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Hitting the Golden TORget: Curcumin’s Effects on mTOR Signaling

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Abstract: The polyphenol natural product curcumin possesses a plethora of biological and pharmacological properties. For years, much interest has been placed in the development and use of curcumin and its derivatives for the prevention and treatment of cardiovascular, diabetic, and neurodegenerative diseases, as well as cancer. Increasing evidence suggests that curcumin displays amazing molecular versatility, and the number of its proposed cellular targets grows as the research continues. The mammalian target of rapamycin (mTOR) is a master kinase, regulating cell growth/proliferation, survival, and motility. Dysregulated mTOR signaling occurs frequently in cancer, and targeting mTOR signaling is a promising strategy for cancer therapy. Recent studies have identified mTOR as a novel target of curcumin. Here we focus on reviewing current knowledge regarding the effects of curcumin on mTOR signaling for better understanding the anticancer mechanism of curcumin. The emerging studies of mTOR signaling and clinical studies on curcumin with cancer patients are also discussed here.

Keywords: Curcumin, mTOR, Akt, Cancer, Cell proliferation, Cell death.

CURCUMIN

Phytochemicals are plant-based molecules that are non-nutritive in nature, but possess beneficial pharmacological actions in the human body when ingested [1]. Over the past decades, interest in the potential use of phytochemicals as chemopreventive and chemotherapeutic agents has grown both scientifically and publically, with the greatest amount of attention being paid to their potential application in the fight against cardiovascular disease and cancer [1]. Turmeric, a spice produced from the rhizome of the perennial Asian herb Curcuma longa, has been especially popular in this growing interest in botanical pharmacology, owing to its distinguished and long history as a therapeutic agent in Oriental medicine for several thousand years. In this capacity, turmeric has been utilized topically for the treatment of open wounds, skin tumors, and inflammatory conditions, while orally ingested forms have been used to remedy gastrointestinal disorders and other inner ailments [1, 2].

Analyses of turmeric have identified its major chemical constituents as a family of polyphenolic compounds called the curcuminoids, which include curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], curcumin II (demethoxycurcumin), and curcumin III (bis-demethoxycurcumin) [1, 2]. Curcumin is the most biomedically potent chemical component in turmeric, and it comprises approximately 2-8% of most turmeric preparations [1, 2]. Hundreds of scientific studies conducted over the past 30 years have extensively studied the chemical, biochemical, pharmacological, and clinical properties of curcumin [1, 2]. The vast majority of these investigations have specifically attempted to elucidate the potential chemopreventive and chemotherapeutic value of this phytochemical in the battle against human diseases, particularly neurodegenerative disorders, inflammatory conditions, gastrointestinal disorders, cardiovascular diseases, and cancer [1, 2].

Curcumin (molecular weight: 368.37) possesses a conjugated double bond heptadienone linker containing a bis-α,β-unsaturated β-diketone moiety [1]. This linker joins together the molecule’s two methoxyphenol rings [1]. The diketone moiety undergoes tautomerization in a pH-dependent fashion [1]. The bis-keto tautomer displays potent Bronsted acid activity, while the enol tautomer functions as a powerful Lewis base [3]. Curcumin is insoluble in water, but is soluble in organic solvents such as acetone, ethanol, and dimethylsulfoxide (DMSO) [2]. In neutral/alkaline solutions the compound displays a dark red color, while in acidic solutions it adopts a vibrant yellow color [2]. Curcumin is extremely unstable in phosphate-based buffers, normal cell culture media, and most alkaline solutions, while it is extremely stable in acidic solutions [1, 2, 4].

A number of clinical trials have addressed the safety and pharmacokinetics of curcumin in humans. The safety and tolerability of curcumin at high doses are well established [5-7]. In patients with high-risk or pre-malignant lesions, oral dose ranging from 500 to 8,000 mg/day for 3 months are well tolerated [6]. However, the in vivo bioavailability of curcumin is poor, due to a combination of poor aqueous solubility, poor gastrointestinal absorption, efficient first pass metabolism, and rapid elimination [8, 9]. After oral dosing, curcumin is metabolized into several chemical species, including curcumin glucuronide, curcumin sulfate, hexahydrocurcumin, tetrahydrocurcumin, and dihydrocurcumin [10]. One of the current major initiatives in the field of curcumin study has been the manipulation and optimization of the compound’s pharmacokinetic characteristics via the development of curcumin derivatives and curcumin-drug vehicle combinations that display greatly enhanced absorption and systemic bioavailability, and many of these studies appear to be very promising [10, 11].

