Epigenetic regulation of high glucose-induced proinflammatory cytokine production in monocytes by curcumin

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Abstract

Diabetes is a proinflammatory state. We have previously shown increased monocyte proinflammatory cytokines in patients with Type 1 and Type 2 diabetes. High glucose induces proinflammatory cytokines via epigenetic changes. Curcumin, a polyphenol responsible for the yellow color of the spice turmeric, is known to exert potent anti-inflammatory activity in vitro. Recent studies indicate that it may regulate chromatin remodeling by inhibiting histone acetylation. In this study, we aimed to test the effect of curcumin on histone acetylation and proinflammatory cytokine secretion under high-glucose conditions in human monocytes. Human monocytic (THP-1) cells were cultured in presence of mannitol (osmolar control, mannitol) or normoglycemic (NG, 5.5 mmol/L glucose) or hyperglycemic (HG, 25 mmol/L glucose) conditions in absence or presence of curcumin (1.5–12.5 μM) for 72 h. Cytokine level, nuclear factor κB (NF-κB) transactivation, histone deacetylases (HDACs) activity, histone acetylases (HATs) activity were measured by western blots, quantitative reverse transcriptase-polymerase chain reaction, enzyme-linked immunosorbent assay, immunofluorescence staining. HG significantly induced histone acetylation, NF-κB activity and proinflammatory cytokine (interleukin 6, tumor necrosis factor α and MCP-1) release from THP-1 cells. Curcumin suppressed NF-κB binding and cytokine release in THP-1 cells. Also, since p300 histone acetyltransferase is a coactivator of NF-κB, we examined its acetylation. Curcumin treatment also significantly reduced HAT activity, level of p300 and acetylated CBP/p300 gene expression, and induced HDAC2 expression by curcumin. These results indicate that curcumin decreases HG-induced cytokine production in monocytes via epigenetic changes involving NF-κB. In conclusion, curcumin supplementation by reducing vascular inflammation may prevent diabetic complications.
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Keywords: Acetylation; Deacetylation; p300; HDAC2 and NF-κB

1. Introduction

Diabetes is a proinflammatory state. Several studies indicate that hyperglycemia is one of the key factors that contribute to diabetic complications [1–3]. High glucose leads to the activation of the master switch of inflammation, transcription factor nuclear factor κB (NF-κB) and significantly induces the expression of several inflammatory cytokines [4–8]. Although the mechanisms are not fully resolved so far, some recent studies have shown that in diabetic conditions, specific transcription mechanisms and nuclear chromatin remodeling events occur, which regulate monocyte activation via the NF-κB pathway [9,10].

NF-κB plays an important role in the regulation of proinflammatory genes that are associated with several inflammatory diseases, including atherosclerosis, insulin resistance, metabolic syndrome and diabetes and its complications [11–15]. NF-κB consists of homo- or heterodimers of different sub-units, such as p50, p52, p65/RelA, RelB and c-

Rel, with p65/RelA and p50 being the most common and well studied [16,17]. In most unstimulated cells, NF-κB resides in the cytoplasm in an inactive latent form complexed with its inhibitor subunit, inhibitory κBα factor (IκBα). Multiple extracellular stimuli, including inflammatory cytokines can induce NF-κB activation by promoting IκBα phosphorylation and its proteasomal degradation [14,15]. The released p65-p50 dimer then translocates to the nucleus, where it binds to the promoters of NF-κB-dependent inflammatory genes to induce their expression. p65 protein is a key transcriptionally active component of NF-κB. NF-κB activation is reciprocally regulated by RelA/p65 acetylation and deacetylation, which are mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs)[18,19].

Histone acetylation/deacetylation is an important epigenetic event that plays an important role in inflammation [20,21]. Acetylation by HAT of specific lysine residues on the N-terminal tail of core histones, results in uncoiling of the DNA and increased accessibility to transcription factor binding. In contrast, histone deacetylation by HDAC represses gene transcription by promoting DNA winding thereby limiting access to transcription factors [21,22]. Altered HAT and HDAC activities can lead to several diseases [23–26] including cancer, diabetes, cardiac hypertrophy and asthma [27–29].

The antioxidant and/or anti-inflammatory effects of dietary polyphenols, have all been shown to play a role in either controlling

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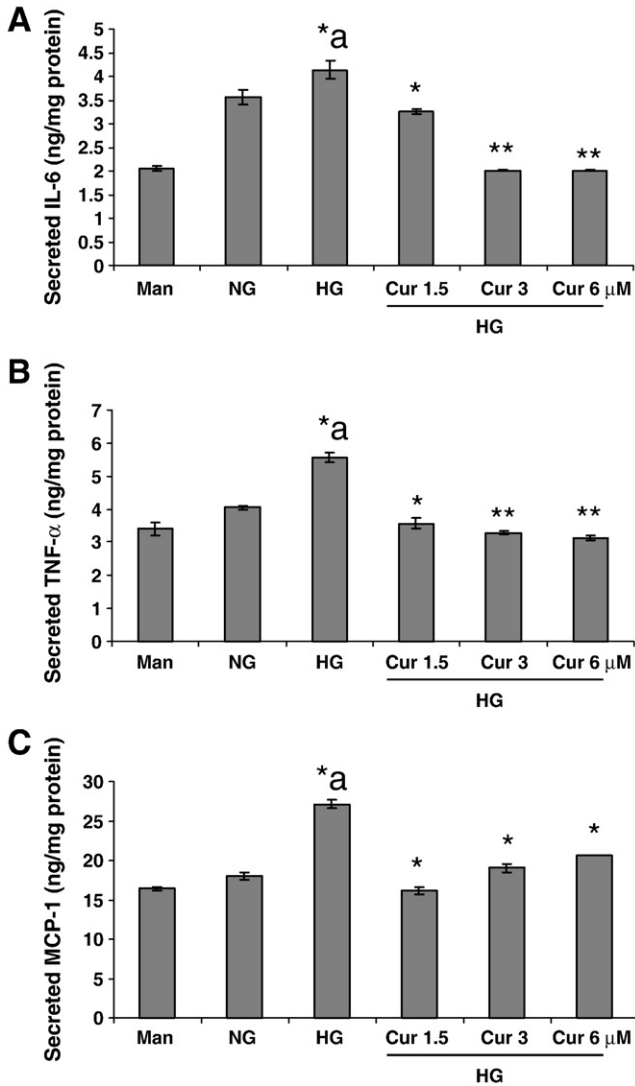 *E-mail address:* sridevi.devaraj@ucdmc.ucdavis.edu (S. Devaraj).

