosphorylation. The study concluded that curcumin is a potential drug for the treatment of metastatic cancer because it inhibits cancer cell metastasis at different levels, particularly inhibition of transcription factors, cell adhesion molecules and cell surface markers, whereas it enhances cell adhesion ability and cell-cell tight junctions to prevent epithelial to mesenchymal transition (EMT). Leu *et al*. also reported the suppression of FAK activity by curcumin (50). Their results indicated that the activity of FAK was directly inhibited by curcumin in a colon cancer cell line. The reduction of Src kinase activity was medicated by curcumin, which then inhibited FAK phosphorylation and its activity, inhibiting colon cancer metastasis.

Curcumin regulates microRNAS (miRNAs) expression. miRNAs are non-coding RNAs that regulate the translation and degradation of target messenger RNAs (mRNAs). Therefore, miRNAs regulate cellular proliferation, differentiation and apoptosis, closely associated with cancer (51-53). miRNAs regulate several proteins' expressions by binding to mRNA three prime untranslated region (3'UTR), which then degrades mRNA or inhibits mRNA translation (53). miRNAs have a dual effect in cancers, either acting as tumor promoters or tumor suppressors (53). miR-21 is up-regulated in almost all cancers, including breast, lung, colon, liver and prostate (51, 52). miR21 promotes tumor cell proliferation, invasion and metastasis by targeting tumor suppressors, including programmed cell death protein 4 (PDCD4) and phosphatase and tensin homologue (PTEN) (51, 52). AP-1 controls the regulation of pri-miR-21 promoter and the results of Mudduluru *et al*. showed that curcumin inhibited the miR21 expression and activity in colorectal cancer cell lines by inhibiting AP-1 binding to pri-miR-21 promoter in a dose-dependent manner (51). Curcumin also induced the expression of PDCD4 *in vitro*. Migration and invasion assays showed that curcumin treatment of colorectal cancer cells significantly decreased cell migration and invasion. They also conducted an *in vivo* metastasis study where colorectal cancer cells were inoculated in the upper chorioallantoic membrane (CAM) of 10-day-old chicken embryos. The results demonstrated that curcumin significantly inhibited metastasis of colorectal cancer cells both *in vitro* and *in vivo* and the authors concluded that curcumin can inhibit tumor invasion and metastasis by inhibiting miR21 expression *via* AP-1 and stabilizing PDCD4 in colorectal cancer.

As mentioned earlier, pro-inflammatory cytokines CXCL1 and -2 are up-regulated in breast cancer cells and curcumin significantly down-regulates CXCL1 and -2. Kronski *et al*. found that miR181b-mediated CXCL1 and -2 expression through direct binding to 3'UTR that mediated curcumin-related down-regulation of CXCL1 and -2 in breast cancer cells (MDA-MB-231) (53). They also suggested that miR181b induces apoptosis and inhibits the expression of MMPs *in vitro*, thus inhibiting invasion of cancer cells. Lastly, they showed overexpressing of miR181b inhibits metastasis of breast cancer cells *in vivo* (immunodeficient mice). They concluded that curcumin inhibits metastasis of breast cancer cells by up-regulating miR181b and down-regulating CXCL-1 and -2.

Curcumin regulates DnaJ-like heat shock protein 40 (HLJ1) expression. HLJ1 is a heat shock protein (HSP) associated with progress of cancers and regulating cancer cell invasion and metastasis (54). HLJ1 is able to inhibit lung cancer cell proliferation and invasion; high HLJ1 expression is associated with reduced cancer recurrence and long overall survival of patients with non-small cell lung cancer (NSCLC). Chen *et al*. showed that curcumin inhibited tumor cell invasion and metastasis in both *in vitro* (CL1-5 cell, a high invasive lung cancer cell line) and *in vivo* (mouse model) through up-regulating HLJ1. The possible mechanisms suggested by their results were a) curcumin up-regulates HLJ1 though up-regulating JunD (an AP-1 compound) in a time- and concentration-dependent manner; and b) activation of HLJ1 by curcumin stimulates up-regulation of E-cadherin (act as an invasion suppresser), which, in turn, inhibits lung cancer cell invasion and metastasis.

Combination Therapies of Curcumin and Other Medications

As mentioned above, there are many possible molecular mechanisms for anti-invasive and anti-metastatic activities of curcumin that have been reported in *in vitro* and *in vivo* studies. As curcumin affects cancer cells at different levels and through different signaling pathways, the possible combination or synergistic effects of curcumin and other anticancer drugs on metastatic cancers have been studies and are explored in this section.

Duarte *et al*. conducted *in vitro* and *in vivo* studies to identity the effects of using curcumin in combination with cisplatin in head and neck squamous cell carcinoma (HNSCC) treatment (55). They used two HNSCC cell lines and treated them with curcumin or cisplatin alone or in combination. In that work, the *in vivo* study consisted of intravenous tail vein injection of liposomal curcumin or with intraperitoneal cisplatin, into nude mice growing xenograft HNSCC tumors. Results demonstrated that the administration of curcumin and suboptimal concentrations of cisplatin showed a significant cancer suppressive effect. Additionally, the team showed curcumin inhibited NF-κB activation and cisplatin induced apoptosis though p53 pathway. Since curcumin and cisplatin destroy cancer cells *via* different pathways, this combination therapy potentially improves cancer treatment by synergistic anti-cancer effects of these agents, minimizing the adverse side-effects of cisplatin. LoTempio *et al*. had also previously suggested that curcumin suppresses the growth of HNSCC cell lines *in vitro* and reduces tumor volume *in vivo via* topical application (56).

