

Review Article

Curcumin, a component of turmeric: From farm to pharmacy

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Abstract

Curcumin, an active polyphenol of the golden spice turmeric, is a highly pleiotropic molecule with the potential to modulate the biological activity of a number of signaling molecules. Traditionally, this polyphenol has been used in Asian countries to treat such human ailments as acne, psoriasis, dermatitis, and rash. Recent studies have indicated that curcumin can target newly identified signaling pathways including those associated with microRNA, cancer stem cells, and autophagy. Extensive research from preclinical and clinical studies has delineated the molecular basis for the pharmaceutical uses of this polyphenol against cancer, pulmonary diseases, neurological diseases, liver diseases, metabolic diseases, autoimmune diseases, cardiovascular diseases, and numerous other

chronic diseases. Multiple studies have indicated the safety and efficacy of curcumin in numerous animals including rodents, monkeys, horses, rabbits, and cats and have provided a solid basis for evaluating its safety and efficacy in humans. To date, more than 65 human clinical trials of curcumin, which included more than 1000 patients, have been completed, and as many as 35 clinical trials are underway. Curcumin is now used as a supplement in several countries including the United States, India, Japan, Korea, Thailand, China, Turkey, South Africa, Nepal, and Pakistan. In this review, we provide evidence for the pharmaceutical uses of curcumin for various diseases. © 2013 BioFactors, 00(00):000–000, 2013

Keywords: autophagy; cancer stem cells; curcumin; microRNA

1. Introduction

Since ancient times, natural agents derived from fruits, vegetables, spices, legumes, and cereals have been preferred as

potential therapeutics for most chronic diseases because of their safety, affordability, long-term use, and ability to target multiple cell signaling pathways [1]. Curcumin is one such agent that was described about two centuries ago as the

Abbreviations: ACF, aberrant crypt foci; AKT, AKT8 virus oncogene cellular homolog; ALDH, aldehyde dehydrogenase; AML, acute myeloid leukemia; AP-1, activator protein-1; Atgs, autophagy-related genes; Bcl-2, B-cell lymphoma-2; CAM, chorionallantoic membrane; CDF, difluorinated-curcumin; CML, chronic myelogenous leukemia; CSC, cancer stem cell; Erbb3, erythroblastic leukemia viral oncogene homolog 3; ERK, extracellular signal-regulated kinase; ESR1, estrogen receptor 1; EZH2, enhancer of zeste homologue 2; FOLFOX, 5-Fluorouracil plus oxaliplatin; GSH, glutathione; GSH-Px, glutathione peroxidase; GSK-3 β , glycogen synthase kinase-3 β ; GST, glutathione S-transferase; HAT, histone acetyltransferase; HIV, human immunodeficiency virus; IGF, insulin-like growth factor; IKK β , I κ B kinase beta; IL, interleukin; LC3, microtubule-associated protein 1 light chain 3; MDA, malondialdehyde; miRNA, microRNA; MT1-MMP, membrane type-1 matrix metalloproteinase; mTOR, mammalian target of rapamycin; NB-UVB, narrowband ultraviolet B; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; OncomiRs, oncogenic microRNAs; p70S6K, p70 ribosomal protein S6 kinase; Pcd4, programmed cell death protein 4; PhK, phosphorylase kinase; PI3K, phosphoinositide 3-kinase; PPAR- γ , peroxisome proliferator-activated receptor-gamma; PSA, prostate specific antigen; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; Sp1, specificity protein 1; STAT3, signal transducers and activators of transcription protein 3; TGF- β , transforming growth factor beta; TNF- α , tumor necrosis factor- α ; VCAM1, vascular cell adhesion molecule 1; Wnt, wint; WT1, wilms' tumor 1

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FIG 1 Various curcumin-based products being marketed in various countries. These preparations include, but are not limited to, capsules, tablets, ointments, energy drinks, soaps, and cosmetics.

“yellow coloring-matter” from the rhizomes of *Curcuma longa* (turmeric) [2]. Traditionally, turmeric has been used to flavor food preparations, especially in South Asian cuisine [3]. In Asian medicines, curcumin has been used for the treatment of acne, psoriasis, dermatitis, and diaper rash [4].

Besides curcumin, more than 300 different components, including phenolics and terpenoids, have been identified in turmeric [5,6]. Although curcumin is one of the major components, research during the past decade has revealed that some of the activities of turmeric are independent of curcumin. For instance, curcumin-free aqueous turmeric extract suppressed benzo[a]pyrene-induced tumorigenesis in mice [7]. Curcumin-free turmeric also inhibited 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in rats [8]. Studies from our laboratory using cell-based assays have indicated that curcumin is less potent than turmeric containing equivalent amounts of curcumin in inhibiting cancer growth [9]. Likewise, whole turmeric had higher peroxisome proliferator-activated receptor-gamma (PPAR- γ) ligand binding activity than did pure curcumin [10]. Turmeric was also more effective than curcumin in suppressing streptozotocin-induced diabetic cataracts in rats [11] and in reducing blood glucose levels in type 2 diabetic KK-A mice [10]. Overall, these studies provide sufficient

evidence that whole turmeric may exhibit superior activities compared with curcumin alone.