Fig. 1. Curcumin treatment suppresses cytokine release in HG-induced THP-1cells. THP-1 cells (1×10^5 cells/ml) were cultured in presence of osmolar control (19.5 mmol/L mannitol) or NG (5.5 mmol/L glucose) or HG (25 mmol/L) conditions in absence or presence of curcumin for 72 h as described in Methods. After that, the media was saved. Cytokines were measured with a Milliplex MAP Assay Kit (Millipore) according to the manufacturer's instructions. Values were calculated based on a standard curve constructed for the assay. Results were shown as mean±S.D. of five different experiments. *a:$P<.05$ compared to NG; *$P<.05$; **$P<.01$ compared to HG.

NF-κB activation or chromatin remodeling through modulation of HDAC activity and subsequently inflammatory gene expression [30,31]. Recent studies have reported that curcumin is an inhibitor of p300-HAT [32].

Curcumin (diferuloylmethane) is a polyphenol responsible for the yellow color of the curry spice turmeric. It has been used in a variety of diseases in traditional medicine. Modern scientific research has demonstrated its anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-thrombotic and cardiovascular protective effects [33]. Since NF-κB regulates expression of a wide variety of genes that are intimately involved in the process of inflammation, inhibition of NF-κB by curcumin may be an interesting prospect for controlling chronic inflammatory diseases involving the NF-κB signaling pathway [34]. However, very little is known about the specific molecular transcription mechanisms and nuclear chromatin remodeling events regulated by curcumin treatment under diabetic conditions especially as it relates to monocyte proinflammatory cytokines.

Thus, we hypothesize that curcumin suppresses the proinflammatory cytokine secretion through the NF-κB signaling pathway via altering histone acetylation/histone deacetylation balance under high-glucose conditions in human monocytes.

2. Materials and methods

2.1. Materials

Anti-HDAC2 antibody was procured from Active Motif (Carlsbad, CA, USA) and anti-NF-κBp65, anti-phospho NF-κBp65, anti-acetyl CBP/p300 and anti-phospho IκBα were procured from Cell Signaling Technology (Beverly, MA, USA), respectively. Anti-acetyl p65 was procured from Abcam (Cambridge, MA , USA). Transcription enzyme-linked immunosorbent assay (ELISA) kit (TransAM NF-κBp65), HAT and HDAC assay kit (colorimetric) were purchased from Active motif (Carlsbad, CA, USA) and Biovision (Mountain View, CA, USA), respectively. Anti-p300 antibody and Milliplex MAP ELISA Kit were purchased from Millipore (Temecula, CA, USA). Curcumin was purchased from Sigma (St. Louis, MO, USA). The BCA protein assay kit was purchased from Pierce. Novex pre-cast Tris-glycine gels were obtained from Invitrogen (Carlsbad, CA, USA). All other chemicals, unless otherwise stated, were obtained from Sigma (St. Louis, MO, USA).

2.2. Cell culture and treatment with curcumin

Human monocytic (THP-1) cell line was obtained from American Type Culture Collection (Manassas, VA, USA). THP-1 cells were cultured in RPMI medium containing 10% fetal bovine serum and 1% antibiotics at 37°C and 5% CO_2. Curcumin (dissolved in dimethyl sulfoxide [DMSO]) was used for the treatment of cells. The final concentration of DMSO used was 0.1% (v/v) for each treatment. THP-1 cells (1×10^5 cells/ml) were cultured in presence of osmolar control (19.5 mmol/l mannitol) or normoglycemic (NG, 5.5 mmol/L glucose) or hyperglycemic (HG, 25 mmol/L glucose) conditions in absence or presence of curcumin (1.5–12 μM) for 72 h after which media was saved for cytokine release measurement and cells were washed with phosphate-buffered saline (PBS) and then harvested.
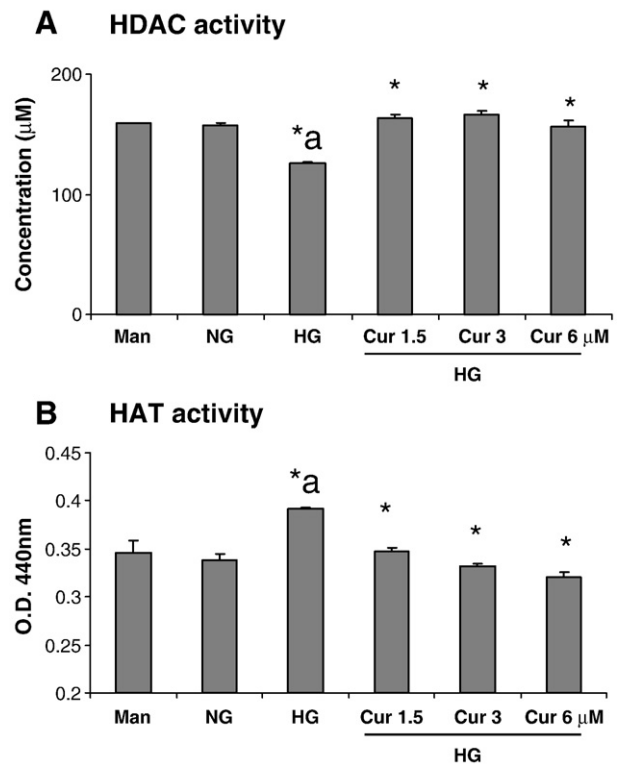

Fig. 2. Modulation of HDACs and HATs activity by curcumin treatment in HG-induced THP-1 cells. Following treatment of cells with various concentrations of curcumin for 72 h as described in Methods, cells were harvested and nuclear lysates were prepared; 10 and 50 μg of nuclear lysate protein from each group were taken for determination of HDACs and HATs activity, respectively. The experiment was done according to the manufacturer's instructions. Absorbance was taken at 405 nm and 440 nm by using ELISA reader. Results were shown as mean±S.D. of five different experiments. *a:$P<.05$ compared to NG; *$P<.05$ and **$P<.01$ compared to HG.

2.3. Cytokine release measurement

Cytokines were measured with a Milliplex MAP Assay Kit (Millipore) according to the manufacturer's instructions. Values were calculated based on a standard curve constructed for the assay.

2.4. Trypan blue exclusion assay for cellular viability

The cytotoxicity of curcumin to cultured high glucose-induced THP-1 cells was measured by Trypan blue assay. The effect of curcumin on growth inhibition was assessed as the percentage of inhibition in cell growth where vehicle-treated cells were taken as 100% viable. Cytotoxic effect by curcumin (0.5–12.5 μM; 72 h) treatment was not observed. Curcumin treatment was cytotoxic at ≥25 μM. To be in the physiologic range (0.75–10 μM), only concentrations of curcumin <6 μM were used for all further experiments.