Without question, the most intensive study of curcumin has been exploration of its pharmacodynamic profiles, namely its mechanisms of action, pharmacological actions, and pharmacological effects, and how these profiles are potentially translated into clinical use and responsiveness. Those studies have been carried out extensively in vitro (human cell lines) and in vivo (both animals and humans), and have been thoroughly reviewed [1, 2, 9, 10, 12, 13]. Consistent with the strong pre-clinical evidence of its pharmacological activities, curcumin has been in early clinical trials for...
treatment of a variety of human diseases, including rheumatoid arthritis [14, 15], ulcerative colitis, Alzheimer’s disease [15], multiple myeloma [15], pancreatic cancer [15, 16], and colon cancer [2, 15]. Here we only focus on reviewing some clinical trials related to cancer.

A phase I study evaluated the toxicology, pharmacokinetics, and biologically effective dose of curcumin in 25 patients with various types of high-risk or pre-malignant lesions [6]. After an initial dose of 0.5 g curcumin daily, the dose was increased to as much as 8 g daily for 3 months. There was no treatment-related toxicity up to 8 g/day. Histological improvement of precancerous lesions was observed in 1 of 2 patients with recently resected bladder cancer (decreased dysplasia and inflammation), 1 of 6 patients with intestinal metaplasia of the stomach (fewer goblet cells), 1 of 4 patients with cervical intraepithelial neoplasm (decreases in hyperkeratosis, parakeratosis), 2 of 7 patients with oral leukoplakia, and 2 of 6 patients with Bowen’s disease, indicating a biologic effect of curcumin in the chemoprevention of cancer [6].

Two clinical phase I dose-escalation studies have investigated the use of curcumin therapy in patients with advanced colorectal cancer [7, 17]. In the pilot study, 15 patients received an oral capsule of Curcuma extract at doses between 440 and 2,200 mg/day, containing 36-180 mg of curcumin, for up to 4 months [7]. The compound was well tolerated, and dose-limiting toxicity was not observed. The lymphocytic biomarker glutathione S-transferase (GST) activity showed a 59% decrease with ingestion of low-dose (440 mg/day) of curcuma extract, and radiologically stable disease was demonstrated in 5 patients for 2-4 months of the study period [7]. In a subsequent study in a similar population, Sharma et al. further explored the pharmacology of curcumin administered in capsules compatible with curcumin doses between 0.45 and 3.6 g/day for up to 4 months [17]. Three biomarkers of the potential activity of curcumin were measured in patient blood leukocytes: GST activity, levels of deoxyguanosine adduct M(1)G, and PGE2 activity of curcumin were measured in patient blood leukocytes: capsules compatible with curcumin doses between 0.45 and 3.6 g/day for up to 4 months [17]. Three biomarkers of the potential activity of curcumin were measured in patient blood leukocytes: GST activity, levels of deoxyguanosine adduct M(1)G, and PGE2 production induced ex vivo. In blood samples taken 1 h after the dose on Days 1 and 29, consumption of 3.6 g of curcumin daily decreases inducible PGE2 production by 62% and 57%, respectively, when compared with levels observed immediately before administering the drug [17]. Consequently, the 3.6 g dose was chosen for further evaluation in phase II trial in cancers outside the gastrointestinal tract [17].

In another clinical trial, 25 patients with advanced pancreatic cancer received 8 g curcumin orally every day, and 21 of them exhibited evaluable responses [16]. Of note, one patient achieved complete disease stabilization for 18 months, and another patient had a brief, but marked, tumor regression (73%) accompanied by 4- to 35-fold increases in serum cytokine levels (IL-6, IL-8, IL-10 and IL-1 receptor antagonists) [16]. Downregulated expression of NF-kB, COX-2, and phosphorylated signal transducer and activator of transcription 3 (Stat3) by curcumin in peripheral blood mononuclear cells from patients were also observed [16]. In an interesting, but uncontrolled study of 62 patients with oral cancerous lesions, topical curcumin application produced remarkable symptomatic relief in patients. Dry lesions were noted in 70% of the cases, and a reduction in lesion size and pain was observed in 10% of patients [18]. Currently, a search on the web of http://clinicaltrials.gov shows that there are 17 clinical trials investigating the preventive or therapeutic efficacy of curcumin in cancer patients. Five studies have completed, and 12 are ongoing. Hopefully, these ongoing trials over the next few years will provide a better understanding of the anticancer potential of curcumin in patients with different cancers.