Yin *et al*. conducted *in vitro* (A549 cells) and *in vivo* (A549 transplanted xenograft model) studies to identify possible effects of combining curcumin and docetaxel in lung cancer (57). Docetaxel is considered the first-line chemotherapy for lung cancer; however, it causes severe toxicity even at therapeutic doses and yields poor response rates as monotherapy. Results showed that curcumin synergistically increased the efficacy of docetaxel after 4 days of initial treatment; simultaneous administration of curcumin and docetaxel showed little toxicity to normal tissues, including bone marrow and liver at therapeutic doses.

Shakibaei *et al*. used an alginate-based 3D scaffold to screen the possible anti-tumor-effects of combining curcumin and 5-fluorouracil (5-FU) on colorectal cancer cells (HCT116) (58). Overexpressed tumor promoting factors (*e.g*., CXCR4, MMP-9, NF-κB) in HCT116 cells promote proliferation, invasion and metastasis. Shakibaei *et al*. demonstrated that curcumin was able to further enhance the ability of 5-FU to decrease cancer cell proliferation and invasion. Curcumin increased the sensitivity of 5-FU through down-regulating NF-κB activation and NF-κB-regulated gene products. The combination therapy of curcumin and 5-FU was proposed as a potential treatment for colorectal cancer and may overcome the drug resistance in cancer cells.

Paclitaxel is a chemo-agent to treat breast cancer. However, because of paclitaxel-induced drug resistance through NF-κB activation, paclitaxel is not effective in treating advanced breast cancers (59). Aggarwal *et al*. have investigated curcumin therapy by using paclitaxel (Taxol)-resistant breast cancer cells and a human breast cancer xenograft animal model (60). Results showed that curcumin significantly inhibited paclitaxel-induced NF-κB activation and metastatic proteins *in vitro* and significantly decreased the incidence of breast cancer metastasis to the lungs through suppressing NF-κB, COX-2 and MMP-9.

Conclusion

Curcumin is well-known for its anti-cancer activities in all different stages of cancer progress: transformation of normal cells to cancer cells; cancer cell proliferation; and tumor invasion. There are many research studies that have shown curcumin significantly inhibits metastasis in various types of cancers by regulating different signaling pathways. The potential anti-metastatic mechanisms of curcumin include inhibition of transcription factors and their signaling pathways (*e.g*., NF-κB, ApP-1 and STAT3), inflammatory cytokines (*e.g*., CXCL1, CXCL2, IL-6, IL-8), multiple proteases (*e.g*., uPA, MMPs), multiple protein kinases (*e.g*., MAPKs, FAK), regulation of miRNAs (*e.g*., miR21, miR181b) and heat shock proteins (HLJ1).

In addition to identify its potential therapeutic effects, the safety of curcumin and its appropriate therapeutic dose should also be determined. A phase I clinical trial conducted by Bayet-Robert *et al*. suggested that the recommended dose of curcumin is 6 g/day for seven consecutive days every three weeks in combination with a standard dose of docetaxel in patients with advanced and metastatic breast cancer (61). A phase I clinical study conducted by Cheng *et al*. in patients with high-risk or pre-malignant lesions concluded that curcumin is not toxic to humans up to 8 g/day when taken orally for 3 months (62). Dhillon *et al*. conducted a phase II trial in twenty-one advanced pancreatic cancer patients by giving 8 g oral dose of curcumin daily until disease progression (63). They reported two patients changed cytokine levels after curcumin treatment. One patient had ongoing stable disease for more than 18 months and slow improvement over 1.5 years. Another patient had a brief but marked tumor regression (73%).

Curcumin has been used as a dietary spice for centuries and its potential in cancer therapy supported by numerous research studies. However, there are few studies indicating possible negative effects of curcumin in cancer treatment. An *in vivo* study conducted by Yan showed curcumin enhanced metastatic growth of Lewis lung carcinoma (LLC) in mice (64). Curcumin treatment significantly increased metastatic tumor cross-sectional area by 46% and volume by 70%. Curcumin increased plasma concentration of angiogenin (angiogenic factor), VEGF, IL-1β and monocyte chemotactic protein-1 (MCP-1), which is believed to have contributed to the enhanced metastatic growth of LLC in mice. Yan *et al*. conducted another study to investigate the effects of curcumin on bone microstructure in non-tumor-bearing and LLC-bearing mice (65). They reported the possibility of a combined effect of cancer-induced osteolysis and curcumin-stimulated bone loss in patients treated with curcumin.

The future studies on anti-metastasis activities of curcumin should be directed towards efficacy and safety of curcumin in preventing cancer metastasis in animal models and clinical trials. There are still two major problems of using curcumin in cancer treatment/prevention that limit its clinical application. Curcumin has low bioavailability due to low water solubility, rapid metabolic rate and systemic clearance; it has also low chemical stability (10). Curcumin nanoparticles have shown potential to improve its anti-cancer activities through enhanced cellular uptake, localization to targeted areas and improved bioavailability. Liposomes, polymeric micelles and polymeric nanoparticles are the common nano-carries employed for encapsulation of curcumin (10). Further effort to modify curcumin nanoparticles for targeting cancer cells (*e.g*., coating nanoparticles with antibodies/peptides that bind to overexpressed receptors on the surface of cancer cells) should also be envisaged in future studies.

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