Curcumin is a highly pleiotropic molecule with the potential to modulate the biological activity of a number of signaling molecules [12]. Chemically, pure curcumin is a diferuloyl methane molecule (1,7-bis (4-hydroxy-3-methoxyphenol)-1,6-heptadiene-3,5-dione) containing two ferulic acid residues joined by a methylene bridge. However, commercially available curcumin also contains approximately 17% demethoxycurcumin and 3% bisdemethoxycurcumin. Curcumin is now marketed in several countries including the United States, India, Japan, Korea, Thailand, China, Turkey, South Africa, Nepal, and Pakistan in the form of capsules, tablets, ointments, energy drinks, soaps, and cosmetics (Fig. 1). Curcumin has shown efficacy against a number of human ailments; more than 65 human clinical trials of curcumin have been completed, and more than 35 other clinical trials are under way to further evaluate its efficacy. How a single molecule can possess such diverse activities has been an enigma over the years; however, studies have indicated that bis- α , β -unsaturated β -diketone, two methoxy groups, two phenolic hydroxy groups, and two double-conjugated bonds may contribute to the biological activities of curcumin [12]. Furthermore, recent

studies have indicated that curcumin can target numerous newly identified signaling pathways including those associated with microRNA (miRNA), cancer stem cells (CSCs), and autophagy. In the following sections, we provide evidence for the pharmaceutical uses of curcumin from both preclinical and clinical studies; we also discuss the newly identified signaling pathways modulated by curcumin.

2. Pharmaceutical Uses of Curcumin

2.1. *In Vitro* Preclinical Studies

Studies from cell-based models have indicated the pleiotropic nature of curcumin. At the molecular level, curcumin has been shown to modulate numerous signaling molecules either directly by binding to them or indirectly by modulating the expression of other proteins. The indirect targets of curcumin include transcription factors, enzymes, growth factors, receptors, inflammatory mediators, protein kinases, drug resistance proteins, adhesion molecules, cell-survival proteins, chemokines and chemokine receptors, invasive and angiogenic proteins, and cell-cycle regulatory proteins [13]. Curcumin, through its α -, β -unsaturated β -diketone moiety, carbonyl and enolic groups of the β -diketone moiety, methoxy and phenolic hydroxyl groups, and phenyl rings, has been shown to directly interact with numerous signaling molecules. More specifically, this polyphenol has been shown to directly interact with protein kinases, protein reductases, histone acetyltransferase (HAT), histone deacetylase, sarcoplasmic (endoplasmic) reticulum Ca^{2+} ATPase, DNA methyltransferases 1, FtsZ protofilaments, carrier proteins, inflammatory molecules, cell survival proteins, glyoxalase I, xanthine oxidase, proteasome, human immunodeficiency virus (HIV) 1 integrase, HIV1 protease, and metal ions. Curcumin can also bind directly to DNA and RNA [12]. For the complete list of curcumin's molecular targets, please see articles that we and others have published [14–17,12]. In this section, we discuss newer targets of curcumin associated with miRNA, CSCs, and autophagy pathways (Table 1).

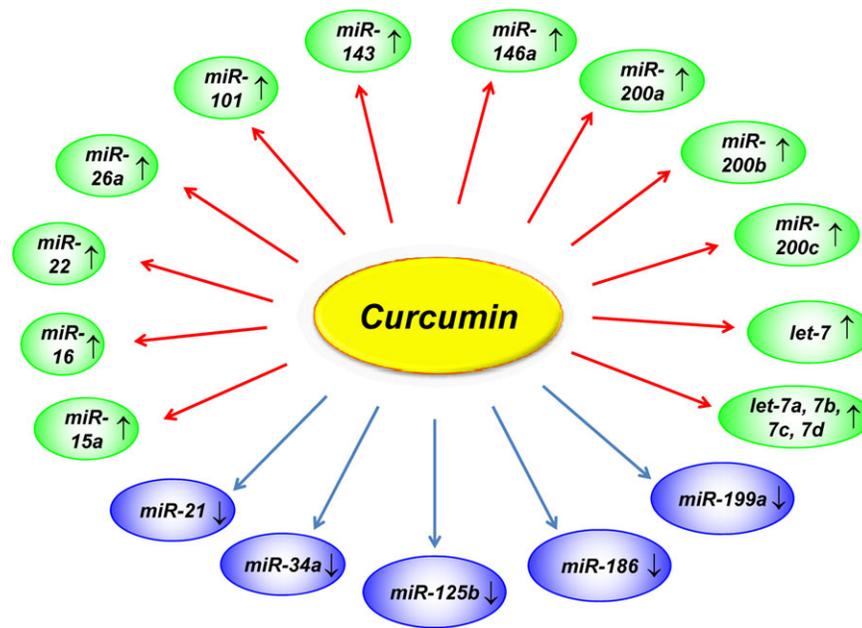
2.1.1. Curcumin and miRNA.