Preclinical studies in cell culture and in animal models strongly support that curcumin has the ability to exert anti-cancer actions, including inhibiting cell proliferation/growth and motility [2, 13, 19], inducing cell death, and inhibiting angiogenesis [2, 13, 19, 20]. These studies also suggest that curcumin possesses amazing molecular versatility as evidenced by the large number of proposed drug receptors for the compound, including plasma membrane-bound receptors, proteases, transporters, apoptotic factors, kinases, transcription factors, and adhesion molecules [2, 12]. While it remains a possibility that curcumin is in fact interacting with this large range of molecules, a more plausible explanation is that curcumin predominately targets only a few key cell regulators, and these actions spill over to affect many different pathways, factors, and processes within the cell. Identifying these key cellular regulators, however, remains challenging due to the seemingly diverse molecular promiscuity of the compound. Recently, the mammalian target of rapamycin (mTOR) has been identified as a novel molecular target of curcumin, which may in fact represent one of these central targets due to the fact that mTOR stands at the center of numerous key cellular processes (cell growth/proliferation, survival and motility), almost all of which are affected to some degree by curcumin.
phosphorylation of the 40S ribosomal subunit, which has been suggested to increase the translational efficiency of a class of mRNA transcripts with a 5'-terminal oligopyrimidine sequence [43]. Recently, it has been further proposed that the mechanism by which S6K1 regulates translation is likely by phosphorylation of eIF4B at Ser 422 [44], which causes it to associate with eIF3 and phosphorylation of the 40S ribosomal subunit, which has been shown to promote cap-dependent translation [47, 48]. Phosphorylation of 4E-BP1 by mTORC1 stimulates protein synthesis through the release of eIF4E from 4E-BP1, allowing eIF4E to associate with eIF4G and other relevant factors to promote cap-dependent translation [47, 48].

mTOR truly functions as a master cellular regulator. It acts as a sensor of growth factor stimuli [25], nutrient [23, 49], energy [49], redox [50], and oxygen levels [51, 52] (Fig. 1). mTOR controls numerous cellular processes such as cell proliferation [53], cell growth (size) [23, 54], cell motility [55], cell survival [56, 57], and metabolism [53]. In humans, dysregulated mTOR signaling is implicated in a wide range of disease processes [53, 58, 59], including tuberous sclerosis [53, 60], diabetes [61, 62], obesity [62], lymphangiomyelomatosis [63], and most types of cancer [58, 62, 64, 65]. mTORC1 and mTORC2 are essential complexes, as downregulation of Raptor, Rictor, and mSin1 leads to embryonic lethality [66, 67]. mTORC1 regulates, to varying degrees, translation [53, 68, 69], translation initiation [25, 48], nutrient transport [56, 70], autophagy [71-73], and ribosome biogenesis [74, 75]. mTORC2 controls actin cytoskeleton organization, cell shape [76, 77], and cell motility via its effects on the Rh-type GTPases [78], focal adhesion proteins, and protein kinase C (PKC) [77]. mTORC2 also regulates cell survival via activation of Akt and serum- and glucocorticoid-inducible kinase 1 (SGK1) [32, 78] and the subsequent regulation of its numerous cellular targets, including the Foxo family of transcription factors [79] and the apoptosis-regulating Bcl-2 family of proteins such as BAD [80].