2.5. Detection of transcription factor NF-κBp65 using ELISA

Following treatment of cells with various concentration of curcumin for 72 h, cells were harvested and nuclear lysates were prepared. The commercially available kit for NF-κB/p65 (Active Motif, Carlsbad, CA, USA) contains the specific oligos with the specific consensus sequence for NF-κB/p65 binding. Five micrograms of nuclear lysate protein from each group was taken for quantification of NF-κB activity. The experiment was done according to the manufacturer's instructions. Absorbance was taken at 450 nm by using ELISA reader (Multiscan MCC/340, Fisher Scientific).

2.6. Measurement of HDAC and HAT activity using ELISA

Following treatment to cells with various concentrations of curcumin for 72 h, cells were harvested and nuclear lysates were prepared. 10 and 50 μg of nuclear lysate protein from each group were taken for determination of HDACs and HATs activity, respectively. The experiment was done according to the manufacturer's instructions. Absorbance was taken at 405 nm and 440 nm.

2.7. Preparation of nuclear and cytoplasmic lysates

After treatment of cells with curcumin the medium was aspirated and the cells washed twice in PBS (10 mM, pH 7.4). Nuclear lysates were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce). The lysates were collected and cleared by centrifugation, and the supernatant was aliquoted and stored at −80°C. The protein content in the lysates was measured by BCA protein assay (Pierce), as per the manufacturer's protocol.

2.8. Western blot analysis

For Western blot analysis, 12 μg of protein from each treatment was resolved over 10% Tris-glycine polyacrylamide gels (Novex), transferred onto nitrocellulose membranes and subsequently incubated in blocking buffer [5% nonfat dry milk/1% Tween 20; in 20 mmol/L Tris-buffered saline (pH 7.6)] for 2 h. The blots were incubated with appropriate primary antibody (HDAC 2, p300, acetylated p300/CBP, p-NF-κBp65, NF-κBp65 and acetylated p65) washed and incubated with appropriate secondary horseradish peroxidase–conjugated antibody (Amersham Biosciences). The blots were detected with chemiluminescence (ECL kit, Amersham Biosciences) and autoradiography, using XAR-5 film (Eastman Kodak). Equal loading of protein was confirmed by stripping the blots and reprobing with TATA binding protein (Abcam, Cambridge, MA, USA).

2.9. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNAs were extracted from cultured cells using TRIZOL reagent (Invitrogen). Template cDNAs were obtained by reverse transcription (RT) using QuantiTect reverse transcription kit (Qiagen). Detection of cDNAs was done by PCR reactions using primers designed for candidate transcript detection. HDAC2: sense:5′-GGGAA-TACTTTCCTGGCACA-3, Antisense;5′ACGGATTGTGTAGCCACCTC-3′,p300:forward:5′-GGTCCACTCCAATCCAG-3′, Reverse: 5′CTCAAGATGTCTCGGAA-3′. Real-time RT-PCR experiments were done in triplicate using SYBR Green chemistry and a Mastercycler ep gradient S (Eppendorf). The quantity in each sample was normalized to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts. RT-PCR data were calculated by Delta-Delta CT method.

2.10. Immunostaining

After treatment of cells with curcumin, the cells were centrifuged and medium was aspirated. Cells were washed twice in PBS (10 mM, pH 7.4) and placed on L-lysine coated slides, the slides were air-dried, fixed with 4% formaldehyde for 30 min at 4°C and stained overnight at 4°C with HDAC2, p300, acetylated p65 antibody (1:1000
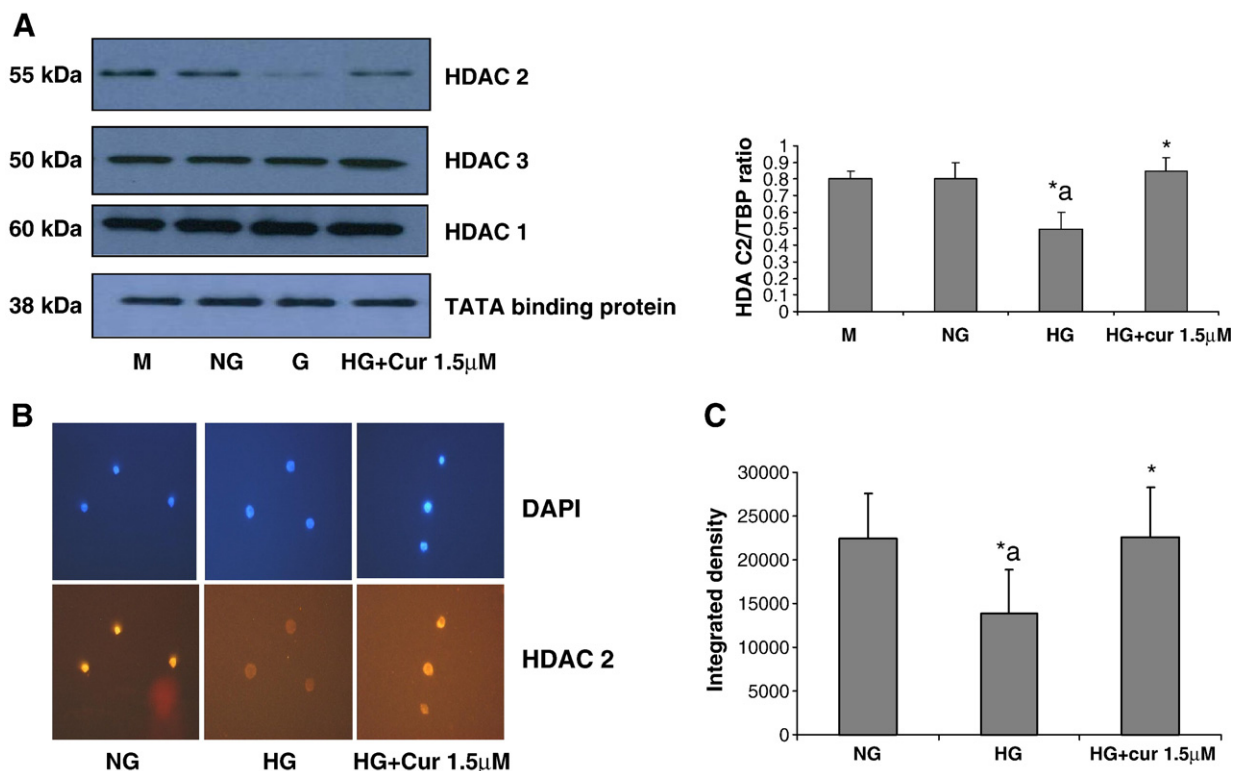