MicroRNAs are small (~22 nucleotides), endogenous, single-stranded noncoding RNAs that negatively regulate gene expression by binding to the 3' untranslated region of target mRNA and inducing mRNA degradation or inhibiting translation [18]. MicroRNAs play crucial roles during normal physiological processes and may possess both oncogenic and tumor suppressor activities. Extensive research throughout the past two decades has indicated that miRNAs could regulate various stages in tumorigenesis and thus represent an attractive target for cancer chemoprevention. Curcumin has been shown to alter the expression of miRNAs, which may lead to either abrogation of tumor growth or sensitization of cancer cells to chemotherapeutic agents. For instance, in human pancreatic cancer cells, curcumin treatment was associated with up-regulation in miRNA-22 and down-regulation in miRNA-199a

expression [19]. Curcumin mediated up-regulation of miRNA-22 suppressed the expression of its target genes specificity protein 1 (Sp1) transcription factor and estrogen receptor 1 (ESR1). Furthermore, inhibition of miRNA-22 by anti-sense oligonucleotide enhanced Sp1 and ESR1 expression, suggesting that Sp1 and ESR1 are the target genes of miRNA-22. Authors of this study speculated that modulation of miRNA expression may be an important mechanism by which curcumin mediates its effects on cell growth and apoptosis. However, the role of miRNA in the anti-growth and anti-apoptotic effects of curcumin was not demonstrated experimentally.

Zhang et al. [20] reported that curcumin can induce apoptosis in A549 cells through down-regulation of miRNA-186. In a subsequent study, the group found that silencing miRNA-186 promoted apoptosis whereas overexpressing miRNA-186 significantly inhibited curcumin-induced apoptosis in multidrug-resistant human lung adenocarcinoma cells [21]. MicroRNA-21 is overexpressed in many tumors and has been associated with tumor progression. In one study, the potential of curcumin in regulating miRNA-21, tumor growth, invasion, and *in vivo* metastasis in colorectal cancer was investigated [22]. Curcumin treatment reduced miRNA-21 promoter activity and expression in a dose-dependent manner by inhibiting activator protein-1 (AP-1) binding to the promoter and inducing the expression of the tumor suppressor programmed cell death protein 4 (Pdc4). Treatment of Rko and HCT116 cells with curcumin was associated with cell cycle arrest in the G2/M phase. Curcumin also inhibited tumor growth, invasion, and *in vivo* metastasis in the chicken-embryo-metastasis chorionallantoic membrane (CAM) assay. Curcumin significantly inhibited miRNA-21 expression in primary tumors generated *in vivo* in the CAM assay by Rko and HCT116 cells. The authors of this study concluded that curcumin inhibits the transcriptional regulation of miRNA-21 via AP-1; suppresses cell proliferation, tumor growth, invasion, and *in vivo* metastasis; and stabilizes expression of the tumor suppressor Pdc4 in colorectal cancer [22].

Although curcumin has shown promise as an anticancer agent, the poor bioavailability of this polyphenol limits its use in this capacity. Difluorinated-curcumin (CDF), an analogue of curcumin, has been shown to possess enhanced bioavailability compared with curcumin in pancreatic tissues [23,24]. Previous studies have shown an association between attenuated expression of miRNA-200 [25,26], increased expression of miRNA-21 [27–31], and aggressiveness of numerous tumors. Therefore, up-regulating miRNA-200 and down-regulating miRNA-21 might be potentially useful to overcome resistance of cancer cells to cancer therapeutics. In one study, CDF was found to up-regulate miRNA-200b and miRNA-200c and to down-regulate miRNA-21 in both gemcitabine-sensitive (BxPC-3) and gemcitabine-resistant (MIAPaCa-E and MIAPaCa-M) cell lines, which were associated with induction of apoptosis [32]. Furthermore, the combination of CDF with gemcitabine was found to be much more effective than was either agent alone, suggesting that CDF-mediated alterations in specific miRNAs could be a novel approach for the treatment of patients with pancreatic cancer.


FIG 2

MicroRNA targets of curcumin. Curcumin down-regulates oncogenic microRNA and up-regulates tumor suppressive microRNA. The targets shown in orange are up-regulated (↑) whereas those in blue are down-regulated (↓) by curcumin.

The loss of expression of miRNA-200a, -200b, and -200c in chemoresistant pancreatic cancer cells BxPC-3, MIAPaCa-2, and MIAPaCa-2-GR has been associated with loss of phosphatase and tensin homolog (PTEN) and overexpression of membrane type-1 matrix metalloproteinase (MT1-MMP). Because overexpression of MT1-MMP and loss of PTEN contribute to aggressive behavior in tumor cells, agents with the potential to up-regulate expression of miRNA-200a, -200b, and -200c may have potential as cancer therapeutics. In one study, CDF was found to significantly up-regulate miRNA-200 and PTEN while significantly down-regulating expression of MT1-MMP [33]. Furthermore, forced overexpression or silencing of miR-200c, followed by CDF treatment of MIAPaCa-2 cells, altered the morphology of the cells, colony formation, and the expression of MT1-MMP and PTEN. The authors of this study suggested that CDF could be useful as a therapeutic agent against pancreatic cancer [33].

In another study, CDF was found to induce let-7 and miRNA-143 and to down-regulate miRNA-21 expression, consistent with the attenuation of Ras expression and its activity in pancreatic cancer cells [34]. Because loss of expression of let-7 and miRNA-143 as well as increased expression of miRNA-21 and Ras are often correlated with tumor aggressiveness, these observations suggested CDF as a novel agent for the treatment of pancreatic cancer. The inhibition in growth of human pancreatic cancer cells by CDF was correlated with increased expression of let-7a, let-b, let-c, let-d, miRNA-26a, miRNA-101, miRNA-146a, miRNA-200b, miRNA-200c, and decreased expression of enhancer of zeste homologue 2 (EZH2), a histone methyltransferase and central epigenetic regulator of cell survival, proliferation, and CSC function [35].

