Targeting Proteasomal Pathways by Dietary Curcumin for Cancer Prevention and Treatment

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Abstract: Curcumin, a major component of the golden spice turmeric (Curcuma longa), has been linked with the prevention and treatment of a wide variety of cancers through modulation of multiple cell signaling pathways. Since the first report from our laboratory in 1995 that curcumin can inhibit activation of the proinflammatory transcription factor NF-κB by inhibiting the 26S proteasomal degradation of IkBα, an inhibitor of NF-κB, this yellow pigment has been shown to inhibit the proteasomal activities of the proteasome. The carbonyl carbons of the curcumin molecule directly interact with the hydroxyl group of the amino-terminal threonine residue of the proteasomal CT-L subunit of 20S proteasome, and cellular 26S proteasome. Curcumin is also a potent inhibitor of COP9 signalosome and associated kinases, casein kinase 2 and protein kinase D, all linked to the ubiquitin-proteasomal system (UPS). Curcumin can also directly inhibit ubiquitin isopeptidases, a family of deubiquitinas (DUBs) that salvage ubiquitin for reuse by the 26S proteasome system. The inhibition of this enzyme by curcumin is mediated through α,β-unsaturated ketone and two sterically accessible β-carbons. Regulation of the UPS pathway by curcumin has been linked to regulation of cancer-linked inflammatory proteins (such as COX-2 and iNOS), transcription factors (NF-κB, STAT3, Sp, AP-1, GADD153/CHOP, HIF-1α), growth factors (VEGF, HER2), apoptotic proteins (p53, Bcl-2, survivin, DNA topoisomerase II, HDAC2, p300, hTERT) and cell cycle proteins (cyclin D1, cyclin E, cyclin B, p21, p27) associated with the prevention and therapy of cancer. Interestingly, the effect of curcumin on 26S proteasome appears to be dose-dependent, as low doses (≤1 μM) increase proteasome activity whereas high doses (≥10 μM) inhibit the proteasome activity. In this review, we discuss in detail how modulation of these targets by curcumin is linked to prevention and treatment of cancer.

Keywords: Cancer, cell death, curcumin, degradation, inflammatory protein, prevention, proteasome, transcription factor, treatment, ubiquitination.

1. INTRODUCTION

Under normal physiological conditions, most proteins in the human body constantly undergo turnover. Highly regulated protein synthesis is followed by highly regulated protein degradation. The ubiquitin-proteasomal system (UPS) is one of the major pathways for proteasomal degradation. For a given protein to be targeted for degradation, the protein undergoes ubiquitination catalyzed by ubiquitin-conjugating enzymes E1, E2, and E3 and is then degraded by the proteasome. The proteasome is a multicatalytic protease complex crucial for extra-lysosomal degradation of most intracellular proteins. The 26S proteasomal complex is composed of a 20S proteasome core capped with the 19S regulatory particle (Fig. 1). The 20S proteasome is a cylinder-shaped particle composed of four stacked rings, two outer α and two inner β subunits with seven distinct subunits per ring. The α1-α7 subunits are covalently associated with the β1-β7 subunits [1, 2]. The α subunits are the sites for binding the 19S regulatory complex, and they control substrate entrance via a dynamic gating process [3]. The β subunits are more diverse, with a terminal threonine residue responsible for the proteolytic activities. The β1, β2, and β5 subunits are catalytic and have three distinct substrate specificities: caspase-like (C-L), trypsin-like (T-L), and chymotrypsin-like (CT-L), respectively.

Alternative β forms (denoted β1i, β2i, and β5i) are expressed in lymphoid tissues when exposed to pro-inflammatory cytokines, in particular interferon γ. The proteasome assembled with these alternative subunits is known as the immunoproteasome, and their substrate specificity is altered relative to the normal proteasome [1].

The proteasome complex is responsible for degradation of target proteins involved in two distinct and successive steps, polyubiquitination and degradation of the protein by proteasome. Ubiquitination of the target proteins is mediated by the sequential action of an E1 Ub-activating enzyme, an E2 Ub-conjugating enzyme, and an E3 Ub-ligase. Once ubiquitinated, the target proteins bind to a subunit in the 19S lid component, Rpn11, a metallo-deubiquitinase that removes polyubiquitin chains from substrates and unfolds in an energy-dependent manner before being fed into the catalytic core chamber of the 20S complex to be degraded into peptides of 3-22 amino acids in size.