CURCUMIN AND mTOR: THE GOLDEN COUPLE

The worlds of curcumin and mTOR first collided in studies conducted in rhabdomyosarcoma (RMS) cell lines [20]. Curcumin inhibited proliferation and induced apoptosis of RMS cells in a concentration-dependent manner (2.5-40 μM) [20]. Curcumin inhibition of cell proliferation was related to the arrest of cells in the G1/G0 phase of the cell cycle [20]. Curcumin also blocked the basal and IGF-1-stimulated cell motility of these RMS cells [20]. It has been well documented that RMS is a type of cancer that possesses as one of its major genetic/molecular features the upregulation and dysregulation of the IGF-1R/PI3K/Akt/mTOR signal transduction cascade [20, 55, 81]. Of note, rapamycin, the first identified inhibitor of mTORC1 signaling, successfully inhibits the proliferation/growth, motility, and survival of RMS cells [55, 57, 82, 83]. This information drove the authors to investigate whether or not curcumin might be acting on some component of the IGF-1R/PI3K/Akt/mTOR pathway. Subsequent experiments demonstrated that curcumin inhibited the IGF-1-stimulated, mTORC1-mediated phosphorylation of S6K1 and 4E-BP1 at low concentrations (~2.5 μM), and the IGF-1-stimulated, mTORC2-mediated phosphorylation of Akt at higher concentrations (~40 μM) in RMS cells [20]. Similar results were also observed in prostate (DU145), breast (MCF-7), and cervical (HeLa) cancer cell lines [20].

Upon the publication of these results, numerous other investigations began confirming the interaction of curcumin with the mTOR pathway. Curcumin treatment of human prostate cancer cells (PC3) lead to a dose- and time-dependent decrease in the transcriptional expression of the p53 ubiquitin E2 ligase murine double minute 2 (MDM2), and this was mediated via curcumin action on the PI3K/mTOR/ETS2 pathway [84]. Curcumin induced G2/M cell cycle arrest and autophagy in two human malignant glioma cell lines (U87-MG and U373-MG) [85]. These effects were
related to curcumin inhibition of mTORC2 (as indicated by the phosphorylation of Akt) and activation of the ERK pathway, as reconstitution of Akt activity and inhibition of ERK (use of PD98059) prevented curcumin-mediated autophagy [85]. Curcumin treatment also prevented growth of these tumor cells in vivo by inducing autophagy [85]. Curcumin was able to decrease the transcriptional and translational expression of mTOR, Raptor, and Rictor in human colorectal cancer cells (HCT116) [86]. Lim et al. suggested that curcumin-mediated inhibition of mTOR signaling could be the result of its function as a protonophoric uncoupler and activator of F$_{0}$F$_{1}$-ATPase, thus leading to 5′-AMP-activated protein kinase (AMPK) activation and its induction of mTOR inhibition [87]. Curcumin was able to attenuate irradiation-mediated activation of Akt and mTOR phosphorylation in human intestinal microvacular endothelial cells and this correlated with curcumin-mediated induction of apoptosis [88]. The compound was able to block basal and nicotine-stimulated mTORC1 signaling in human head and neck squamous cell carcinoma cell lines (PCI15a and SCC40) and this correlated with inhibition of cell proliferation, invasion, and migration [89]. A study conducted in adenoid cystic carcinoma cells suggested that curcumin inhibitory action on both mTOR signaling and the NF-κB pathway may be related to cross-talk via the PI3K/Akt/IKb kinase network [90]. Curcumin was able to inhibit mTORC1 signaling in autosomal dominant polycystic kidney disease, even in the presence of a deletion of the gene encoding polycystin-1 (PC1), a molecular genotype that results in an activated mTOR pathway phenotype [91]. Curcumin also inhibited mTORC1 signaling in human leiomyosarcoma cells (SKN), an event that was correlated with decreased cell growth/proliferation and induction of apoptosis [92], and this activity of curcumin was enhanced when it was administered in combination with epigallocatechin-3-gallate (EGCG) [93]. The compound also successfully abrogated mTORC1 signaling in squamous cell carcinoma cells in vivo [94]. Curcumin, as well as rapamycin and several other compounds, successfully enhanced the efficiency of reprogramming somatic cells into induced pluripotent stem cells, more than likely via inhibition of the IGF-1/mTOR signaling pathway [95].