In MCF-7 breast cancer cells, curcumin was found to up-regulate the expression of miRNA-15a and miRNA-16 and to down-regulate B-cell lymphoma-2 (Bcl-2) expression [36]. Furthermore, silencing miRNA-15a and miRNA-16 by specific inhibitors restored the expression of Bcl-2, thus suggesting that curcumin suppresses Bcl-2 expression in MCF-7 cells through up-regulation of miRNA-15a and miRNA-16. In Y79 retinoblastoma cells, curcumin up-regulated tumor-suppressor miR-22 [37]. Transfection of Y79 cells with miR-22 was found to inhibit the cell proliferation and reduced the migration of retinoblastoma cells. Furthermore, erythroblastic leukemia viral oncogene homolog 3 (ErbB3) was found to be the target gene of miR-22. In esophageal cancer cells, curcumin was found to down-regulate Notch-1-specific miRNA-21 and miRNA-34a and to up-regulate tumor suppressor let-7a miRNA in association with inhibition of proliferation of tumor cells [38].

A high level of Wilms' tumor 1 (WT1), an oncogene that is detected in most cases of human acute myeloid leukemia (AML) and chronic myelogenous leukemia (CML), is associated with poor long-term prognosis [39]. Curcumin was found to up-regulate the expression of miRNA-15a/16-1 and to down-regulate the expression of WT1 in leukemic cells and in primary AML cells [40]. The up-regulation of miRNA-15a/16-1 by curcumin was an early event upstream to WT1 down-regulation. Furthermore, anti-miRNA-15a/16-1 oligonucleotides partly abrogated the down-regulation of WT1 induced by curcumin in leukemic cells and promoted the growth of curcumin-treated K562 and HL-60 cells. Overall, these observations suggested that curcumin down-regulates the expression of WT1 partly by up-regulating miRNA-15a/16-1, which

TABLE 1

MicroRNA

- Up-regulated the expression of miRNA-22 and down-regulated miRNA-199a expression in human pancreatic cancer cells (19).
- Induced apoptosis in A549 cells through down-regulation of miRNA-186 (21, 20).
- Inhibited miRNA-21 expression via AP-1; suppressed cell proliferation, tumor growth, invasion, and *in vivo* metastasis; and stabilized expression of the Pcd4 in colorectal cancer (22).
- Up-regulated miRNA-200b and miRNA-200c expression, down-regulated miRNA-21 expression, and induced apoptosis in pancreatic cancer cell lines (32).
- Up-regulated miRNA-200 and PTEN and significantly down-regulated MT1-MMP expression in chemoresistant pancreatic cancer cells (33).
- Induced let-7 and miRNA-143 and down-regulated miRNA-21 expression, and attenuated the expression and activity of Ras in pancreatic cancer cells (34).
- Inhibited the growth and increased the expression of let-7a, let-b, let-c, let-d, miRNA-26a, miRNA-101, miRNA-146a, miRNA-200b, miRNA-200c, and decreased the expression of EZH2 in human pancreatic cancer cells (35).
- Suppressed Bcl-2 expression through up-regulation of miRNA-15a and miRNA-16 in breast cancer cells (36).
- Up-regulated miR-22 and inhibited the proliferation and migration of retinoblastoma cells (37).
- Down-regulated miRNA-21 and miRNA-34a, up-regulated let-7a, and inhibited the proliferation of esophageal cancer cells (38).
- Down-regulated WT1 expression by up-regulating miRNA-15a/16-1 and inhibited the proliferation of leukemic cells (40).

Cancer stem cells

- Inhibited the self-renewal of ALDH expressing breast CSCs through suppression of Wnt/ β -catenin signaling (62).
- Inhibited the growth, self-renewal, and clonogenicity of brain CSCs by blocking the Hedgehog signaling pathway (65).
- In combination with dasatinib or FOLFOX decreased the expression of CD133, CD44, CD166, and ALDH in colon CSCs (68, 67).
- Inhibited STAT3 phosphorylation, cell viability, and tumorsphere formation of colon CSCs (66).
- Inhibited pancreatosphere formation, and attenuated the expression of CD44 and EpCAM in gemcitabine-resistant pancreatic cancer cells (64).
- Reduced CD44 and CD166, inhibited growth, induced apoptosis, and attenuated colonosphere formation of chemoresistant colon cancer cells (69).

Autophagy

- Triggered autophagy in a caspase-independent manner in human CML cells (75).
- Increased the levels of beclin 1 and LC3-II and reduced the viability of leukemia cells (76).
- Increased LC3-II/LC3-I expression and induced the formation of autophagosomes in mesothelioma cell line (77).
- Induced autophagic cell death in malignant glioma cells through inhibition of the AKT/mTOR/p70S6K pathway and activation of the ERK1/2 pathway (78).
- Induced autophagy in a mice model bearing U87-MG cells (78).
- Degraded beclin-1 and induced LC3 expression in cutaneous T-cell lymphoma (79).
- Induced cell death in colon cancer cells through ROS-dependent activation of autophagy (80).
- Induced autophagy through induction of ROS production in oral cell carcinoma (81).
- Induced autophagy in glioma-initiating cells (82).