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Several small molecules have been identified that can inhibit the proteasome activity. These include peptide boronates (bortezomib, also called velcade or PS-341), peptide epox ketones (carfilzomib or ONX 0912), peptide aldehydes (MG-132, MG-115, and  N-acetyl-L-leucyl-L-leucyl-L-norleucinal [ALLN]), peptide vinyl sulfones (NLVS and YL3VS), β-lactone inhibitors (lactacystin, clasto-lactacystin β-lactone, also called omuralide), and salinosporamide (also known as NPI-0052 or marizomib) (Fig. 2). These inhibitors either inhibit only the CT-L activity of the proteasome, or they inhibit the CT-L, T-L, and C-L activity of the proteasome by covalent modification of the N-terminal threonine of the catalytically active β subunits [4]. The boron atom in bortezomib binds the catalytic site of the 26S proteasome with high affinity and specificity [5-7]. While carfilzomib (like bortezomib) predominantly inhibits CT-L activity of the proteasome, carfilzomib (unlike bortezomib) irreversibly inhibits the 26S proteasome [8]. Marizomib inhibits all three protease activities in the proteasome (CT-L, T-L, and C-L) [4, 9]. Epoxomicin forms an irreversible, selective, and highly specific adduct only with the N-terminal threonine of the β5 subunit [10]. The β lactones were among the first proteasome inhibitors identified, and their ability to covalently modify the N-terminal threonine of the proteasome blocks the proteolytic activity and results in the proteasome being irreversibly inhibited [11]. Lactacystin, which is isolated from Streptomyces lactacystinaeus, as a proteasome inhibitor binds to the catalytic subunits of the 20S proteasome. Omuralide, the transformed product of lactacystin, is also a specific inhibitor of 20S proteolytic activity [12].

It is believed that inhibition of proteasomes leads to intracellular aggregation of unwanted proteins, which triggers cell death [13] and upregulates autophagy [14]. The latter process delivers proteins to lysosomes for destruction [15]. How proteasome inhibitors such as bortezomib induce apoptosis is not understood. Likewise, how bortezomib selectively induces apoptosis in tumor cells is also unknown. Bortezomib inhibits the accumulation of the proapoptotic proteins bax and p53, and this has been shown to be critical for its ability to induce apoptosis in cells [16, 17]. Recently, it was reported that Cas, a docking protein, is required for induction of apoptosis by proteasome inhibitors [18]. The question of whether proteasome inhibitors have any role in cancer first emerged from studies with bortezomib, which was approved for the treatment of multiple myeloma in 2003 [7]. Since then, several proteasome inhibitors, including car filzomib and marizomib, have entered clinical trials.

2. MECHANISM OF REGULATION OF PROTEASOME BY CURCUMIN

Curcumin (diferuloylmethane), a major component of the dietary spice turmeric, has been shown to modulate the UPS through several pathways. One mechanism by which curcumin affects UPS is through suppression of the protease activities of the proteasome, with the CT-L mechanism being the most prominent [19]. These researchers showed that carbonyl carbons of the curcumin molecule interact with the hydroxyl group of the amino-terminal threonine residue of the proteasomal CT-L subunit, and they found that curcumin inhibited the CT-L activity of purified rabbit 20S proteasome (IC₅₀ 1.85 μM) and cellular 26S proteasome.

A second mechanism by which curcumin affects UPS is in its role as a potent inhibitor of COP9 signalosome (CSN) [20, 21], an important regulator of the UPS. CSN is a novel protein complex (molecular mass of 450 kDa) that controls the stability of many proteins such as IkBα, c-Jun, and p53 via serine/threonine protein kinase activities [22]. The human core CSN consists of eight subunits (CSN1 to CSN8), mostly identified as components of signal transduction pathways through its kinase activity [20]. Even though it possesses structural similarities with eight non-ATPase subunits of the 19S lid of the proteasome, its functions seem...
Fig. (2). The molecular structures of curcumin and other proteasome inhibitors.

to be different. The CSN has two important known functions, deneddylation and phosphorylation, with the former being postulated to control the S-phase kinase-associated protein 1 (SKP1)-Cullin/CDC53-F-box protein (SCF) ubiquitin E3 ligase functions. The SCF Ub ligases interact with specific Ub-conjugating enzymes in the ubiquitination of different substrates. The CSN seems to function as an interface between signal transduction and ubiquitin-dependent proteolysis [23]. Therefore it is not surprising that there is increasing evidence for a functional cooperation between CSN and the Ub-26S proteasome system in regulating the stability of important cellular proteins and removal of tumor-related proteins. Thus inhibition of CSN by curcumin may play a role in its ability to inhibit AP-1, c-Jun N-terminal kinase (JNK), and NF-κB activation through inhibition of phosphorylation of c-Jun and IkBα [20]. Phosphorylation of c-Jun and IkBα leads to their degradation by UPS. Uhle et al. showed that casein kinase 2 (CK2) and protein kinase D (PKD) are associated with CSN and that curcumin inhibits the activity of these kinases [21].

Evidence that CSN mediates its effects through ubiquitin-independent degradation was found by Berse et al. by using the transcriptional regulators and substrates of the ubiquitin system inhibitors of differentiation (Id)-1 and Id3. They showed that Id1 and Id3, but not Id2 and Id4, bind to the CSN subunit CSN5. Id3 physically interacts with the CSN complex. Recombinant Id3 is not phosphorylated by the CSN-associated kinases CK2 and PKD. However, it inhibits c-Jun and CSN2 phosphorylation by the isolated CSN complex and by recombinant CK2. As an inhibitor of CSN-associated kinases, curcumin induces ubiquitination and proteasome-dependent degradation of transiently expressed Id3 in HeLa cells. Proteasome-dependent degradation of endogenous Id1 in HeLa cells is also stimulated by treatment with curcumin [24].