Fig. 3. Effect of curcumin on HDAC2 gene expression level in HG-induced THP-1 cells. (A) Following treatment of cells with various concentrations of curcumin for 72 h as described in Methods, cells were harvested and nuclear lysates were prepared. protein was subjected to SDS-PAGE as detailed in Materials and Methods Section. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for TATA binding protein (TBP). The immunoblot shown here are representative of three independent experiments with similar results. (B) Cells were fixed with 4% formaldehyde for 30 min at 4°C and stained overnight at 4°C with p300 as described in Methods. Data from a typical experiment of three are shown (original magnification×400). *a:P<.05 compared to NG; *P<.05 compared to HG. (C) In order to measure immunostaining intensity of HDAC2, p300 and acetylated p65, images were captured with a Nikon eclipse TE200 camera (Japan). The signal intensity was measured using ImageJ software. *a:P<.05 compared to NG; *P<.05 compared to HG.

dilution). After being air dried, slides were incubated with appropriate secondary antibody for 60 min. The slides were washed as described above, air-dried, mounted with mounting medium and then examined with a fluorescence microscope at 400× magnification. In order to measure immunostaining intensity of HDAC2, p300 and acetylated p65, images were captured with a Nikon eclipse TE200 camera (Japan). The signal intensity was measured using ImageJ software.

2.11. Chromatin immunoprecipitation assays

ChIP assays were performed using Chip-IT Express (Active Motif) according to the manufacturer's instructions. After treatment of cells with curcumin, the cells were centrifuged and medium was aspirated. Cells were washed twice in PBS (10 mM, pH 7.4) and fixed with fresh Fixation solution (37% formaldehyde) for 5 s at room temperature, followed by glycine stop-fix solution. Cells were washed twice with cold PBS, PBS was poured off and discarded and cells were scraped, pelleted by centrifugation for 10 min at 2,500 rpm at 4°C. Cells were resuspended in 1 ml of ice-cold lysis buffer followed by 30 min incubation on ice. Pellets were spun down for 10 min at 5000 rpm. Chromatin were sheared using 25% power sonication using our optimized condition (10 pulses of 20 s each with a 30-s rest on ice between pulses) to an average DNA size of 600 bp and lysates were cleared by centrifugation at 14 000 for 10 min at 4°C. For each ChIP, one-tenth of the total sonicated chromatin volume (500 µl) was used. Immunoprecipitations were performed overnight at 4°C with 5 µl

of the p300 antibody. Chromatin–antibody complexes were captured to magnetic beads (25 µl), and chromatin was eluted as described in manufacturer's instructions. The cross-links were reversed and DNA purified by proteinase K. DNA was analyzed by PCR.

2.12. PCR

DNA concentration was measured spectrophotometrically at 260 nm. DNA was subjected to PCR. The antibodies against p300 were purchased from Upstate Biotechnology. Primer sequences for the amplification of interleukin (IL)-6 and tumor necrosis factor α (TNF-α). IL-6 were: forward: 5′-TTGCGATGCTAAAGGACG-3′ and reverse: 5′-TGTGGAGAAGGAGTTCATAGC-3′. TNF-α forward:5′-CCCTCCCAGTTC-TAGTTCTATC-3′and reverse: 5′-GGGGAAAGAATCATTCAACCAG-3′. PCR was performed after a 4 min denaturation at 94°C, and repeating the cycles of; 94°C, 55°C and 72°C each for 40 s; the number of cycles was specific for each primer set. PCR products were electrophoresed in a 1.5% agarose gel containing ethidium bromide.

2.13. Statistical analysis

Each experiment was performed at least three times. Results are expressed as the mean±SD. Statistical analysis was performed using Student's t-test, and statistical significance is expressed as $*P<.05$ and $**P<.01$.
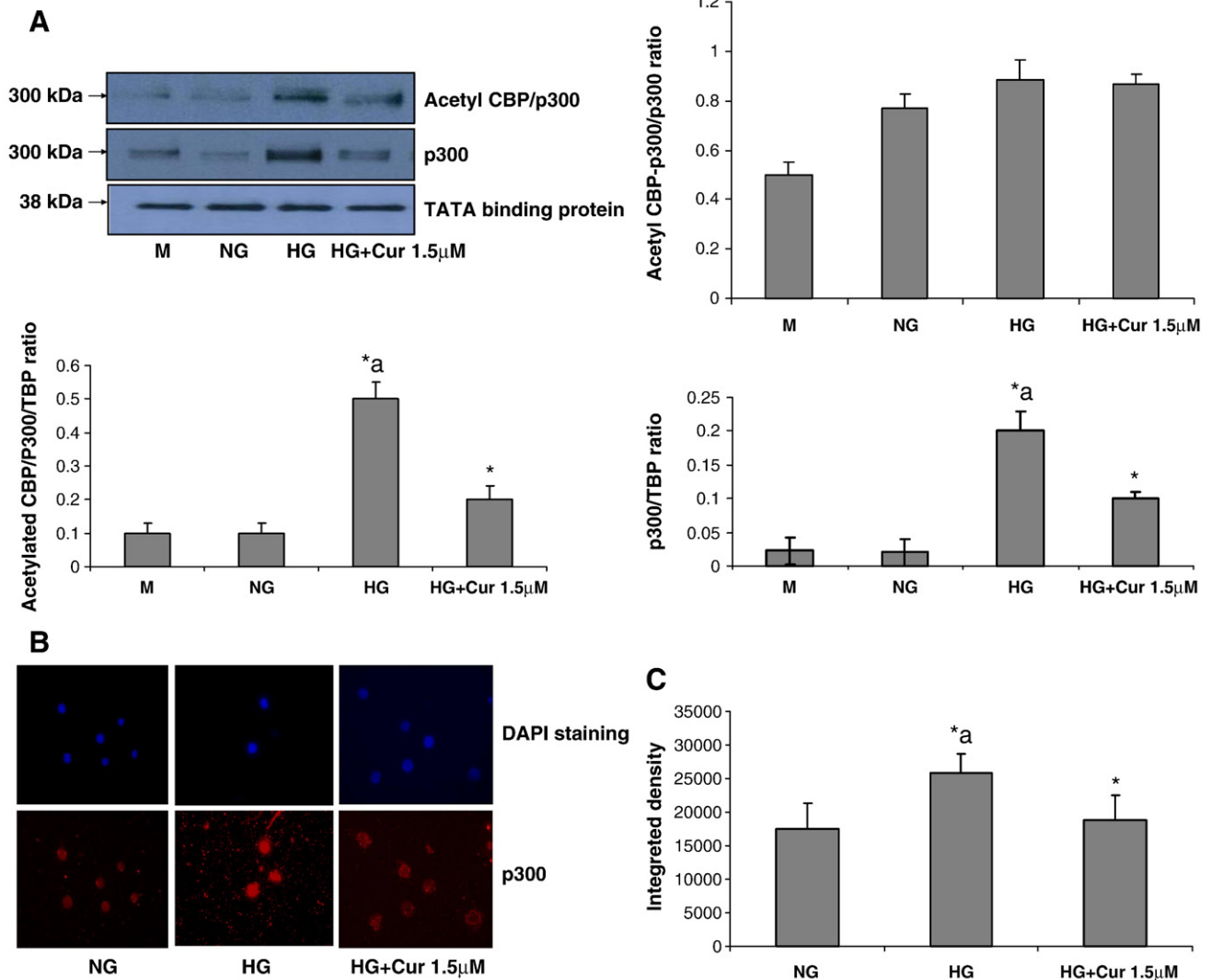