To date, at least two major studies [26, 96] have been undertaken to determine the mechanism of action by which curcumin disrupts mTORC1 and mTORC2 signaling. The first was conducted by Yu et al. utilizing human prostate cancer cells (PC3) [96]. This study demonstrated that curcumin inhibited the mTORC2- and PDK1-mediated phosphorylation of Akt and the mTORC1-mediated phosphorylation of S6K1 and 4E-BP1 at similar concentrations [96]. This study also found that curcumin stimulated the phosphorylation and activity of AMPK, which serves as a positive regulator of TSC2 [96]. Use of a PI3K-rescue agent and a phosphatidylinositol-dependent kinase 1 (PDK1) in vitro kinase assay revealed that curcumin action on the complexes of mTOR was independent of PI3K and PDK1, while overexpression of Akt did not prevent curcumin inhibition of mTORC1-mediated S6K1 and 4E-BP1 phosphorylation [96]. Overexpression of wild-type, dominant-negative AMPK and pretreatment of cells with the AMPK inhibitor compound C did not block curcumin abrogation of mTORC1-mediated phosphorylation of its two major downstream targets [96]. Genetic and siRNA knockdown of TSC2 also failed to interfere with curcumin-mediated inhibition of mTORC1 activity [96]. Finally, use of the serine/threonine protein phosphatase inhibitors calyculin A and okadaic acid was able to reverse curcumin inhibition of both mTORC1 and mTORC2 signaling, suggesting that curcumin might be acting directly as an activator of protein phosphatase 2A (PP2A), thus leading to inhibition of the mTOR pathway [96]. This is indeed a possibility because PP2A functions as the major phosphatase responsible for the dephosphorylation of the mTORC1 substrates S6K1 [97, 98] and 4E-BP1 [99], and the mTORC2 substrate Akt [97, 100].

The second study was carried out primarily in RMS cells by Beevers et al. [26]. This study also showed that the IGF-1R, the PDK1, and the AMPK/TSC2 pathways did not play a role in curcumin-mediated inhibition of mTORC1 and mTORC2 signaling [26]. However, PP2A was found not to be involved in curcumin action on the mTOR pathway, as the use of okadaic acid, expression of dominant-negative PP2A, and shRNA-mediated downregulation of PP2A was unable to prevent curcumin inhibition of mTORC1 and mTORC2 signaling in rhabdomysarcoma cells [26]. Whether the discrepancy between the studies [26, 96] is related to different cell lines or experimental conditions used remains to be defined. Of interest, Beevers et al. observed that low concentrations of curcumin (2.5 μM) blocked the kinase activity of the mTORC1 by disrupting mTOR-Raptor interaction and that higher concentrations of the compound (40 μM) inhibited the kinase activity of the mTORC2 by disrupting mTOR-Rictor interaction [26]. This concentration-dependent effect of curcumin on the two complexes of mTOR fits data concerning the nature of the two complexes. The interaction of mTOR with Raptor in the mTORC1 is a weak and dynamic association, facilitating the large amount of control that is needed over mTORC1 activity [23], while the mTOR-Rictor interaction in the mTORC2 appears to be much stronger and more static association [77]. At that time (2008), these results suggested that curcumin was the first, and currently only, identified compound to exert activity against both complexes of mTOR specifically by disrupting complex partner interactions.

CONCLUSIONS

mTOR is a master kinase that controls cell proliferation/growth, survival, and motility. Over the past 5 years, mTOR has emerged as an exciting and novel molecular target for curcumin, particularly in cancer cell lines. It appears that curcumin inhibits both mTORC1 and mTORC2, but in a concentration-dependent manner. There exist some discrepant findings regarding the mechanism by which curcumin inhibits mTOR signaling pathways. The findings have opened the doors to many different avenues of research into the interactions between curcumin and mTOR and the cellular processes and factors affected by this interaction. Obviously, much work remains to be done, including identifying the exact mechanism by which curcumin disrupts mTOR activity and complex formation/stability and whether or not these results can translate over into other disease states and/or normal human tissue as well. Obviously, the ultimate aim of these molecular studies would be to attempt to replicate these discoveries within in vivo cancer models in animals. Greater demonstration of successful replication of these results in animal models may provide the necessary stimulus to induce the initiation of clinical trials in humans using various formulations/derivatives of curcumin as chemotherapeutic and/or chemopreventive agents against many types of cancer.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Curcumin Inhibition of mTOR


