

Curcumin a potent cancer preventive agent: Mechanisms of cancer cell killing

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Abstract: There is no doubt that diet could effectively improve health and halt cancers. Dietary phytochemical compounds and their derivatives represent a cornucopia of effectively anticancer compounds. This review discusses existing data on the anticancer activities of curcumin, and then offers possible explanations for and mechanisms of its cancer-preventive action. This review also offers insights into the molecular mechanism and targets through which curcumin modulates cell cycle, apoptotic signals, anti-apoptotic proteins, miRNAs, Wnt/beta-catenin signaling, protein kinases, nuclear factor- κ B, proteasome activation, epigenetic regulation including DNA methylation and histone modification. Finally, this review provides explanations for how curcumin reverses the multi-drug resistance (MDR) of cancer cells.

Keywords: cell cycle arrest, apoptotic signals, miRNAs, proteasomes, DNA methylation, histone modification

Introduction

Compounds of natural origin could lead to new, innovative therapeutic agents for cancer. Mans et al. have enlisted several examples of naturally derived anticancer compounds [1, 2], for example, vincristine, which is derived from the periwinkle plant *Vinca rosea*; etoposide, which is derived from the mandrake plant *Podophyllum peltatum*; and taxol, which is derived from the pacific yew *Taxus brevifolia*. A number of promising new agents are in clinical development based on their selective molecular targets in the field of oncology [3]. Yet, due to the depth of our understanding for the etiology of many diseases that enabled us to design and synthesis of drug molecules for specific molecular targets. Therefore, we can shift the attention from the chemically synthetic drugs to the purely natural ones [4, 5]. Curcumin, a yellow pigment obtained from the rhizomes of *Curcuma longa* (Family: *Zingiberaceae*), is a major component of turmeric and is commonly used as a spice and food coloring agent [6]. *C. longa* has been used in traditional remedy for a wide range of ailments, including wound healing, urinary tract infection, and liver ailments [7]. Various metabolites of curcumin have been reported, including dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocur-

cumin (HHC), octahydrocurcumin (OHC), curcumin glucuronide, and curcumin sulphate [8]. Curcumin has been speculated to have promising chemotherapeutic and preventive activities, which could approve avenues for alternative treatments for many diseases. Recently, much attention has been directed to study the medical applications of curcumin in the treatment of human cancers, since curcumin has been shown to exhibit antitumor and apoptosis activities in a wide spectrum of human cancer cell lines [6], that is aside its prospective role as a potential immunomodulatory effector both *in vivo* and *in vitro* studies [9–11]. Currently, several clinical trials have been applied curcumin for treatment of pancreatic cancer [12], multiple myeloma [13, 14], Alzheimer's [15], and colorectal cancer [16]. Curcumin is also known to activate and regulate dendritic cells and inhibit IL-1, IL-6, and TNF- α along with inhibition of NF- κ B activation [17]. Curcumin could prevent production of interleukin-8 (IL-8), monocyte inflammatory protein-1 (MIP-1 α), monocyte chemoattractant protein-1 (MCP-1), IL-1 β , tumor necrosis factor- α (TNF- α), 4- β -phorbol-12- β -myristate-13- α acetate (PMA), or lipopolysaccharide (LPS)-stimulated monocytes and macrophages [18]. The exact molecular mechanisms of curcumin-induced apoptosis in cancer cells are varied and depend on the cell type and dose used in the experiments [19, 20].

Cell Cycle Arrest

The cell cycle, arranged in the following phases, leads to cell growth and division:

- In G₁ phase, the cell grows and chromosomes prepare for replication.
- In S phase, DNA replicates and chromosomes duplicate.
- G₂ phase represents the gap between DNA synthesis and mitosis.
- In M phase (mitosis), nuclear and cytoplasmic division occurs, yielding two daughter cells.