AKT, AKT8 virus oncogene cellular homolog; ALDH, aldehyde dehydrogenase; AP-1, activator protein-1; CML, chronic myelogenous leukemia; CSC, cancer stem cell; EpCAM, epithelial cell adhesion molecule; ERK, extracellular signal-regulated kinase; EZH2, enhancer of Zeste homologue 2; FOLFOX, 5-Fluorouracil plus oxaliplatin; LC3, microtubule-associated protein 1 light chain 3; MT1-MMP, membrane type-1 matrix metalloproteinase; mTOR, mammalian target of rapamycin; p70S6K, p70 ribosomal protein S6 kinase; Pcd4, programmed cell death protein 4; PTEN, phosphatase and tensin homolog; Ras, rat sarcoma; STAT3, signal transducers and activators of transcription protein 3; Wnt, wint; WT1, Wilms' tumor 1.

TABLE 2
Clinical efficacy of curcumin

Disease	Pts (Number)	Dosage	Duration	Reference
Cholecystitis	67	0.1–0.25 g/day	3 months	101
Cancer lesions	25	8 g/day	3 months	109
Cancer lesions	75	1 g/day	7 days	116
Prostate cancer	85	0.1 g/day	6 months	115
Pancreatic cancer	20	1.5 g/day	6 weeks	114
Pancreatic cancer	25	8 g/day		110
Pancreatic cancer	21	8 g/day		111
Colorectal cancer	44	2 and 4 g/day	1 month	112
Colorectal cancer	126	1.08 g/day	10–30 days	113
Ulcerative colitis	89	2 g/day	6 months	117
Ulcerative colitis	1	0.5 g/day	2–10 months	118
Vitiligo	10	twice/day	12 weeks	119
Diabetes	72	0.6 g/day	8 weeks	120

contributes to the antiproliferation effects of curcumin in leukemic cells.

It is clear from the above discussion that tumor cells commonly have up-regulated expression of oncogenic microRNAs (oncomiRs) and down-regulated expression of tumor-suppressive microRNAs. Curcumin has been shown to down-regulate oncomiRs (*e.g.*, miR-21) and to up-regulate tumor-suppressive microRNAs (*e.g.*, let-7) (Fig. 2). Thus, alteration in the expression of microRNA could contribute to the anticancer activities of curcumin. However, further studies are required to determine whether curcumin modulates miRNA expression in clinically relevant animal models and in patients.

2.1.2. Curcumin and CSCs.

Cancer stem cells are a subpopulation of undifferentiated cancer cells that have the ability to self-renew and to generate tumors through the processes of self-renewal and differentiation [41,42]. Recent studies have indicated that CSCs may be responsible for tumor relapse and are a major culprit in the development of resistance to therapy [43,44]. The first conclusive observation showing the existence of CSCs in human AML was published in 1997 [45]. Since then, studies have demonstrated that diverse cancer types, including breast [46,47], pancreatic [48,49], brain [50,51], colon [52–54], liver [55], head and neck [56], ovarian [57,58], and melanoma [59,60], are also driven and sustained by CSCs [61]. The most common pathways that regulate self-renewal of CSCs include wint (Wnt), Notch, Hedgehog, signal transducers and activators of

transcription protein 3 (STAT3), phosphoinositide 3-kinase (PI3K)/ AKT8 virus oncogene cellular homolog (AKT), glycogen synthase kinase-3 β (GSK-3 β), and HAT. Therefore, therapeutic strategies that selectively target CSCs while limiting mistargeting to normal stem cells are needed to reduce the risk of cancer relapse and recurrence.

Curcumin has been shown to selectively target CSCs without a deleterious effect on normal stem cells in a number of preclinical studies. For instance, the inhibition of self-renewal of aldehyde dehydrogenase (ALDH)-expressing breast CSCs by curcumin was mediated by suppression of Wnt/ β -catenin signaling [62]. On the contrary, curcumin had little effect on differentiated cells [62]. Curcumin inhibited CD133-positive medulloblastoma, glioblastoma, and pancreatic and colon CSC proliferation that was dependent on insulin-like growth factor (IGF), STAT3, Hedgehog, and EZH2 [63–66]. Nanoparticle-encapsulated curcumin has been reported to inhibit growth, self-renewal, and clonogenicity of brain CSCs by blocking the Hedgehog signaling pathway [65]. Combinations of dasatinib and curcumin were found to inhibit growth, invasion, and colonosphere formation of 5-Fluorouracil plus oxaliplatin (FOLFOX)-resistant colon cancer cells [67]. The combination therapy also reduced the CSC population as evidenced by the decreased expression of CSC-specific markers (CD133, CD44, CD166, and ALDH) that further confirmed curcumin's efficacy against CSCs [67]. Curcumin, alone and together with FOLFOX, decreased the expression of CSC markers (CD44 and CD166) and reduced colonosphere formation of colon cancer cells [68].