A third mechanism by which curcumin could modulate UPS is by inhibiting ubiquitin isopeptidases, a family of cysteine proteases (deubiquitinases; DUBs) that salvage ubiquitin for its reuse by the 26S proteasome system [25]. Curcumin contains α,β-unsaturated ketone and two sterically accessible β-carbons that mediate the inhibition of this enzyme. Such compounds are known to induce cell death through a p53-independent mechanism [19, 26].

Interestingly, curcumin exhibits a biphasic hormetic dose-response with respect to proteasome activity. Whereas at lower concentrations curcumin was found to activate the proteasomal pathway, at high concentrations it inhibited this pathway. Curcumin treatment of human keratinocytes (up to 1 μM for 24 h) increased CT-L activity by 46% compared with that in untreated cells. However, higher concentrations of curcumin were inhibitory, and at 10 μM the proteasome activity decreased to 46% of its initial value [27].

Curcumin could also induce proteasomal malfunction through the generation of oxidative stress [28-30], which is known to inhibit proteasome function [31, 32]. Since curcumin also exhibits anti-oxidant activity [30, 33, 34], this fact might explain the biphasic role of curcumin in controlling UPS. A wide variety of cell signaling proteins that have been linked with tumorigenesis have been shown to be regulated by curcumin through the activation of the UPS pathway (Tables 1 and 2).
Table 1. Targets Upregulated by Curcumin through Inhibition of Proteasome.

<table>
<thead>
<tr>
<th>Mechanism</th>
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<tr>
<td>Inhibits degradation of IkBα that leads to inhibition of NF-κB activation</td>
<td>[35]</td>
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<tr>
<td>Inhibits COP9 signalosome kinase (CSN, has homology to 26S proteasome lid)</td>
<td>[20]</td>
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<tr>
<td>Inhibits p53 degradation through inhibition of CSN</td>
<td>[36]</td>
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<tr>
<td>Inhibits cellular ubiquitin isopeptidases (DUBs)</td>
<td>[25]</td>
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<tr>
<td>Inhibits DNA topoisomerase II degradation through inhibition of CSN</td>
<td>[37]</td>
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<tr>
<td>Inhibits degradation of myotubes</td>
<td>[38]</td>
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<tr>
<td>Blocks estrogen receptor α (ERα) degradation</td>
<td>[39]</td>
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<tr>
<td>Inhibits degradation of the hepatitis C virus nonstructural 2 (NS2) protein</td>
<td>[40]</td>
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<tr>
<td>Uregulates the CHOP/GADD153 through inhibition of 20S core proteasome</td>
<td>[41]</td>
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<tr>
<td>Increases proteasome activity at ≥ 1 μM but inhibits the activity at ≤ 10 μM</td>
<td>[27]</td>
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<tr>
<td>Reverses inhibition of NK cell activation through inhibition of proteasome system</td>
<td>[42]</td>
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<tr>
<td>Protects from coxsackievirus B3-induced cytopathic effect by inhibiting its replication</td>
<td>[43]</td>
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<tr>
<td>Blocks sepsis-induced muscle proteolysis in rats</td>
<td>[44]</td>
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<td>Restores corticosteroid function by inhibiting proteasomal degradation of HDAC2</td>
<td>[45]</td>
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<tr>
<td>Decreases cellular c-Jun resulting in a reduction in VEGF production</td>
<td>[46]</td>
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<tr>
<td>Induces apoptosis through inhibition of proteasome activity in colorectal cancer</td>
<td>[19]</td>
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<tr>
<td>Reduces the infective viral particle through inhibition of proteasomal pathway</td>
<td>[47]</td>
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<tr>
<td>Reverses cachexia-induced weight loss by upregulating muscle-specific MAFbx and MURF-1</td>
<td>[48]</td>
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<tr>
<td>Induces cytotoxicity by accumulation of ubiquitinated proteins and cyclin B</td>
<td>[49]</td>
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<tr>
<td>Induces down-regulation of inhibitor protein of paraparosis, AIP-1/Alix</td>
<td>[50]</td>
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<td>Exerts antifibrinolytic activity by inhibition of TGFβ degradation in systemic scleroderma</td>
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<td>Induces degradation of HIF-1 ARNT</td>
<td>[52]</td>
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<tr>
<td>Downregulates cyclin E which mediates G1 to S transition</td>
<td>[53]</td>
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<tr>
<td>Decreases in AP-1 and involucrin gene expression in keratinocytes</td>
<td>[54]</td>
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<tr>
<td>Promotes the degradation of COX2 in HeLa cells</td>
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<tr>
<td>Decreases the expression of Sp1, leading to decreased hTERT expression</td>
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<td>Promotes the degradation of iNOS after LPS stimulation</td>
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<tr>
<td>Promotes the degradation of STAT3 in osteosarcoma cell lines</td>
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<tr>
<td>Promotes the clearance of toxic intracytoplasmic aggregates of PHOX2B</td>
<td>[59]</td>
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AIP-1/Alix, ALG-2-interacting protein 1; CHOP/GADD153, C/EBP homologous protein (CHOP) and growth arrest- and DNA damage-inducible gene 153 (GADD153); CK2, casein kinase 2; COP9, constitutive photomorphogenic 9; CSN, COP9 signalosome; DUBs, deubiquitinases; HDAC2, histone deacetylase 2; HSP, heat shock protein; IkBα, inhibitor of NF-κB; JEV, Japanese encephalitis virus; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PIF, proteolysis-inducing factor; TGIF, transforming growth factor-β-induced factor; TNF, tumor necrosis factor.

