

Epigenetics of Curcumin: A Gifted Dietary Therapeutics Compound

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Received date: Nov 25, 2014, Accepted date: Dec 25, 2014, Published date: Dec 30, 2014

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Abstract

Purpose of review: Curcumin (CUR), an active polyphenol extracted from the rhizome of *Curcuma longa*, is a highly pleiotropic molecule having diverse biological activities. The purpose of this review is to present the facts that would assist to understand the Curcumin in regulation of epigenetics and future applications.

Recent findings: The recent growth in the understanding of the epigenetics is leading to a medical revolution that assures a new age of health and disease management. Compounds that have the potential to regulate epigenetics are of great pharmacological importance. Curcumin is one of the best-studied natural bioactive compounds known to interact with several molecular targets inside the cell and influences a range of biological processes. We have analyzed the findings of recent studies on effects of CUR on biological pathways. Accumulating studies suggest that CUR might be a promising agent to treat various human diseases that can occur due to alterations in epigenetics.

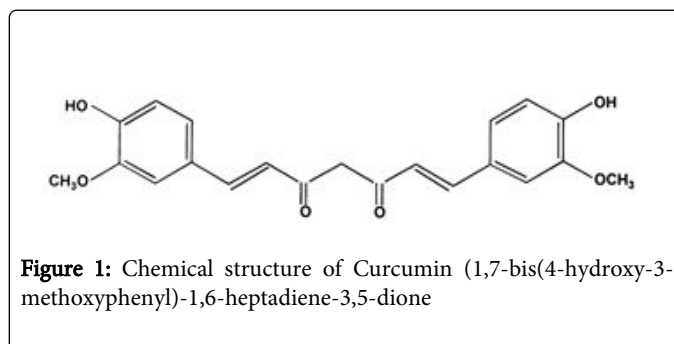
Summary: This review summarizes the actions of CUR on different chromatin modifiers, including, histone acetyl-transferases, histone deacetylases, and DNA methyl-transferases. Taking together, we have discussed the novel therapeutic potential of CUR, and we strongly believe that through future studies we will be able to effectively use CUR to improve the human health.

Keywords: Curcumin; Epigenetics; Drug-response; Histone modifications; Signaling pathways

Introduction

Chromatin is a dynamic structure and dynamicity is facilitated by altering the covalent modifications of histones [1]. This is carried out by a wide variety of chromatin modifying enzymes [2,3]. The combination of modifications (“marks”) produced by chromatin modifying enzymes represents a code that controls downstream processes, such as, transcription, DNA repair, and apoptosis [4-7]. It is well established that mutations in chromatin-modifying machineries that disturb the spatial-temporal patterns of gene expression can contribute to the pathogenesis of human diseases. As a result, the scientific community has focused on identifying the small molecular inhibitors for many of the histone-modifying machineries and using them for targeted therapeutics. Hence, a number of bioactive dietary components are of particular interest in the field of epigenetics [8], including, curcumin (CUR).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), chemical structure shown in figure 1, is the major bright yellow color pigment extracted from turmeric [9]. Owing to its wide range of epigenetic targets, CUR has been extensively studied to better understand its diverse pharmacological activities and it has been used in several worldwide clinical trials [10]. This review summarizes the epigenetic targets of CUR and we discuss how CUR can be effective in treating various ailments by targeting epigenetic machineries and associated factors.



Effect of curcumin on Epigenetics

Over the past decade, knowledge regarding CUR biochemistry and its targeted pathways has grown tremendously, and it has been considered as a promising epigenetic modifier. Here, we will focus on how CUR alters post-translational modifications in histone proteins along with DNA methylation.

Histone acetylation

CUR attenuates histone acetylation levels causing histone hypoacetylation [11-14]. CUR is an inhibitor of p300/CREB-1 binding protein (CBP) HAT activity. It was established in vitro, that CUR covalently inhibited the acetylation of histones H3 and H4 by p300/CBP [15]. Moreover, the binding site on p300/CBP led to a conformational change, resulting in a reduction of binding efficiency

of histones H3 and H4, and acetyl CoA [14,16], leading to a decrease in acetylation levels of these histones. Similarly, CUR inhibited the acetylation of histones and p53 *in vivo* through specific inhibition of p300/CBP in cervical cancer cells [17]. The inhibition of p300 by CUR also decreased acetylation on RelA protein causing defect in downstream nuclear processes [18]. Furthermore, CUR induced hypoacetylation of histones H3 and H4, leading to suppression of differentiation in astrocytes and has been implicated in determining stem cell fate through modulation of acetylation levels [19]. CUR not only affects the protein factors that are involved in histone acetylation but also regulates their expression. A study has revealed that, in mouse spermatids, the expression levels of several HATs, such as, CBP, Cdy1, and Myst4 [20-22] were significantly downregulated in response to CUR exposure [23]. The decrease in expression of HATs led to reduction in acetylation levels of histone H4 [23] in mouse spermatids.