Curcumin and GO-Y030, a curcumin analogue, have been reported to inhibit STAT3 phosphorylation, cell viability, and tumorsphere formation in colon CSCs [66]. In gemcitabine-resistant pancreatic cancer cells, CDF significantly inhibited the sphere-forming ability (pancreatospheres), which was associated with attenuation of CSC markers (CD44 and EpCAM) [64]. In another study, CDF, together with 5-fluorouracil and oxaliplatin, was found to be more potent than was curcumin alone in reducing CD44 and CD166 in chemoresistant colon cancer cells that was associated with the inhibition of growth, induction of apoptosis, and disintegration of colonospheres [69].

In summary, the above studies suggest the potential of curcumin in modulating stem cell fate, which may contribute to its anticancer activities.

2.1.3. Curcumin and autophagy.

Autophagy is an evolutionarily conserved self-catabolic process that involves sequestration of organelles and long-lived proteins into autophagosomes and their subsequent delivery to and degradation in lysosomes [70–72]. Autophagy is altered in cancer cells and is involved in both cell survival and cell death pathways [73]. To date, 35 autophagy-related genes (Atgs) have been discovered in yeast, all of which have mammalian homologues [74].

Accumulating evidence over the past 5 years has indicated that curcumin can induce autophagy in cancer cells. For instance, in human CML cells, curcumin triggered autophagy in a caspase-independent manner [75]. In CML cell line K562, curcumin inhibited the viability of cells in a dose- and time-dependent manner [76]. The induction of cell death in these cells by curcumin was associated with the formation of the apoptosome, the collapse of mitochondrial membrane potential, and caspase-3 activation. Curcumin increased the protein levels of beclin 1 and microtubule-associated protein 1 light chain 3 (LC3)-II. Furthermore, autophagy inhibitors bafilomycin A1 and the pan-caspase inhibitor suppressed curcumin-induced K562 cell death. Overall, these results suggested that both apoptotic and autophagic mechanisms contribute to the curcumin-induced death of K562 cells [76].

In another study, curcumin dose-dependently reduced cell viability but did not induce apoptosis in a malignant pleural mesothelioma cell line. Instead, curcumin increased LC3-II/LC3-I expression and induced the formation of autophagosomes. These changes were attenuated by gene silencing of *atg5*, thus suggesting that induction of autophagy may be involved in the reduction of cell viability by curcumin [77]. In U87-MG and U373-MG malignant glioma cells, curcumin induced cell cycle arrest at the G2/M phase [78]. Non-apoptotic autophagic cell death in these cells by curcumin was mediated through inhibition of the AKT/ mammalian target of rapamycin (mTOR)/ p70 ribosomal protein S6 kinase (p70S6K) pathway and activation of the extracellular signal-regulated kinase (ERK)1/2 pathway. Curcumin also induced autophagy in the subcutaneous xenograft mice model bearing U87-MG cells, as

evidenced by substantially increased expression of a marker of autophagy [78]. The degradation of beclin-1 by curcumin has been associated with an accumulation of the autophagy-specific marker LC3 in cutaneous T-cell lymphoma [79]. Curcumin induced cell death in HCT116 colon cancer cells through reactive oxygen species (ROS)-dependent activation of autophagy [80]. In oral cell carcinoma, curcumin induced autophagy that was mediated through induction of ROS production. Use of autophagy inhibitor rescued cell death, which further confirmed the induction of autophagy by curcumin in oral cell carcinoma [81]. In glioma-initiating cells that are believed to initiate glioblastoma, curcumin-induced autophagy led to tumor suppression because of differentiation events [82].

Some other cancer types in which curcumin has been shown to induce autophagy alone or in combination with other agents include oesophageal cancer [83], melanoma [84], prostate cancer [85], hepatocellular carcinoma [86], and osteosarcoma [87].

In summary, the above discussion highlights that curcumin-induced autophagy is associated with cancer cell growth suppression and death. Future studies using animal models will further confirm the role of autophagy in the anticancer activities of curcumin.

2.2. Animal-Based Preclinical Studies

Curcumin has been most extensively investigated for its safety and efficacy in animal models. Animal studies have demonstrated the potential of curcumin against such diseases as cancer, lung diseases, neurological diseases, liver diseases, metabolic diseases, autoimmune diseases, cardiovascular diseases, and numerous other inflammatory diseases [88,89]. Although most of these studies have been conducted in rodents, the efficacy of curcumin has also been demonstrated in other animals such as monkeys [90], horses [91,92], rabbits [93–95], and cats [96]. In a recent study, curcumin exhibited anti-inflammatory activities in osteoarthritic-affected dogs [97]. In another study, curcumin was found to specifically bind to the aggregated A β molecules in various animals including monkeys, dogs, and bears [98]. In rabbits, administration of curcumin was found to reduce the contents of lipid and thio-barbituric acid reactive substances in the liver and plasma induced by pure cholesterol [94]. Liver glutathione peroxidase (GSH-Px) and catalase activities were significantly decreased in purely cholesterol-fed rabbits, but the addition of curcumin to the pure cholesterol diet enhanced liver GSH-Px activity [94]. Curcumin has also been shown to improve cardiac function via up-regulating the expression of sarcoplasmic reticulum Ca²⁺-ATPase in a rabbit model [99]. In another study, topical application of curcumin was useful in reducing experimental corneal neovascularization in rabbit eyes [100].