Table 2. Targets Downregulated by Curcumin through Activation of Proteasome.

<table>
<thead>
<tr>
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<tr>
<td>Decreases cellular c-Jun resulting in a reduction in VEGF production</td>
<td>[24]</td>
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<tr>
<td>Induces the degradation of cyclin D1 in prostate and breast cancer cells</td>
<td>[25]</td>
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<tr>
<td>Induces the degradation of C/EBPβ and β factor leading to decrease in involucrin</td>
<td>[26]</td>
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AP-1, activator protein 1; ARNT, aryl hydrocarbon receptor nuclear translocator; C/EBP, CCAAT/enhancer binding protein; CBP, cAMP response element-binding (CREB)-binding protein; COX2, cyclooxygenase-2; HATs, histone acetyl-transferases; HIF-1, hypoxia-inducible factor-1; hTERT, human telomerase reverse transcriptase; Id1, inhibitors of differentiation 1; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; PHOX2B, paired-like homebox 2b; Sp, specificity protein; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor.
3. PROTEASOMAL REGULATION OF CANCER-LINKED TRANSCRIPTION FACTORS BY CURCUMIN

One of the first proteins is reported to be affected by curcumin was IκBα, an inhibitor of nuclear factor (NF)-κB. IκBα is found in the cytoplasm of most mammalian cell types bound to the p65 subunit of NF-κB, and it is known to undergo phosphorylation, ubiquitination, and degradation through the UPS pathway. Our group showed that curcumin could inhibit this pathway by inhibiting the degradation of IκBα [35]. NF-κB is known to control the expression of proteins linked to cellular transformation (such as c-myc), tumor cell survival (Bcl-2, Bcl-xL, X-linked inhibitor of apoptosis protein [XIAP], cellular FLICE inhibitory protein [cFLIP], inhibitor of apoptosis protein [IAP]-1, IAP2, survivin), proliferation (cyclin D1), inflammation (cyclooxygenase [COX]-2, 5-lipoxygenase, tumor necrosis factor [TNF]-α, interleukin [IL]-1, IL-6, IL-8, chemokines), invasion (urokinase-type plasminogen activator, matrix metalloproteinase 9, adhesion molecules), and angiogenesis (TWIST, vascular endothelial growth factor [VEGF]). Owing to downregulation of NF-κB by curcumin, all these proteins were found to be downregulated by this agent [70-72]. Thus curcumin could play a critical role in cancer through UPS-mediated downregulation of the NF-κB pathway (Fig. 3).

Activator protein (AP)-1 is another transcription factor, consisting of c-jun and c-fos, that has been closely linked to cellular proliferation (Fig. 4). Curcumin has been shown to block activation of AP-1 [60, 73], and these inhibitory effects are very likely mediated through suppression of ubiquitin-proteasomal pathway modulation of c-jun as described above [20, 21]. Curcumin suppresses AP-1-dependent differentiation and activates apoptosis in human epidermal keratinocytes [60]. Thus inhibition of AP-1 by curcumin could account for its anti-proliferative effects against cancer cells.

Another transcription factor that plays a major role in cancer is signal transducer and activator of transcription 3 (STAT3), which is constitutively active in most tumor cells and controls the expression of various proinflammatory (such as COX-2), cell survival (survivin), cell proliferative (such as cyclin D1), and angiogenic (VEGF) gene products. Our group was the first to demonstrate that curcumin could inhibit both constitutive and inducible activation of STAT3 by IL-6 and epidermal growth factor [74]. A novel diketone analogue of curcumin, FLLL32, was found to induce the degradation of STAT3 through activation of the ubiquitin-proteasome pathway in an osteosarcoma cell line [68]. These authors showed that increased ubiquitination of STAT3 was induced by FLLL32, followed by its degradation.

Another transcription factor affected by curcumin through UPS is the tumor-suppressor protein p53. Through inhibition of CSN, inhibition of p53 phosphorylation by curcumin leads to stabilization of the protein in tumor cells [20, 36]. Although numerous groups have shown the upregulation of p53 by curcumin through inhibition of UPS [36, 37, 59, 75], one group [76] found downregulation of p53 by...
curcumin. The reason for these opposite results, however, is unclear at present. Tsvetkov et al., however, showed that curcumin induced ubiquitin-independent degradation of WT p53. Bech-Otschir showed that upregulation of p53 by curcumin is mediated through the inhibition of CSN [36]. Because curcumin has been shown to induce apoptosis, upregulation of p53 has been linked to enhanced apoptosis by this agent.