Histone deacetylation

It has been demonstrated that CUR treatment causes a decrease in the mRNA expression of several HDACs including HDAC 1, 3, and 8 in Raji cells, thereby leading to significantly higher levels of histone H4 acetylation. Furthermore, western blots confirmed that CUR exposure significantly reduced the protein levels of HDAC 1, 3, and 8 in a dose-dependent manner [24]. Although little is known about the molecular mechanisms behind the inhibitory action of CUR on HDACs, it has been suggested that CUR can modulate HDAC activity by regulating their expression. Reports suggest that in monocytes, CUR exposure causes decrease in the expression of HDAC2 [25]. Similarly in medulloblastoma cells, CUR blocks HDAC activity by decreasing HDAC4 expression [26]. However, through molecular docking experiments, it has been suggested that CUR can stably bind to human HDAC8 toward the entrance domain [27], and thereby, also affect the enzymatic function of HDACs.

Histone phosphorylation

It has been shown that CUR treatment increases the phosphorylation of histone H3 at Ser 10 residue in a dose-dependent manner [28] in mouse models. CUR exposure causes down-regulation of Aurora A transcript levels, which has been correlated with the reduction in histone H3 phospho-Ser10 levels [29] of human bladder cancer cells. Furthermore, CUR treatment has been proposed to alter the activity of histone phosphatases and/or kinases. We have also shown that CUR reduces the global levels of histone H3 acetylated at lysine-9 (H3K9ac) S10 phosphorylation in yeast cells [30]. There are reports suggesting that CUR can effectively target different signaling pathways, including MAPK, Akt, p53, androgen receptor (AR), Ras, and estrogen receptor (ER) pathways [31,32] in different human cell lines. Although not much is known about the effect of CUR on histone phosphorylation, we propose that because CUR interferes with various kinase pathways of the cells, it might regulate the phosphorylation state at several other residues of histone proteins [31,32].

DNA methylation

CUR has been shown to have the potential to inhibit DNMT1, leading to hypomethylation of various genes [33-35] in various human cell lines. It has been shown that CUR makes covalent interaction with DNMT1 and blocks the catalytic thiolate of C1226 of this enzyme to exert its inhibitory effect [35]. Another study demonstrated that CUR reduces global DNA methylation levels in a leukemia cell line at very low concentrations. CUR also inhibits enzymatic activity of M.Sss1

(methyltransferase Sss1, an analog of DNA methyltransferase I) *in vitro* [36,35]. Through genome wide studies, it has also been suggested that curcumin-induced changes in methylation occur only in a subset of partially-methylated genes [37] in colorectal cancer cells. CUR also reduces the hypermethylation of FANCF gene promoter, leading to an increase in the expression of FANCF in SiHa cells [39]. It was found that CUR causes the reversal of the methylation status of the first 5 CpGs in the upstream of Nrf2 gene with subsequent induction of Nrf2 [36]. CUR is also able to restore the expression of Nrf2 via promoter CpGs demethylation in TRAMP C1 prostate cancer cells treated at a concentration of 2.5 μ M. Furthermore, another study revealed that CUR exposure led to demethylation of the first 14 CpG sites of the CpG island in Neurog1 gene, which consequently led to the restoration of its expression in human prostate LNCaP cells [39].

Effect of CUR on Various Biological Processes

CUR can influence a wide range of molecular targets by either directly interacting with different molecules or indirectly modulating the signaling pathways. This section will illustrate the biological effects of CUR on various cellular pathways.

DNA Repair

It has been demonstrated that CUR can inhibit the DNA repair process in different model systems. CUR exposure causes impaired activation of ATR-Chk1 signaling [40] different cancer cell lines. Furthermore, the detailed study revealed that CUR could effectively induce histone hypoacetylation at the DNA double-strand break (DSB) sites by inhibiting specific HATs, thus inhibiting the recruitment of key repair factors at the DSB sites [40]. It was also demonstrated that CUR suppresses homologous recombination in DNA repair by inhibiting the expression of BRCA1 gene through impairing histone acetylation at its promoter [40]. We have also found that CUR activates DNA damage response in yeast cells [30]. Interestingly, Lu et al. discovered that CUR has the potential to cause DNA damage in combination with reducing the expression levels of DNA damage response genes, such as, BRCA1, ATM, ATR, 14-3-3 σ , and DNA-PK, leading to impaired DNA damage response [41] in mouse-rat hybrid retina ganglion cells.