Fig. 4. Effect of curcumin on p300 and acetylated CBP/p300 gene expression level in HG –induced THP-1 cells. (A) Following treatment of cells with various concentrations of curcumin for 72 h as described in Methods, cells were harvested, and nuclear lysates were prepared. Protein was subjected to SDS-PAGE as detailed in Materials and Methods section. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for TBP. The immunoblot shown here is representative of three independent experiments with similar results. (B) Cells were fixed with 4% formaldehyde for 30 min at 4°C and stained overnight at 4°C with p300 as described in Methods. Data from a typical experiment of three are shown (original magnification×400). $*a$:$P<.05$ compared to NG; $*P<.05$ compared to HG. (C) In order to measure Immunostaining intensity of p300, images were captured with a Nikon eclipse TE200 camera (Japan). The signal intensity was measured using ImageJ software $*a$:$P<.05$ compared to NG; $*P<.05$ compared to HG.

3. Results

3.1. Cytoinflammatory cytokine secretion is decreased by curcumin treatment under hyperglycemic conditions in monocytes

We first examined the effect of hyperglycemia (HG, 25 mmol/L glucose) compared to NG (5.5 mmol/L glucose) on cytokine release from monocytes by ELISA (Fig. 1A-C). Under HG, inflammatory cytokine IL-6, TNF-α and MCP-1 release were significantly increased compared to NG. Mannitol was used as hyperosmolar control and did not significantly affect cytokine release. As shown in Fig. 1A–C, inflammatory cytokines from high glucose-induced THP-1 cells were significantly down-regulated by curcumin (1.5-, 3- and 6-µM) treatment.

3.2. Curcumin treatment significantly modulates HAT and HDACs activity in monocytes under hyperglycemia

Next, we studied its specific regulation mechanisms. Acetylation of histone protein is associated with increased binding of the transcription factor NF-κB [35]. HDACs and HATs play an important role in regulating proinflammatory response. Thus, to obtain further insights into the mechanisms of curcumin-induced down-regulation of inflammatory cytokines, as a first step, we examined whether curcumin treatment modulates HDACs activity and HAT activity using ELISA. As shown in Fig. 2, under HG conditions, there was a
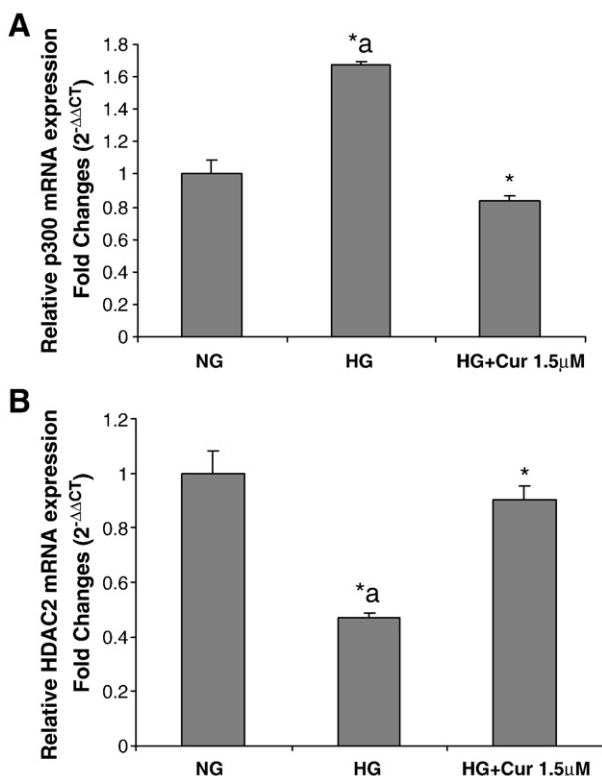

Fig. 5. Effect of curcumin on HDAC2 and p300 mRNA level in HG-induced THP-1 cells. Following treatment of cells with various concentrations of curcumin for 72 h as described in Methods, cells were harvested and total RNA were prepared. Template cDNAs are obtained by RT using QuantiTect reverse trasnscription kit (Qiagen). Detection of cDNAs was done by PCR reactions using primers designed from for candidate transcript detection. HDAC2 and p300. Real-time RT-PCR experiments was done in triplicate using SYBRR Green chemistry and a Mastercycler ep gradient S (Eppendorf). The quantity of each sample was normalized to the levels of GAPDH transcripts. Quantitative PCR data were calculated by Delta–Delta CT method. Values are expressed as mean±standard deviation (n=3; technical replicates). Data represent mean±SD. *a:P<.05 compared to NG; *P<.05 compared to HG.

significant increase in the HATs activity and decrease in HDACs activity when compared to normal glucose-treated cells (P<.01). Furthermore, curcumin treatment results in a significant down-regulation of HAT and up-regulation of HDAC activity (P<.01).