The 5th subunit of COP9 signalosome (also known as Jab1 or COPS5) is implicated in regulating p53 activity and is overexpressed in various tumors [36, 77]. However, the precise roles of CSN5 in the p53 network are unclear. Zhang et al. showed that CSN5 is a critical regulator of both p53 and MDM2. Curcumin was found to downregulate not only CSN5 but also MDM2, which results in p53 stabilization. Importantly, CSN5 interacts with p53 and antagonizes the transcriptional activity of p53. CSN5 expression leads to p53 degradation, facilitating MDM2-mediated p53 ubiquitination and promoting p53 nuclear export. Additionally, CSN5 expression results in stabilization of MDM2 through reducing MDM2 self-ubiquitination and decelerating the turnover rate of MDM2.

Growth arrest and DNA damage induced gene-153 (GADD153) is in the CCAAT/enhancer binding protein (C/EBP) family of transcription factors that are induced in response to cellular stress, especially by endoplasmic reticulum stress [78]. These factors are involved in apoptosis. Curcumin has also been shown by Dikshit et al. to induce the transcription factor C/EBP-homologous protein (CHOP)/GADD153 through the inhibition of 20S core proteasome [42]. They suggested that curcumin-induced proteasomal malfunction might be linked with both anti-proliferative and anti-inflammatory activities. In contrast, another report found that curcumin could inhibit the EGCG-induced activation of C/EBP (C/EBPα and C/EBPβ) [56]. Because curcumin-dependent suppression of C/EBP factor is inhibited by treatment with the proteasome inhibitor MG132, this suggests that the proteasome function is required for curcumin action.

Hypoxia-inducible factor-1 (HIF-1), another transcription factor, is composed of HIF-1α and aryl hydrocarbon receptor nuclear translocator (ARNT; HIF-1β). This transcription factor plays a key role in cell survival and angiogenesis in hypoxic tumors [79, 80]. Choi et al. found that curcumin inhibited HIF-1 activity, leading to downregulation of gene products regulated by HIF-1. They showed that this downregulation was mediated through ARNT and that curcumin stimulated the proteasomal degradation of ARNT via oxidation and ubiquitination processes [57]. This effect can be noted not only in vitro but also in vivo. In mice bearing Hep3B hepatomas, curcumin retarded tumor growth and suppressed ARNT, erythropoietin, and VEGF in tumors, thus suggesting that the anti-cancer activity of curcumin is attributable to HIF-1 inactivation by ARNT degradation [57].
Specificity protein (Sp) was perhaps the first transcription factor to be identified. Gene products regulated by various specificity proteins have been linked with cancer through modulation of cellular proliferation, invasion, and angiogenesis [81-83]. Chadalapaka et al. found that curcumin induced the proteasome-dependent downregulation of specificity protein (Sp)-1, Sp3, and Sp4 in bladder cancer cells, which led to downregulation of expression of survivin, VEGF, and VEGFR1 [63]. They showed that curcumin-dependent inhibition of NF-kB-dependent genes, such as bcl-2, survivin, and cyclin D1, was also due in part to the loss of Sp proteins. They found that curcumin also decreased bladder tumor growth in athymic nude mice, accompanied by decreased Sp1, Sp3, and Sp4 protein levels in tumors. The downregulation of human-telomerase reverse transcriptase (hTERT) by curcumin in lung cancer cells may also be linked to UPS-dependent degradation of Sp1 [66]. This effect of curcumin was reactive oxygen species (ROS)-mediated, as N-acetyl cysteine (NAC) reversed the downregulation of Sp1.

Overall, curcumin could manifest its effects against cancer through proteasomal modulation of several transcription factors.

4. PROTEASOMAL REGULATION OF CANCER-LINKED INFLAMMATORY PROTEINS BY CURCUMIN

Inflammation plays a major role in tumorigenesis in a wide variety of cancers. Thus downregulation of inflammatory pathways has a great potential in both prevention and treatment of cancer. From the above account it is clear that through proteasomal modulation of NF-kB, AP-1, STAT3, and Sp, curcumin could downregulate the expression of proinflammatory proteins including COX-2, 5-lipoxygenase, TNF-α, IL-1, IL-6, IL-8, inducible nitric oxide synthase (iNOS), chemokines, and others.

COX-2 is degraded by the UPS linked to the regulator CSN, which controls the stability of many proteins. Neuss et al. showed that the proteasome-dependent degradation of COX-2 was stimulated by curcumin through inhibition of CSN-associated kinases [62]. Thus this is another mechanism of downregulation of this proinflammatory enzyme.

Another enzyme that plays a critical role in both prevention and treatment of cancer is iNOS. Curcumin was found to promote the degradation of iNOS [67]. The degradation of iNOS protein was due to ubiquitination and proteasome-dependency, since it was almost completely blocked by MG132. In addition, curcumin has been shown to decrease the lipopolysaccharide-induced iNOS upregulation at the transcription level. These results suggest that curcumin could regulate iNOS at the post-translational level as well.