Signaling pathways

CUR displays promising pharmacological activities that are believed to be mediated through the regulation of cell signaling pathways, including, MAPK, JAK/STAT, Wnt/ β -catenin, and AMPK pathways [42-45] in human cells. Curcumin has been demonstrated to modulate the MAPK signaling pathway by decreasing p38 MAPK activation and reducing inflammation [46] in a murine model. CUR has been shown to inhibit the activation of JAK-STAT pathways through the inhibition of JAK1 and JAK2 phosphorylation in microglia cells [47]. Another study revealed that CUR can also inhibit the phosphorylation of Akt and the activation of mTOR (mammalian target of rapamycin) in human prostate cancer PC-3 cells [48]. In esophageal cancer cell lines, CUR has been shown to inhibit the activation of Notch-1 signaling by downregulating the expression levels of Notch-1 specific micro-RNAs, including, miR-21 and miR-34a [49]. Interestingly, CUR can activate AMPK pathway by down-regulating Erk1/2, p38, and COX-2 in colon cancer cells [50]. Furthermore, CUR exposure, together with radiotherapy, has been shown to enhance tumor cell death and reduce radio-resistance in mice with fibro-sarcomas through the inhibition of radiation-induced Erk and NF- κ B expression [51].

Apoptosis

CUR has been shown to reduce the expression of anti-apoptotic factors of the Bcl-2 family, including, Bcl-2 and Bcl-XL, as well as increase the expression of pro-apoptotic factors, such as, Bax, and procaspases-3, -7, -8, and -9, leading to the induction of apoptosis [52] in various human cell lines. Furthermore, CUR can also induce apoptosis by mitochondrial pathway via cytochrome C release in different cancer cells including mantle cell lymphoma [53] and multiple myeloma cells [54]. CUR promotes apoptosis by inhibiting the Akt signaling pathways in cancer cells [55-57]. Another study has revealed that CUR induces apoptosis in various human melanoma cells through the Fas receptor/caspase-8 pathway [58]. CUR has also been shown to activate the TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis [59]. Due to the ability of CUR to target apoptotic pathway, it acts as an effective anti-cancer agents.

Therapeutic Potential of Curcumin

CUR has been consumed as a dietary supplement for centuries and has been widely used in Indian Ayurveda medicines [60]. Due to the diverse cellular targets, CUR is being used to treat as mentioned below.

CUR is an effective agent against various human diseases

CUR has been demonstrated to possess numerous pharmacological activities against a broad range of human diseases due to its anti-microbial [61,62], anti-cancerous [63,64], hepato-protective [65,66], anti-thrombotic [67], cardio-protective [68,69], and anti-arthritis properties [70]. Furthermore, CUR is also protective against neurodegenerative diseases, including, Parkinson's and Alzheimer's diseases [71,72]. Additionally, CUR pretreatment has been associated with a considerable decline in liver fibrosis and injury in response to external stimuli [73,74]. CUR is effective in reducing blood glucose levels by increasing pancreatic β -cell function in diabetes mouse model [75]. The anti-diabetic effects of curcumin have also been attributed to its ability to decrease macrophage infiltration [76], increase antioxidant capacity, decrease IL-1 β , VEGF, and NF- κ B activities [77,78], and through enhancing the PPAR- γ ligand-binding activity [79] in various mouse models of diabetes. Several studies demonstrated that CUR is effective against diabetes for example, in type 2 diabetic KK-A(y) mice; dietary turmeric extract reduced the blood glucose levels [79]. In diet-induced obesity mice and ob/ob male mice, dietary curcumin (3%) for 6 weeks improves glycemic status and insulin sensitivity [76]. In another mouse diabetes model, dietary curcumin (0.2%) for 6 weeks was beneficial in improving glucose homeostasis and insulin resistance [80].