High glucose activates transcription factors, such as NF-κB, by recruitment of transcriptional coactivator molecules p300, which possess intrinsic HAT activity, resulting in histone acetylation and DNA unwinding, allowing DNA polymerases access to the DNA leading to proinflammatory gene expression (inflammatory cytokines). HDAC2 forms a bridge with HAT and RelA/p65 to inhibit gene transcription [36]. Thus, we tested the effect the expression level of related HDACs and HAT using immunoblots. As shown in Fig. 3A, under HG conditions, there was a marked down-regulation of HDAC2 when compared with normal glucose-treated cells. This down-regulation of HDAC2 was attenuated by curcumin (1.5-µM) treatment. Similar results were obtained by using Immunofluorescence (Fig. 3B). There was no significant change in HDAC1 and 3 (Fig. 3A). As shown in Fig. 4, HG treatment of THP-1 cells resulted in a marked up-regulation of p300 which is coactivator including intrinsic HAT and acetylated CBP/p300 expression when compared with normal glucose-treated cells. D-Mannitol had no effect on the regulation. In addition, acetylation of Lys1499 p300 has been demonstrated to enhance its HAT activity and affect a wide variety of signaling events [37]. The p300 activation was reduced completely by curcumin (1.5 µM) treatment. No effects were seen with DMSO (0.1%) used as vehicle control. Similar results were obtained by using Immunofluorescence (Fig. 4C). mRNA expression level of HDAC2 and p300 also were affected by HG. The down-regulation of HDAC2 and up-regulation of p300 under HG conditions were abrogated by curcumin treatment (Fig. 5)

3.3. Curcumin inhibits NF-κBp65 activation in high glucose-induced THP-1 cells

Acetylation of histone protein is associated with increased binding of the transcription factor NF-κB [38]. Increased histone acetylation was associated with increased activation of NF-κB, leading to increased acetylation of RelA/p65 subunit of NF-κB [38]. Therefore, we studied the effects of curcumin on acetylation of NF-κBp65, transactivation and translocation of NF-κBp65 in high glucose induced-THP-1 cells. NF-κB activation was analyzed by measuring IκBα phosphorylation and subsequent translocation of NF-κB (p65) to the nucleus and acetylation of NF-κBp65 using Western blot assays of nuclear protein. We observed that curcumin (1.5 µM) treatment of cells resulted in decreased NF-κBp65 in the nuclear fraction (Fig. 6A). We further confirmed the inhibition of NF-κB/p65 transactivation by performing ELISA (Fig. 6B). We also observed that curcumin (1.5 µM) resulted in significantly decreased acetylation and phosphorylation of NF-κBp65 in the nuclear fraction (Fig. 6A). Similar results on acetylation of p65 were obtained by using Immunofluorescence (Fig. 6C). Thus, curcumin appears to suppress inflammatory cytokines through at least in part the NF-κB signaling pathway via inducing HDAC activity (HDAC2) and suppressing HAT activity (p300) under HG conditions.

3.4. Curcumin affect chromatin events at the promoters of inflammatory genes

To confirm the epigenetic regulation of curcumin on inflammation, we next used ChIP assays to further investigate whether p300 can be bound to the promoters of NF-κB-related inflammatory cytokine genes under HG conditions. ChIP assays showed that HG increased the recruitment of p300 to the TNF-α and IL-6 promoters. As shown in Fig. 7, curcumin reduced the binding of p300 to the promoter region of IL-6 and TNF-α. This was associated with decreased IL-6 and TNF transcription in monocytes under HG conditions.
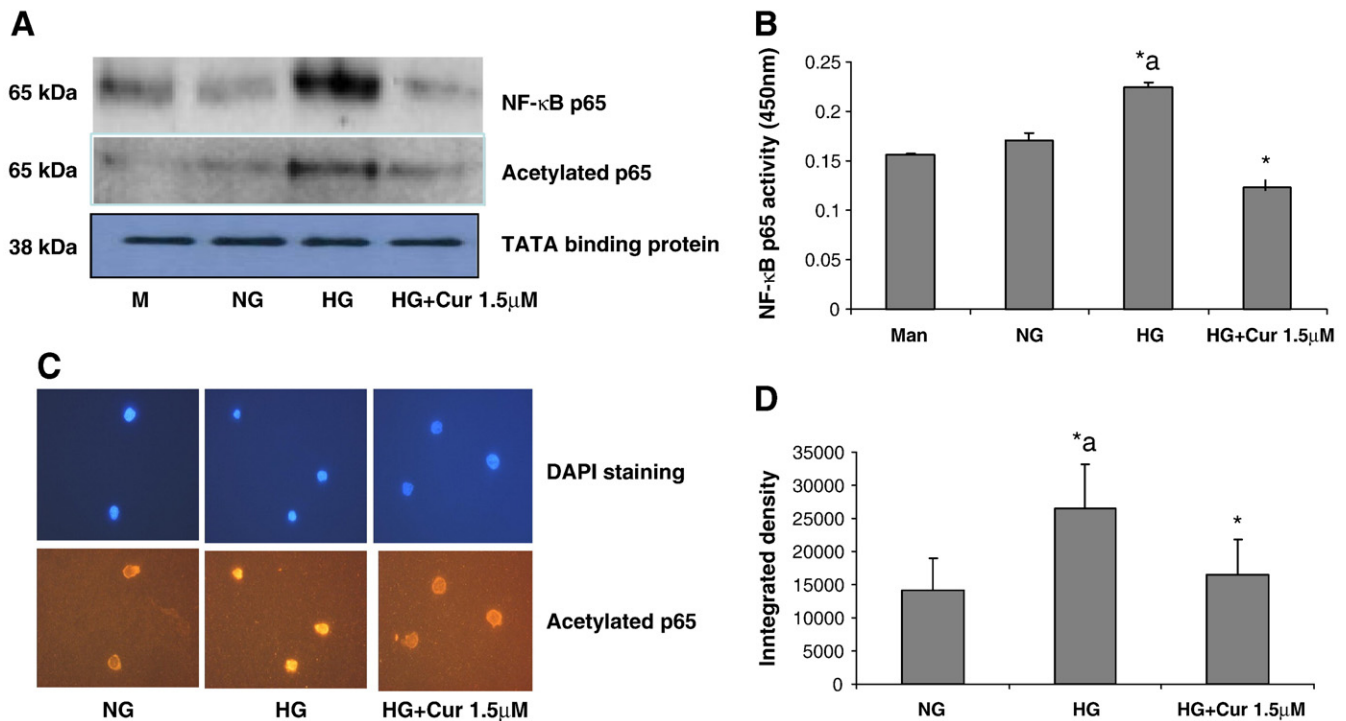
Fig. 6. Suppression of NF-κBp65 activation pathway by curcumin in HG-induced THP-1 cells. (A) Following treatment of cells with various concentrations of curcumin for 72 h as described in Methods, cells were harvested and nuclear lysates were prepared. Protein was subjected to SDS-PAGE as detailed in Methods Section. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for TBP. The immunoblot shown here are representative of three independent experiments with similar results. (B) The commercially available kit for NF-κB/p65 (Active Motif) contains the specific oligos with the specific consensus sequence for NF-κB/p65 binding. Five micrograms of nuclear lysate protein from each group was taken for quantification of NF-κB activity. The experiment was done according to the manufacturer's instructions. Absorbance was taken at 450 nm by using ELISA reader (Multiscan MCC/340, Fisher Scientific). (C) Cells were fixed with 4% formaldehyde for 30 min at 4°C and stained overnight at 4°C with p300 as described in Methods. Data from a typical experiment of 3 are shown (original magnification×400). *a:$P<.05$ compared to NG; *$P<.05$ compared to HG. (D) In order to measure Immunostaining intensity of acetylated p65, images were captured with a Nikon eclipse TE200 camera (Japan). The signal intensity was measured using ImageJ software. *a:$P<.05$ compared to NG; *$P<.05$ compared to HG.