Curcumin was found to induce cell cycle arrest at the G₀/G₁ phase in leukemic cells [21] and S and G₂/M phases in breast cancer cells and human bladder cancer [22, 23]. The major cell-cycle proteins, cyclin-dependent kinase 1, 2, and 4 (CDK 1, 2, and 4), are considered potential molecular targets of curcumin [24]. Curcumin may inhibit the expression of cyclin D1 and CDK4 via acetylation and upregulation of p53, leading to cell cycle arrest at G₁-S phase in cervical cancer [25]. Furthermore, curcumin acts as an ATP-competitive inhibitor; it downregulated the mRNA and the protein expression of cyclin D1 and suppressed transition of the cells from G₁ to S phase, thus, prevents invasion of gastric cancer cells [26]. In human prostate cancer, curcumin mediates cycle arrest at G₁/S phase through induction of CDK inhibitors p16(/INK4a), p21(/WAF1/CIP1), and p27(/KIP1), inhibition of cyclin E and cyclin D1 expression, and hyperphosphorylation of retinoblastoma (Rb) protein, a well-known CDK2 substrate [27]. However, in HCT116 colon cancer cells, the expression levels of CDK2 and its regulatory subunit, cyclin E, were not changed, but the phosphorylation of Rb was declined by curcumin [28]. In Ras-activated cells, curcumin enhanced Erk1/2 expression and inhibited Akt and its downstream molecules (mTOR and S6K1), thus, promotes G₂/M arrest, reflecting a potential role of Ras/Erk1/2 activation in curcumin-induced G₂/M arrest [29]. Treatment of Jurkat cells with different doses of curcumin enhanced JNK and p-JNK expressions without affecting the expressions of ERK1/2 and P38 MAPK or the activity of MMP-2 and MMP-9. Thus, the activation of JNK pathway but not the MMPs is the potential mechanism which might involve in cell proliferation [30].

Induction of Apoptotic Signals

Apoptosis occurs through two major pathways, the extrinsic and intrinsic pathways. Intrinsic apoptosis operates by modulating of mitochondrial membrane potential ($\Delta\Psi_m$) which releases cytochrome *c* and inhibits expression of antiapoptotic proteins Bcl-2 and Bcl-xL.

Curcumin suppresses the Bcl-xL level, mediating imbalance in the mitochondrial membrane potential and enhancing cleavage of procaspases and poly(ADP-ribose) polymerase [31]. The extrinsic apoptosis operates through induction of TNF-related apoptosis and enhanced expression of death receptors and their downstream factors, such as DR4, DR5, tumor necrosis factor receptor apoptosis-inducing ligand (TRAIL), and Fas/FasL [32]. When the apoptotic signal is received, Fas-associated death domain (FADD) binds and recruits the death-induced signaling complex (DISC) forming initiator caspases 8 and 10 [33]. In TNF-related apoptosis inducing ligand (TRAIL)-resistant cell lines, curcumin enhanced apoptosis by upregulating the expression of DR 4 and 5 [34]. However, a recent study concluded that curcumin-induced production of reactive oxygen species did not affect total expression of DR5 but it enhanced mobilization of DR5 to the plasma membrane [35]. Curcumin induced upregulation of Fas, FasL, and DR5 expression [36] and inhibited TNF- α -induced activation of NF- κ B, including NF- κ B-P65, and thus mediates apoptosis [37]. Curcumin also inhibited the TNF- α -induced production of IL-6/IL-8 in HaCaT cells [37]. Curcumin could also induce apoptosis through p38-dependent upregulation of FasL in Huh7 cells [38]. The endoplasmic reticulum (ER) is pointed as a third subcellular compartment implicated in apoptotic execution [39, 40]. Gadd153, growth arrest and DNA damage-inducible protein, is activated by endoplasmic reticulum (ER) stress, resulting in the induction of oxidative stress, generation of reactive oxygen species (ROS), and disturbing iron homeostasis, which in turn mediates cellular-damage/apoptosis [41, 42]. Curcumin can mediate DNA damage whether through topoisomerase inhibition [43, 44] or via generation of ROS [45]. Like other proteasome inhibitors, curcumin upregulates the expression of the growth arrest- and DNA damage-inducible genes GADD45 and 153 [46–48].