The high efficacy of CUR in inhibiting cell proliferation and inducing cell death in different cancer cell lines makes it a promising anti-cancer drug [81-83]. Several studies conducted on patients with cancer, show that CUR has anti-proliferative and pro-apoptotic effects on pancreatic carcinoma, liver carcinoma, and leukemia [83,84]. Aberrant p300/CBP activity has been implicated in cancer progression and CUR inhibits p300/CBP HAT activity [85]. Moreover, CUR also reduces the cardiac ischemia-reperfusion injury by decreasing the expression of key molecules such as toll-like receptor 2 (TLR2), MCP-1, and CD68 [86]. Genome wide microarray study indicated that CUR mediates differential expression of genes involved in the protection of cardiac hypertrophy and inflammation [87-89].

Anti-parasitic effect of Curcumin

CUR exhibits anti-parasitic effect through modulating cellular histone acetylation levels [90]. One study demonstrated that CUR specifically hampers the in vivo PfGCN5 HAT activity in *Plasmodium falciparum* [90]. It has been observed that CUR also regulates the defense pathways of *Salmonella typhimurium* [91]. CUR strongly inhibits the proliferation of *Helicobacter pylori* that is a causative agent of gastric ulcers and also implicated in gastric cancers. CUR also effectively blocks the *H. pylori*-induced mitogenic response, leading to the inhibition of NF- κ B activation and subsequent downstream processes [92]. An interesting study demonstrated that the deleterious effects of the fecal parasite, *Eimeria maxima*, were significantly reduced by CUR in chickens [93]. Another animal study showed that the dermatophyte- and fungi-infected guinea pigs were relieved from disease symptoms upon CUR treatment [94]. Furthermore, CUR also possesses anti-leishmanial activity by exhibiting cytotoxic effect on its causative agent *Leishmania donovani* [95,96].

CUR in Clinical Trials

Since CUR possesses promising therapeutic potential [97,98], several clinical trials have already been conducted to investigate its effects on the prevention and/or treatment of various diseases [99-102]. There was no significant treatment-related toxicity was observed in doses up to 8 g/day for 3 months [103,104] on human patients. CUR treatment also effectively revert the general health status of patients with colorectal cancer [105]. In several human clinical trials, CUR has been administered in combination with other agents. For example, when curcumin was used with piperine, the pain was significantly reduced [106]. Similarly, CUR sensitized the effect of gemcitabine in gemcitabine-resistant pancreatic cancer when used at 8 g/day in combination with gemcitabine [107]. Furthermore, CUR increased the efficacy of prednisone in patients with ulcerative colitis [107] and ultraviolet B against skin disorder to yield significant improvements [108]. Interestingly, one clinical trial revealed that CUR was very effective against type 2 diabetes treatment [109].

Future directions and conclusions

Exploring epigenetic properties of CUR in humans will potentially enhance our understanding of its medicinal values. CUR also shows reduced bioavailability issue. Hence, it will be interesting to discover and characterize novel CUR derivatives that are more stable or more readily absorbed upon administration. For example, curcumin-encapsulated/curcumin-derived exosomes are more stable and can be directed towards target sites. Moreover, CUR can directly bind and alter multiple cell signaling cascades, which can be harnessed to combat selected pathologies including cancer. Future research in this area will provide further insights into the use of CUR and its analogues as efficacious agents to target different diseases. CUR is also shown to be effective on age-related symptoms. Interestingly, CUR has been reported to enhance the lifespan in *Caenorhabditis elegans* and *Drosophila* spp., but its efficacy on humans warrants future exploration. The efficacy of different drugs has been shown to improve significantly when they were administered in combination with CUR. Hence, new drug combinations with CUR can be explored in the near future. Existing reports strongly suggest that CUR is an effective therapeutic agent but its efficacy on animals and humans are not completely understood. Hence, it is essential to learn more about the pharmacodynamics and pharmacokinetics of CUR in the near future to assess its medicinal values.

Conclusions

In summary, we have reviewed recent experimental evidences regarding the biology of CUR. It has been clearly demonstrated that CUR targets various signaling pathways that eventually affects epigenetics. This property of CUR has motivated researchers for developing therapeutic strategies by targeting different epigenetic factors including HDACs, HATs, and DNMTs. Further examination of CUR as an epigenetic agent is required to fully explore its potential for treating various diseases including cancer. We believe that continuous research on CUR and well-controlled human studies will address the biology as well as the therapeutic potential of this micronutrient.

Acknowledgements

We are thankful to all members of the Laboratory of Chromatin Biology for critical readings, discussions, or ideas for manuscript preparation.

Financial Support and Sponsorship

This research work was supported by the Department of Biotechnology (DBT) and Council of Scientific and Industrial Research (CSIR), Govt. of India to RST. CSIR is acknowledged for fellowship support to GKA.

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