5. PROTEASOMAL REGULATION OF CANCER-LINKED GROWTH FACTORS AND APOPTOTIC PROTEINS BY CURCUMIN

Curcumin can affect the proliferation of cancer cells by modulating growth factors, growth factor receptors, and growth factor signaling through the proteasomal pathway. One of the growth factor receptors modulated through this pathway is human epidermal growth factor receptor (HER/p185 neu)-2 [84]. Curcumin was found to both inhibit the tyrosine kinase activity of p185neu and also to deplete p185neu. Overexpression of p185neu in breast cancer is known to be a poor prognostic factor [84]. Curcumin dose-dependently inhibited p185neu autophosphorylation and transphosphorylation in vitro and depleted p185neu protein in vivo. It dissociated the binding of p185neu with glucose-regulated protein (GRP)-94, a molecular chaperone, and enhanced the depletion of p185neu. This led to a drastic reduction of p185neu protein on the cell membrane and suppression of growth in multiple breast cancer cell lines [84].

Angiogenesis is a prerequisite for solid tumor growth and metastasis. Elucidation of the signaling pathways that control tumor angiogenesis constitutes the basis for rational anti-angiogenic tumor therapy. The production of VEGF in HeLa and HL-60 cells is directed by the CSN. VEGF expression is controlled by NF-kB, AP-1, AP-2, Sp1, and HIF-1. Pollmann and coworkers found that inhibition of CSN kinase by curcumin decreased the cellular c-Jun concentration, resulting in a reduction of the VEGF production. The transcription factors AP-2 and Sp1 act independently of the CSN. Pollman et al. showed that overexpression of p53 reduces VEGF, as it competes with c-Jun for CSN-specific phosphorylation with consequent c-Jun destabilization. This provides the basis for the known anti-angiogenic and antitumorigenic activities of curcumin [54].

The transforming growth factor (TGF)-β signaling pathway plays a key role in the fibrotic process in systemic scleroderma. Curcumin has been demonstrated to exert anti-fibrotic activity, but the mechanism is not understood. Song et al. found that curcumin induced upregulation of TGF-β-induced factor (TGF), a negative regulator of TGF-β signaling [51]. This upregulation of TGF by curcumin resulted from decreased ubiquitination of TGF, which blocks its proteasome-mediated degradation.

Estradiol regulates target cell proliferation and gene transcription through a series of molecular events initiated by the hormone-dependent binding of the estrogen receptor (ER)-α, a member of the nuclear receptor superfamily, to its cognate DNA target. In mammary cells, the effects of estradiol can be antagonized by compounds such as 4-hydroxy-tamoxifen (OH-Tam), a tamoxifen metabolite that is a selective estrogen to its cognate DNA binding to its DNA target. Curcumin, on the other hand, impaired estradiol-induced ERα degradation by the proteasome and also inhibited estradiol-induced phosphorylation of ERα, which is necessary for ERα binding to its cognate DNA target. Thus curcumin can antagonize the action of ERα.

Recently, a new type of nonapoptotic cell death, termed parapoptosis and related to apoptosis, has been reported [85]. Parapoptosis is characterized by a process of vacuolation that begins with physical enlargement of mitochondria and the endoplasmic reticulum. This form of cell death does not involve the apoptotic characteristics of pyknosis, DNA fragmentation, or caspase activation. Parapoptosis is known to re-
quire new protein synthesis, and recent reports have identified ALG-2-interacting-protein-1 (AIP-1/Alix) as an inhibitor of paraptosis [86, 87]. A recent report indicated that curcumin induces paraptosis in malignant breast cancer cells but not in normal breast cells [50]. This report found that superoxide anion and proteasomal dysfunction contribute to the paraptotic changes seen in mitochondria and the endoplasmic reticulum of these cells. Curcumin induced paraptosis in breast cancer cells by promoting vacuolation that results from swelling and fusion of mitochondria and/or the endoplasmic reticulum. Cycloheximide blocked curcumin-induced vacuolation and subsequent cell death, indicating that protein synthesis is required for this process. The levels of AIP-1/Alix protein, a known inhibitor protein of paraptosis, were progressively downregulated in curcumin-treated breast cancer cells, and AIP-1/Alix overexpression attenuated curcumin-induced death in these cells. Proteasomal dysfunction was found to be responsible for downregulation of AIP-1/Alix protein associated with endoplasmic reticulum dilation. They also showed progressive accumulation of polyubiquitinated proteins in curcumin-treated cancer cells but not in normal cells. Because proteasome inhibition has been shown to induce the accumulation of misfolded proteins in the endoplasmic reticulum lumen and to induce endoplasmic reticulum stress, Yoon et al. also examined whether proteins associated with endoplasmic reticulum stress are also differentially modulated by curcumin in cancer versus normal cells. Curcumin treatment significantly increased phosphorylation levels of eIF2α as well as the protein levels of GRP78/94 and CHOP in cancer cells but not in normal cells, indicating that proteasomal dysfunction and/or severe endoplasmic reticulum stress may contribute to the preferential effects of curcumin on malignant breast cancer cells [50].