4. Discussion

Hyperglycemia has been implicated as a major contributor to several diabetic complications [38,39]. Several lines of evidence also point to diabetes being a proinflammatory state. Monocytes are important in orchestrating effects of hyperglycemia-induced inflammation. We and others have shown that HG induces proinflammatory cytokines (IL-1, IL-6 and TNF-α) in monocytes via NF-κB dependent pathway. In correlation with this observation and earlier report, high glucose treatment to the THP-1 cells leads to increased expression of the inflammatory cytokines TNF-α, IL-6, IL-8 and IL-1β in a hyperglycemia-induced oxidative stress, nuclear NF-κB and AP-1 transcription factor-dependent manner [4,10,40]. TNF-α, IL-6, IL-8 and MCP-1 are proinflammatory cytokines and widely recognized markers of vascular inflammation [41,42]. The levels of these cytokines are elevated in diabetic patients [43–46]. We have previously shown increased monocyte proinflammatory cytokines in patients with Type 1 and Type 2 diabetes [47].

The antioxidant and/or anti-inflammatory effects of dietary polyphenols, have all been shown to play a role in either controlling NF-κB activation or chromatin remodeling through modulation of HDAC activity and subsequently inflammatory gene expression [30,31].

Curcumin (diferuloylmethane) is a polyphenol responsible for the yellow color of the curry spice turmeric. It has been used in a variety of diseases in traditional medicine. Modern scientific research has demonstrated its anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-thrombotic and cardiovascular protective effects [33]. Also, curcumin has been shown to be anti-inflammatory via suppression of the NF-κB pathway [34,40]. Recent studies have reported that curcumin

possesses HAT inhibitory activity with specificity for the p300/CREB-binding protein [32]. However, its specific regulation mechanisms at the level of chromatin are not known in diabetic conditions.

The goal of this study was to determine whether curcumin, can be used as a therapeutic agent for inflammation that contributes to diabetes complications. In the present study, we investigated the role of curcumin in regulation of high glucose-mediated proinflammatory cytokines (IL-6 and TNF-α release), chromatin remodeling and posttranslational modification of transcription factor, NF-κB in high glucose-induced THP-1cells.

Production of reactive oxygen species has been implicated as a common factor of hyperglycemic damage [48,49]. Reactive oxygen species alter nuclear histone acetylation and deacetylation (chromatin remodeling) leading to increased NF-κB–dependent gene expression of proinflammatory mediators [50]. NF-κB plays an important role in the regulation of proinflammatory genes that are associated with several inflammatory diseases, including atherosclerosis, insulin resistance, metabolic syndrome and diabetes and its complications [11–15]. NF-κB consists of homo- or heterodimers of different subunits, such as p50, p52, p65/RelA, RelB and c-Rel, with p65/RelA and p50 being the most common and well studied [16,17]. p65 protein is a key transcriptionally active component of NF-κB whose transactivation potential is enhanced by several coactivators, including CREB-binding protein/p300, p/CAF and SRC1 [51] which have histones. These cofactors have HAT activity and play key roles in the transcription machinery [52,53]. The p300/CBP-mediated hyperacetylation of RelA (p65) is critical for NF-κB activation. Five main acetylation sites have been identified within p65. Acetylation at Lys221 enhances DNA binding by p65 and impairs its assembly with

A **TNF-α promoter**
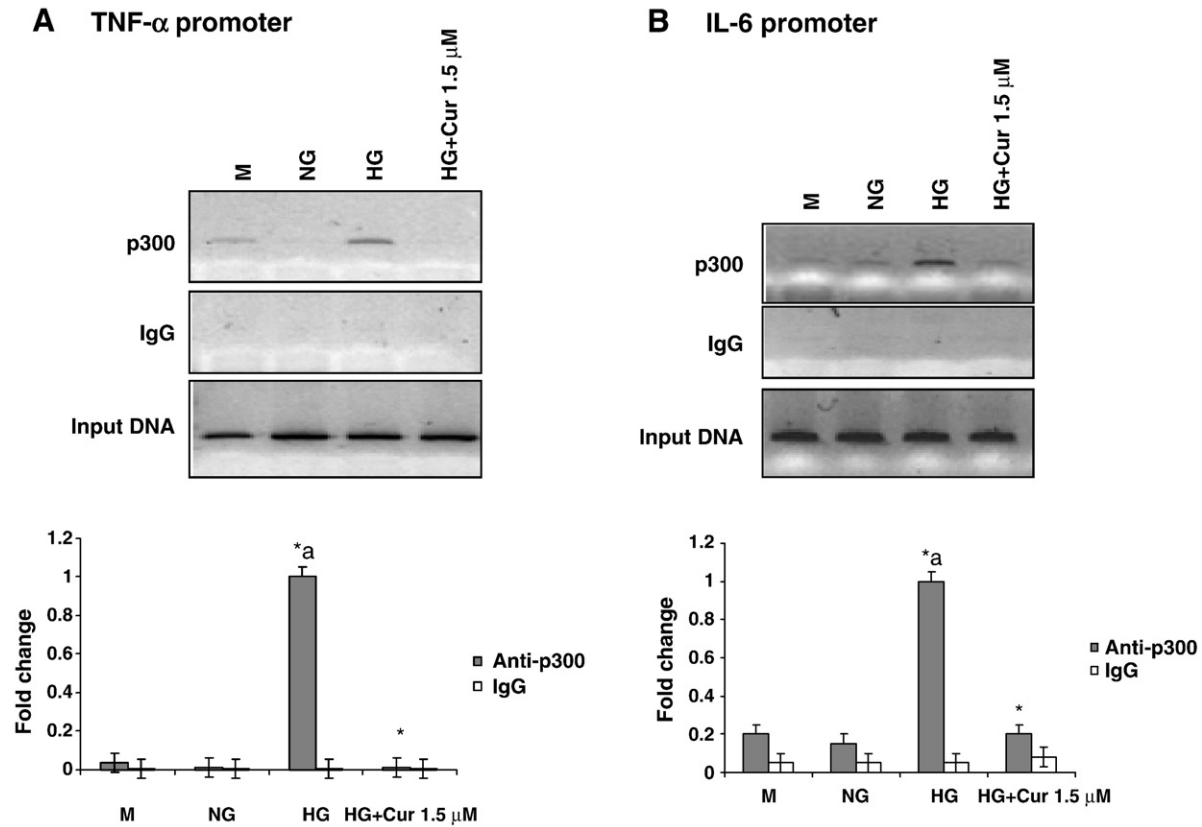
B **IL-6 promoter**