2.3. Clinical Studies

The extensive studies from cell-based and animal models have formed a solid basis for evaluating the safety and efficacy of curcumin against a plethora of human diseases (Table 2).



Curcumin's clinical efficacy against human biliary diseases was first studied in 1937 [101]. In this study, curcumin produced remarkably good results against cholecystitis. Since this initial discovery, observations from more than 65 human clinical trials of curcumin, which included more than 1000 patients, have been published, and more than 35 other clinical trials are under way to further evaluate the efficacy of this polyphenol against human diseases [102]. Among the most common human diseases against which curcumin has exhibited activities include cardiovascular disease, arthritis, uveitis, cancer, ulcerative proctitis, Crohn disease, ulcerative colitis, peptic ulcer, gastric ulcer, idiopathic orbital inflammatory pseudotumor, oral lichen planus, gastric inflammation, vitiligo, psoriasis, acute coronary syndrome, atherosclerosis, diabetes, Dejerine-Sottas disease, diabetic nephropathy, diabetic microangiopathy, lupus nephritis, renal conditions, acquired immunodeficiency syndrome, irritable bowel disease, tropical pancreatitis, β -thalassemia, cholecystitis, and chronic bacterial prostatitis. In these clinical trials, curcumin was used either alone or in combination with other agents such as gemcitabine, soy isoflavones, bioperine, quercetin, mesalamine, acetylcysteine, prednisone, lactoferrin, piperine, docetaxel, sulfasalazine, and pantoprazole. Although the molecular basis for curcumin's efficacy against some of these diseases is still not completely known, this polyphenol has been shown to modulate numerous signaling molecules including proinflammatory cytokines [tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6], apoptotic proteins, nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), cyclooxygenase-2, STAT3, I κ B kinase beta (IKK β), endothelin-1, malondialdehyde, C-reactive protein, prostaglandin E2, glutathione-S-transferase (GST), prostate specific antigen (PSA), vascular cell adhesion molecule 1 (VCAM1), GSH, pepsinogen, phosphorylase kinase (PhK), transferrin receptor, total cholesterol, transforming growth factor beta (TGF- β), triglyceride, creatinine, hemoxygenase-1, antioxidants, aspartate transaminase, and alanine transaminase in human participants. In most of the clinical trials, either a mixture of curcuminoids or turmeric from which curcumin is derived was used; pure curcumin has been used in only a few studies.

Although curcumin has shown efficacy against numerous human ailments, poor bioavailability due to poor absorption, rapid metabolism, and rapid systemic elimination limits its therapeutic efficacy [103]. As a result, numerous approaches including the use of adjuvants [104], nanoparticles [105], liposomes [106], phospholipid complexes [107], and structural analogues [103] have been used to increase the bioavailability of curcumin in human participants. The bioavailability of curcumin has also been shown to be greatly enhanced by reconstituting curcumin with the noncurcuminoid components of turmeric [108].

The safety, tolerability, and nontoxicity of curcumin at high doses have been well-established by human clinical trials. For instance, a phase I study evaluated the toxicology, pharmacokinetics, and biologically effective dose of curcumin in 25 patients with resected urinary bladder cancer, Bowen disease

of the skin, uterine cervical intraepithelial neoplasm, oral leukoplakia, or intestinal metaplasia of the stomach [109]. Curcumin was given orally for 3 months, and biopsy of the lesion sites was done immediately before and 3 months after starting curcumin treatment. There was no treatment-related toxicity with doses up to 8 g/day. However, because of the bulky volume of the drug, doses larger than 8 g/day were unacceptable to patients. Our own group found that curcumin at 8 g/day in combination with gemcitabine was safe and well-tolerated in patients with pancreatic cancer [110,111].

Curcumin has been used against human cancers including colorectal cancer, pancreatic cancer, breast cancer, prostate cancer, multiple myeloma, lung cancer, oral cancer, and head and neck squamous cell carcinoma. In these studies, curcumin was used for both prevention and treatment of cancer. In a recent nonrandomized, open-label clinical trial in smokers, the polyphenol reduced the formation of aberrant crypt foci (ACF), the precursor of colorectal polyps [112]. In this study, 44 smokers were given curcumin orally in two different doses (2 or 4 g/day) for 30 days. The levels of procarcinogenic eicosanoids, prostaglandin E2, and 5-hydroxyeicosatetraenoic acid in ACF or normal flat mucosa were unaffected by the 2 g/day curcumin treatment. Curcumin at 4 g/day, however, significantly reduced ACF formation, and this reduction was associated with a significant five-fold increase in posttreatment plasma curcumin/conjugate levels. Curcumin was well-tolerated at both concentrations. These findings demonstrated the effect of curcumin against ACF formation in smokers [112].

In another recent study, curcumin was administered to patients with colorectal cancer after diagnosis and before surgery [113]. Curcumin (360 mg in capsule form) was given three times a day for 10–30 days. Curcumin administration increased body weight, decreased serum TNF- α level, increased the number of apoptotic cells, and enhanced expression of p53 in tumor tissue. The authors of this study concluded that curcumin treatment can improve the general health of patients with colorectal cancer via the mechanism of increased p53 expression in tumor cells [113].