Whether proteasomal dysfunction is important for induction of the observed paraptotic changes, including mitochondrial and/or endoplasmic reticulum dilation, was also investigated by the authors [50]. They found that treatment of YFP-labeled mitochondria and YFP-labeled endoplasmic reticulum cells with proteasome inhibitors (1 μM MG132, 20 μM lactacystin, or 20 μM ALLN) induced vacuole formation. Interestingly, cellular vacuoles in these cells mainly originated from the endoplasmic reticulum, indicating that proteasomal dysfunction is largely responsible for endoplasmic reticulum-associated events in paraptosis. They concluded that proteasomal dysfunction, rather than endoplasmic reticulum stress itself, is more important for the induction of curcumin-induced paraptosis. Proteasome inhibition, however, was found to be necessary, but not sufficient, for curcumin-induced paraptosis.

6. PROTEASOMAL REGULATION OF CANCER-LINKED CELL PROLIFERATIVE PROTEINS BY CURCUMIN

The unlimited replication potential of cancer cells is one of the major hallmarks of cancer. This may be related to the activity of telomerase, an enzyme activated in 85-90% of human cancers [88-90]. The hTERT is one of the enzyme subunits of telomerase that is overexpressed in cancer cells. Curcumin could downregulate cell proliferation through the UPS-mediated downregulation of Sp1-dependent hTERT [66]. Another mechanism for downregulation of hTERT involves inhibition by curcumin of nuclear localization of hTERT by dissociating the heat shock protein (Hsp)-90 co-chaperone p23 from hTERT [65]. The molecular chaperone complex Hsp90-p23 interacts with hTERT. Curcumin inhibits the telomerase activity by decreasing the level of hTERT expression. Curcumin induced the accumulation of hTERT in the cytoplasmic compartment of the cell due to failure of nuclear import. Cytoplasmic hTERT protein was rapidly ubiquitinated and degraded by the proteasome. Curcumin also decreases the association of p23 and hTERT but does not affect the Hsp90 binding to hTERT. Thus interaction of the Hsp90-p23 complex with hTERT is critical for regulation of the nuclear localization of telomerase, and curcumin downregulates the nuclear pool of hTERT by dissociating the binding of hTERT with p23. Thus, inhibition of nuclear translocation of hTERT by curcumin may provide new perspectives for regulation of telomerase activity during tumorigenic progression.

Cyclin D1 is a critical protein needed by cells for G1 to S transition. Work from our laboratory has indicated that, in addition to downregulating cyclin D1 through UPS-mediated downregulation of NF-κB, curcumin could induce the degradation of cyclin D1 [55]. We found that curcumin-induced downregulation of cyclin D1 was inhibited by lactacystin, an inhibitor of the 26S proteasome, suggesting that curcumin represses cyclin D1 expression by promoting proteolysis. Downregulation of cyclin D1 by curcumin through the proteasomal pathway was also confirmed by other groups [61].

We showed that curcumin could also induce the degradation of cyclin E expression through an ubiquitin-dependent pathway. This downregulation of cyclin E was reversed by the proteasome inhibitors lactacystin and ALLN, suggesting the role of ubiquitin-dependent proteasomal pathway [59]. Downregulation of cyclin E by curcumin was also confirmed by other groups [61, 91].

Curcumin has been shown to induce apoptosis-independent death in esophageal cancer cells. Curcumin induced cell accumulation in G2/M cell cycle phases, distinct chromatin morphology, autophagy and accumulation of poly-ubiquitinated proteins and cyclin B, consistent with a disturbance of the ubiquitin-proteasome system [49]. This effect on a key cell cycle checkpoint regulator was linked with mitotic disturbances and consequent cytotoxicity. Results from our laboratory and others have indicated that curcumin can upregulate cyclin-dependent kinase inhibitors p21 and p27 in multiple human tumor cell lines [36, 59, 92].

Curcumin can promote anti-proliferative effects by destabilizing anti-apoptotic proteins through the UPS pathway. Curcumin was found to sensitize lung cancer cells to cisplatin-induced apoptosis through superoxide anion-mediated Bcl-2 degradation [64]. The mechanism by which curcumin downregulates Bcl-2 and sensitizes cells to cisplatin-induced apoptosis involves proteasomal degradation of Bcl-2. These effects of curcumin on bcl-2 were mediated through ROS, as NAC abolished these effects. Curcumin has also been shown to sensitize non-small cell lung cancer cell anoikis through ROS-UPS-mediated Bcl-2 downregulation [93]. Similarly, UPS-mediated downregulation of NF-κB, Sp, and STAT3
can lead to downregulation of survivin, another anti-apoptotic protein [63, 68, 72].