Suppression of the Antiapoptotic Proteins

Curcumin decreases expression of the antiapoptotic Bcl-2 and Bcl-XL proteins in lung cancer and prostate cancer cell lines [49, 50]. However, an induction of expression of proapoptotic Bax protein was observed in Ehrlich's Ascites carcinoma upon curcumin treatment [51]. Bush and colleagues (2001), reported suppression of X-linked inhibitors of apoptosis (XIAP) protein, a caspase inhibitor, in human melanoma cells in response to curcumin treatment [52]. Several studies showed that curcumin is able to break the apoptosis resistance in multidrug-resistant cancer cells by modulating the expression of many resistance-associated proteins [53, 54].

Modulation of MicroRNAs (miRNAs)

miRNAs are a class of noncoding RNAs that post-transcriptionally inhibit gene expression, and their role in carcinogenesis as tumor suppressors or oncogenes has been widely reported [55]. In MCF-7 and leukemia cells, curcumin upregulating the expression of a miR-15 and miR-16-mediated downregulation of bcl-2 induced apoptosis [56–58], suggesting that the miR-15a/16 family can potentially serve as potential gene targets for bcl-2 overexpressing cancer cells. Curcumin could promote the apoptosis of A549/DDP cells through regulating the expression of miR-186 [59]. Further studies showed that curcumin altered miRNA expression, in particular, significantly downregulated the expression of miR-186 in A549/DDP [60]. miR-22, a tumor-suppressor miRNA, was one of the miRNA which was upregulated by curcumin [61]. Last but not the least, curcumin modulates miRNAs (miR-15a, miR-16, miR-21, miR-22, miR-26, miR-101, miR-146, miR-200, miR-203, and let-7) and their multiple target genes [62].

Modulation of Wnt/ β -Catenin Signaling

Wnt/ β -catenin signaling regulates the proliferation and differentiation of many normal and malignant cells. A number of studies have suggested that curcumin has the potential to target cancer stem cells (CSCs) through regulation of CSC self-renewal pathways including Wnt/ β -catenin pathway [63]. Furthermore, curcumin treatment has found to activate GSK-3 β and reduce expression of β -catenin and its downstream target cyclin D1 [64]. Curcumin as a Wnt inhibitor targets downstream β -catenin activity and effectively represses HBx-mediated regulation of c-MYC and E-cadherin [65]. Curcumin also modulates the Wnt/ β -catenin signaling pathway and, thus, could inhibit LNCaP prostate cancer cells activity [66]. The expression levels of two integral proteins of Wnt signaling, GSK3 β , and E-cadherin were also altered by curcumin treatment, in human breast cancer cells [67].

Modulation of Protein Kinases

It is considered that PKC, mTOR, and EGFR tyrosine kinase are the major upstream molecular targets for curcumin intervention, whereas the nuclear oncogenes such as c-jun, c-fos, c-myc, CDKs, FAS, and iNOS might act as downstream molecular targets for curcumin actions [68]. The oxidant tumor promoter TPA activates PKC by reacting with zinc thiolates present within the regulatory domain, while the oxidized form of cancer chemopreventive agent such as

curcumin can inactivate PKC by oxidizing the vicinal thiols present within the catalytic domain [69]. Due to its ability to inhibit Src, JNK, and Smad3 phosphorylations, curcumin abrogates the TGF β 1-induced tissues overgrowth [70]. Curcumin directly induces a tumor-suppressive miR-203-mediated regulation of the Src-Akt axis in bladder cancer [71]. The downregulation of miR-203 may be due to DNA hypermethylation of its promoter. Another potential mechanism is that curcumin inhibits phosphorylation of Src and stat3 partly through regenerating liver-3 (PRL-3) downregulation [72]. Curcumin may play its anticancer actions partly via suppressing PI3K/Akt signal transduction pathway in several tumor models [73–75]. Curcumin significantly inhibited NF- κ B and attenuated the effect of irradiation-induced prosurvival signaling through the PI3K/Akt/mTOR and NF- κ B pathways in these gut-specific endothelial cells [76].