Fig. 7. Effect of curcumin on chromatin events at the promoters of inflammatory genes. ChIP assays were performed using Chip-IT Express (Active Motif) according to the manufacturer's instructions. Immunoprecipitations were performed overnight at 4°C with 5 μL of p300 antibody. DNA was subjected to PCR. ChIP assays showed the recruitment of p300 to the TNF-α (A) and IL-6 (B) promoters. Results of 1 typical experiment of 3 are shown. Values from ChIP with anti-p300 antibody represents the fold difference relative to those from IgG control antibody. Data are the average of three independent experiments each, and error bars represent standard deviations. *a:P<.05 compared to NG; *P<.05 compared to HG.
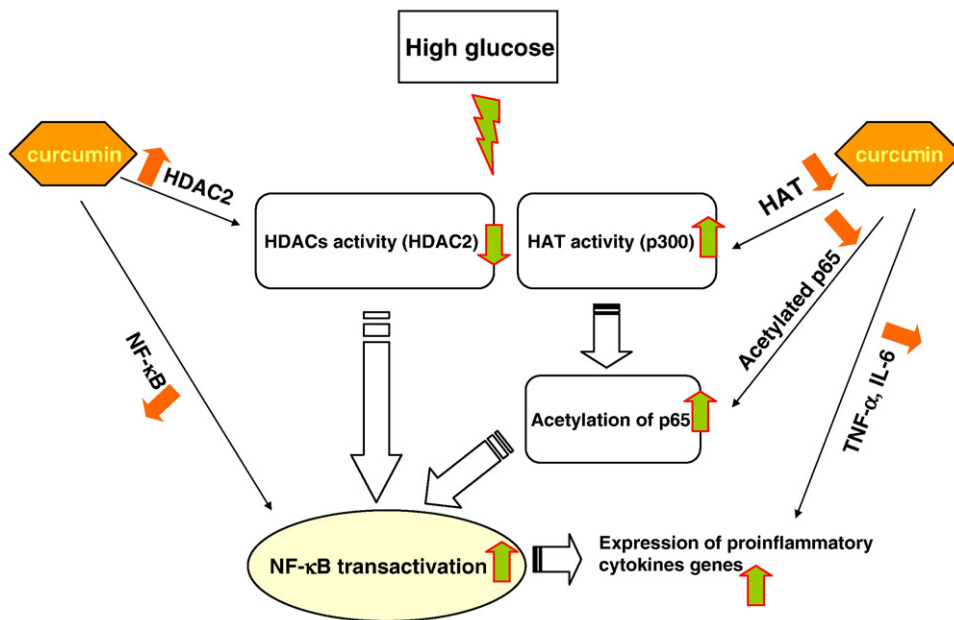

Fig. 8. Proposed epigenetic mechanisms via which curcumin regulates NF-κB signaling pathway leading to decreased expression of proinflammatory genes. High glucose activates the NF-κB signaling pathway leading to proinflammatory gene expression. Curcumin treatment of HG-induced cells activates the HDACs activity, especially HDAC2, and suppresses HAT activity, especially p300, leading to deacetylation of p65 NF-κB, subsequently suppressing proinflammatory cytokine release.

IκBα, whereas acetylation of Lys316 is required for full transcriptional activity of p65 [54]. Therefore, the attenuation of p65 acetylation is a potential molecular target for the prevention of chronic inflammation. In this study, at doses of 1.5 and 3 μM, curcumin treatment inhibited the expression of NF-κB target genes including IL-6, MCP-1 and TNF-α in high glucose-induced THP-1 cells. We also show novel data that curcumin prevents high glucose-induced p65 translocation to the nucleus, confirming that hyperacetylation is critical for NF-κB translocation as well as activity under hyperglycemia. Furthermore, we demonstrate that curcumin inhibits HG-induced p65 acetylation, thereby resulting in suppressed NF-κB transcription activity via hypoacetylation of p65 at Lys310.

There is also some previous evidence to suggest that HDAC corepressor proteins may function to negatively regulate NF-κB transcriptional activity [55–57]. We observed that curcumin can inhibit inflammation through up-regulation of HDAC2 activity in monocytes. Curcumin restored HDAC2 activity in high glucose-induced THP-1 cells. This HDAC2 recruitment may be, at least in part, attenuating NF-κB-mediated chromatin acetylation to exert its corepressor function and subsequent proinflammatory gene expression. Curcumin reduced the binding of p300 to the promoter region of IL-6 and TNF-α genes. Thus, curcumin appears to exert anti-inflammatory effects in monocytes under HG conditions via inhibition of HAT activity, preventing NF-κB-mediated chromatin acetylation and subsequent transcription of cytokines.

Thus, in summary, high glucose activates HAT (p300) which in turn acetylates p65 and suppresses HDAC2 resulting in induction NF-κB activation and increased transcription of IL-6 and TNF-α in monocytes. Curcumin acts at different levels and induces epigenetic changes by increasing HDAC2, decreasing HAT (p300) activity, thereby resulting in decreased NF-κB activation and inflammatory cytokine release (IL-6, TNF) (Fig. 8). All these results were also reproduced using human monocytes. Our results reveal for the first time that in vitro chromatin remodeling by curcumin occurs under diabetic conditions. However, future studies on chromatin events and molecular mechanism in HG conditions are needed in order to understand the effect this compound as natural therapy for chronic inflammation associated with diabetes and its complications.

Acknowledgments

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