Histone acetyltransferases (HATs), and p300/CREB binding protein (CBP) in particular, have been implicated in cancer cell growth and survival, and hence HATs represent novel, therapeutically relevant molecular targets for drug development [94, 95]. Marcu et al. showed that curcumin is a selective HAT inhibitor [58]. They found that curcumin mediates its effect through its α,β-unsaturated carbonyl groups, which function as Michael reaction sites, and that the Michael reaction acceptor functionality of curcumin is required for its HAT-inhibitory activity. They showed that in cells, curcumin promoted proteasome-dependent degradation of p300 and the closely related CBP protein with minimum effect on HAT’s p300/CBP-associated factor or GCN5. In addition to inducing p300 degradation, curcumin inhibited the acetyltransferase activity of purified p300. Radiolabeled curcumin formed a covalent association with p300, and tetrahydrocurcumin displayed no p300 inhibitory activity, consistent with a Michael reaction-dependent mechanism. Finally, curcumin was able to effectively block histone hyperacetylation in both PC3-M prostate cancer cells and peripheral blood lymphocytes induced by the histone deacetylase inhibitor MS-275. These data thus identify curcumin as a novel lead compound for development of possibly therapeutic p300/CBP-specific HAT inhibitors [58].

DNA topoisomerase II (topo II) is an essential enzyme for eukaryotic cell proliferation and plays important roles in many aspects of the DNA process through modulating the topological states. Yun et al. showed that the glucose-regulated destruction domain of DNA topoisomerase IIα binds to CSN [38]. Topo II is targeted to a proteasome-dependent degradation pathway when human tumor cells are glucose-starved through destabilization by the newly identified domain glucose-regulated destruction domain (GRDD). Indeed, the deletion of GRDD conferred stability on topo IIα. Nuclear localization was a prerequisite for GRDD function, because the inhibition of nuclear translocation resulted in the suppression of GRDD-mediated topo II degradation. Furthermore, GRDD was identified as an interactive domain for CSN, which promoted the degradation of topo IIα. Treating cells with curcumin (a CSN-associated kinase inhibitor) inhibited topo IIα degradation induced by glucose starvation [38].

Milacic and his group showed that curcumin inhibits the proteasome activity in human colon cancer cells both in vitro and in vivo [19]. In these cancer cells, curcumin inhibited the CT-L activity of a purified rabbit 20S proteasome (IC50 = 1.85 μM) and cellular 26S proteasome. This led to accumulation of ubiquitinated proteins and several proteasome target proteins, with subsequent induction of apoptosis. Furthermore, treatment of HCT-116 colon tumor-bearing mice with curcumin resulted in decreased tumor growth and was associated with proteasome inhibition, proliferation suppression, and apoptosis induction in tumor tissues. Thus they concluded that proteasome inhibition could be one mechanism for the chemopreventive and/or therapeutic roles of curcumin in human colon cancer. On the basis of their findings, they suggested that curcumin could potentially be used for treatment of both early-stage and late-stage/refractory colon cancer [19].

CONCLUSION

Overall these studies clearly document that curcumin can modulate proteasome activity through a variety of mechanisms. This leads to upregulation and downregulation of large numbers of proteins. Most of these cell signaling proteins have been closely linked with inflammation, tumor cell survival, proliferation, invasion, and angiogenesis of cancer. These studies suggest that curcumin has great potential in both the prevention and treatment of cancer. Several animal studies have indeed shown that curcumin can be used in the prevention of a wide variety of cancers. However, systematic clinical trials are needed to prove its efficacy. Considering the toxicity associated with FDA-approved proteasome inhibitors such as bortezomib, curcumin may provide an excellent alternative, either alone or in combination. Our preliminary animal studies also suggest synergy between curcumin and bortezomib [96], which should also be examined in the clinic for use in patients in multiple myeloma and mantle cell lymphoma.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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LIST OF ABBREVIATIONS

AIP-1/Alix = ALG-2-interacting-protein-1
ALLN = N-acetyl-L-leucyl-L-leucyl-L-norleucinal
AP-1 = Activator protein-1
ARNT = Aryl hydrocarbon receptor nuclear translocator
C/EBP = CCAAT/enhancer binding protein
CBP = p300/CREB binding protein
cFLIP = Cellular FLICE inhibitory protein
CK2 = Casein kinase 2
COX-2 = Cyclooxygenase-2
CSN = COP9 signalosome
DUBs = Deubiquitinases
ERα = Estrogen receptor alpha
GADD153 = Growth arrest and DNA damage induced gene-153
GRP78 = Glucose-regulated protein 78
HATs = Histone acetyltransferases
HIF-1 = Hypoxia-inducible factor-1
hTERT = Human-telomerase reverse transcriptase
IAP-1 = Inhibitor of apoptosis protein-1
Id1 = Inhibitors of differentiation-1
IL-1 = Interleukin-1
iNOS = Inducible nitric oxide synthase
NAC = N-acetyl cysteine
NF-κB = Nuclear factor-kappa B
PKD = Protein kinase D
ROS = Reactive oxygen species
SCF = Signal transduction and activator of transcription 3
Smad = Signal transducer and activator of transcription
TGF-β = Transforming growth factor-beta
TNF-α = Tumor necrosis factor-alpha
topo II = DNA topoisomerase II
UPS = Ubiquitin-proteasomal system
VEGF = Vascular endothelial growth factor
XIAP = X-linked inhibitor of apoptosis protein.

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