Inhibition of Nuclear Factor- κ B (NF- κ B)

NF- κ B is synthesized in the cytoplasm and complexed with its inhibitor I- κ B; thus, NF- κ B is released in an activate form. In order to activate, I- κ B must be phosphorylated followed by a proteasomal degradation of the NF- κ B/p- κ B complex. The free p-NF- κ B then translocates to the nucleus to transcribe and activate genes to synthesize progrowth and antiapoptosis [77]. Treatment with curcumin and resveratrol suppressed NF- κ B-regulated gene products involved in inflammation (cyclooxygenase-2, matrix metalloproteinase (MMP)-3, MMP-9, vascular endothelial growth factor), inhibited apoptosis (Bcl-2, Bcl-xL, and TNF- α receptor-associated factor 1), and prevented activation of caspase-3. Curcumin inhibits the activation of NF- κ B and the expressions of oncogenes including c-jun, c-fos, c-myc, NIK, MAPKs, ERK, ELK, PI3K, Akt, CDKs, and iNOS [69]. Curcumin prevents the entry of NF- κ B into the nucleus thereby decreasing the expression of cell cycle regulatory proteins and survival factors such as Bcl-2 and surviving [24]. The long-term effect of curcumin may contribute to attenuate cancer progression via the downregulation of TNF- α and IL-6 modulated by E26 transformation-specific protein (ETS) and nuclear factor- κ B (NF- κ B). That is may through inhibition binding of nuclear protein with ETS and NF- κ B binding elements of TNF- α and IL-6 promoters, respectively [78]. TNF-induced NF- κ B-regulated gene products involved in cellular proliferation COX-2, cyclin D1, and c-myc, antiapoptosis including inhibitor of apoptosis protein (IAP)1, IAP2, X-chromosome-linked IAP, Bcl-2, Bcl-x(L), and metastasis (vascular endothelial growth factor, matrix metalloproteinase-9, and intercellular adhesion molecule-1) were also downregulated by curcumin [79].

Modulation of Proteasome Activation

Curcumin-induced apoptosis is mediated through the impairment of ubiquitin proteasome system (UPS) [46]. Curcumin can inhibit activation of the proinflammatory transcription factor NF- κ B by inhibiting the 26S proteasomal degradation of I κ B α , an inhibitor of NF- κ B [80]. Furthermore, the inhibition of the 26S proteasomal activity by curcumin is mediated through α,β -unsaturated ketone and two sterically accessible β -carbons. On the other hand, curcumin, a potent inhibitor of the JNK-AP-1 pathway, abrogated the induction of monocyte chemoattractant protein 1 (MCP-1) by MG132, a proteasome inhibitor. The transcriptional activation by proteasome inhibitors was observed not only in MCP-1, but also in other AP-1-dependent genes, including stromelysin and mitogen-activated protein kinase phosphatase 1 [81]. These data revealed that curcumin-mediated proteasome inhibition triggered the expression of MCP-1 and other genes via the multisteps induction of the JNK-c-Jun/AP-1 pathway.

Epigenetic Regulation

In recent years, researchers have extensively documented that epigenetic mechanisms such as DNA methylation and histone modifications regulate many cancer cells activities, and thus, epigenetic regulation has been postulated as an attractive target for the cancer therapeutics [82]. In fact, the human genome has four DNA methyltransferase (*DNMT*) genes, which encoded proteins with distinct functions [83]. The new advantage of epigenetic modifications is that they can be modulated by treatment with HDAC (histone deacetylase) and DNMT (DNA methyltransferase) inhibitors, some of which have already been approved by the FDA for the treatment of myelodysplastic syndromes and acute myeloid leukemia [84]. The U.S. Food and Drug Administration has already approved some HDAC and DNMT inhibitors, such as azanucleoside drugs to treat myelodysplastic syndromes and acute myeloid leukemia [85]. By taking advantage of epigenetic modifications, we can use HDAC and DNMT inhibitors to control various cancer cell activities. Histone tails and their modifications regulate diverse biological processes such as transcription, DNA repair, recombination, cell division and differentiation etc. [86–88]. It is also possible that curcumin may disrupt some cellular processes that function parallel with histone modification. One possible mechanism by which curcumin might exert its numerous effects is through epigenetic modulation by targeting various epigenetic factors, such as HDAC, HAT, DNMTs, and miRNAs [89, 90] which regulates various cellular pathways. A recent study provided that curcumin mediates histone H3, H4, H2A N-terminal