In some cases, curcumin has been used in combination with other agents. For example, a single-blind, randomized, placebo-controlled study evaluated the effects of combinations of oral curcumin and piperine on the pain and markers associated with oxidative stress in patients with tropical pancreatitis [114]. Curcumin administration in patients was associated with a significant reduction in erythrocyte malondialdehyde (MDA) levels and an increase in GSH levels. The pain, however, was not improved by curcumin administration [114].

In another study, the safety and feasibility of combinations of curcumin and gemcitabine were evaluated in 21 patients with gemcitabine-resistant pancreatic cancer. Curcumin at 8 g/day in combination with gemcitabine was safe and well-tolerated [111]. Curcumin has been shown to suppress PSA production in men with increased PSA [115]. Administration of a 1 g curcumin tablet for 1 week increased vitamins C and E levels and decreased MDA and 8-hydroxydeoxyguanosine

contents in the serum and saliva of patients with precancerous lesions [116].

The efficacy of curcumin as maintenance therapy in 89 patients with quiescent ulcerative colitis was evaluated [117]. Results indicated that relapse rates were 4.65% in the curcumin-treated group and 20.51% in the placebo group [117]. Ingestion of oral curcumin at 500 mg/day along with prednisone was associated with clinical and endoscopic remission of the disease in a patient with ulcerative colitis [118].

The efficacy of tetrahydrocurcuminoid in combination with narrowband ultraviolet B (NB-UVB) against vitiligo, a skin disorder, was investigated in one study [119]. Ten patients with focal or generalized vitiligo were treated with either NB-UVB plus topical tetrahydrocurcuminoid cream or with NB-UVB alone. Although NB-UVB and NB-UVB plus tetrahydrocurcuminoid produced significant improvements, the overall degree of repigmentation was slightly better in the combination group, and the tetrahydrocurcuminoid was well-tolerated [119].

The efficacy of a standardized preparation of curcuminoids (NCB-02) against various oxidative stress and inflammatory markers in patients with type 2 diabetes was evaluated [120]. The curcumin treatment significantly improved endothelial function and reduced oxidative stress (MDA) and inflammatory markers (IL-6, TNF α , endothelin-1) in these patients.

In summary, from the observations of some of the clinical trials discussed in this section, the efficacy of curcumin against human diseases seems promising. A search on www.clinicaltrials.gov indicated that curcumin is being evaluated for numerous human diseases including cancer, irritable bowel syndrome, inflammatory conditions, arthritis, neurological conditions, and diabetes. It is expected that these ongoing clinical trials will provide a deeper understanding of curcumin's efficacy and mechanism of action against human diseases.

3. Limitations of Curcumin Use

Curcumin's beneficial activities against human diseases are clear from the above discussion. However, some investigators have reported limitations with the use of this polyphenol. First, curcumin has been shown to inhibit the activity of drug-metabolizing enzymes such as cytochrome P450, GST, and UDP-glucuronosyltransferase *in vitro* and in animal models [121–123]. If this is the case in humans, people taking curcumin as well as drugs metabolized through these enzymes, such as digoxin, acetaminophen, and morphine, are at risk of undesired accumulation of these drugs in the plasma that may lead to toxicity. Second, the observation that curcumin can induce DNA damage in cells [124] raises a concern about the safety of curcumin since the induction of DNA alterations is a common event in carcinogenesis. Third, curcumin was recently found to be an active iron chelator and to induce anemia in mice fed iron-poor diets [125]. Whether curcumin intake produces similar effects in human subjects remains to be elucidated.

Fourth, curcumin at doses ranging from 0.45 to 3.6 g/day for 1–4 months was associated with nausea and diarrhea and caused an increase in serum alkaline phosphatase and lactate dehydrogenase contents in human subjects [126]. Fifth, in patients with high-risk or premalignant lesions, curcumin at doses higher than 8 g/day was unacceptable [109]. Sixth, in one study of patients with advanced pancreatic cancer, 5 of 17 patients receiving curcumin (8 g/day) in combination with gemcitabine reported intractable abdominal pain after a few days to 2 weeks of curcumin intake [127]. Seventh, curcumin has been shown to possess both pro-oxidant and antioxidant activities in cancer cells that may be both good and bad because of the dual role of ROS for cancer [128]. Thus, more studies are needed to evaluate the efficacy of this polyphenol before it can be approved for human use.

4. Conclusions

Since ancient times, curcumin has been used in Asian countries. Modern science has delineated the molecular basis for the pharmaceutical uses of curcumin against human ailments. Multiple studies over the past decade have indicated the safety and efficacy of this polyphenol in rodents, monkeys, horses, rabbits, and cats and have provided a solid basis for evaluating its efficacy in human clinical trials. In human clinical trials, curcumin has been found to be safe at gram doses. Although curcumin's safety and efficacy have already been proven by numerous clinical trials, the polyphenol has not yet been approved for the treatment of any human diseases. Furthermore, because of the fact that turmeric is more effective than curcumin, we believe that by using curcumin alone, we might be limiting ourselves from the various utilities of turmeric. We hope that numerous ongoing studies will help to move this fascinating molecule to the forefront of therapeutics for human use.

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