tail modification leading to a significant growth inhibition, which suggests that curcumin mediates its action through the N-terminal tail regions of histones [91]. Curcumin is found to reduce the expression of positive regulators of DNA methyltransferase 1 (DNMT1), p65 and Sp1, which correlated with a reduction in binding of these transcription factors to the DNMT1 promoter in acute myeloid leukemia cell lines [85]. DNMT1 catalyzes the transfer of methyl groups to DNA, which represents a crucial mediator of DNA methylation. Exposure of MCF7 cells to curcumin resulted in increased global levels of acetylated H3K18 and H4K16 and was less effective in inducing DNA damage markers accompanied by upregulation of DNA damage signalling markers such as γ H2A.X and H3K56Ac [92].

Reverse the Multidrug Resistance of Cancer Cells

Multidrug resistance (MDR) is an obstacle in cancer treatment, often because less drug accumulates in patient's tumor cells owing to enhanced drug efflux [93]. P-glycoprotein (P-gp) is an ATP-dependent drug efflux pump and major player in the development of resistance in cancer cells. Curcumin is found to reverse the MDR of the human gastric carcinoma SGC7901/vincristine cell line [94]. That was associated with decreased P-gp function and expression, and the promotion of caspase-3 activation in MDR cells. Similarly, treatment of drug-resistant KB-V1 cells with curcumin increased their sensitivity to vinblastine that was associated with a decreased amount of P-glycoprotein on the cell plasma membrane [93]. Curcumin can partially reverse the paclitaxel-resistance of SKOV3-TR30 cells through a downregulation of glycogen synthase kinase-3 (GSK-3) [95]. In a short cohort open-labeled study, curcumin decreased multidrug resistance 1 (MDR1) mRNA level in patient leukemic cells, especially in high level of MDR1 gene groups [96]. Thus, curcumin treatment may provide a lead for clinical treatment of leukemia patients in the future.

The Flip Side of Curcumin

Despite its utilization and effectiveness in clinical trials for cancer as shown above, the genotoxicity, poor water solubility, and rapid intestinal and hepatic metabolism of curcumin are the major challenges limiting its clinical utilizes. These challenges represent that the dark side of curcumin is not demonstrated here in detail because of space limitations, and thus, readers are referred to the excellent review of Burgos-Moron et al. (2010) [97]. However, to summarize briefly, unlike the common belief in the scientific literature that there is no major toxicity that has been found in humans, curcumin

is not approved as a safe agent, since such belief was established based on short-term studies. Additionally, numerous analogues of curcumin are synthesized and tested for their bioavailability, selectivity, and stability and it showed encouraging results in cancer treatment [98–100]. A mass of accumulated studies confirmed that curcumin possesses DNA damage and chromosomal alterations both *in vitro* and *in vivo* at concentrations similar to those reported to exert beneficial effect [101, 102]. The increased incidence of carcinomas of the small intestine was observed in mice taking average daily doses of curcumin of about 0.2 mg kg⁻¹ body weight [103, 104]. Moreover, a recent study has also shown that curcumin can mediate lung cancer in mice [105]. Several approaches were established to enhance curcumin's bioavailability, the plasma concentration, and the cellular permeability. The black pepper alkaloid piperine (bioperine) was used in order to increase the bioavailability of curcumin [106]. However, such strategy should be used cautiously, as piperine is a potent inhibitor of drug metabolism and may cause toxicity in people taking specific drugs [107, 108]. Nanoparticles as a new approach for targeting drug delivery have tested and proven for providing curcumin with longer circulation, better permeability, and stronger resistance to metabolic processes. It is important to take into account that any strategy that increases the levels of curcumin in tissues will not only increase the effectiveness of curcumin, but also its toxicity. Overcoming these problems represents a validated and effective therapeutic tool in the battle against cancer.

Conclusion

Curcumin is a potent cancer fighter; through several mechanisms, it can kill a wide range of tumor cell types including MDR cancer cells. Notably, due to the diversity of cell death mechanisms mediated by curcumin, few cancer cells can develop resistance to curcumin-induced cell death. Urgently, clinical trials are required to place this fascinating molecule at the fore front of novel therapeutics and translate this laboratory concept into clinical benefits.

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