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HDAC inhibition prevents NF-κB activation by suppressing proteasome activity: Down-regulation of proteasome subunit expression stabilizes IκBα

Robert F. Place, Emily J. Noonan, Charles Giardina *

Department of Molecular and Cellular Biology, University of Connecticut, 91 North Eagleville Road, U-3125, Storrs, CT 06269-3125, USA

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Abstract

The short chain fatty acid (SCFA) butyrate (BA) and other histone deacetylase (HDAC) inhibitors can rapidly induce cell cycle arrest and differentation of colon cancer cell lines. We found that butyrate and the specific HDAC inhibitor trichostatin A (TSA) can reprogram the NF-κB response in colon cancer cells. Specifically, TNF-α activation is suppressed in butyrate-differentiated cells, whereas IL-1β activation is largely unaffected. To gain insight into the relationship between butyrate-induced differentiation and NF-κB regulation, we determined the impact of butyrate on proteasome activity and subunit expression. Interestingly, butyrate and TSA reduced the cellular proteasome activity in colon cancer cell lines. The drop in proteasome activity results from the reduced expression of the catalytic β-type subunits of the proteasome at both the protein and mRNA level. The selective impact of HDAC inhibitors on TNF-α-induced NF-κB activation appears to relate to the fact that the TNF-α-induced activation of NF-κB is mediated by the proteasome, whereas NF-κB activation by IL-1β is largely proteasome-independent. These findings indicate that cellular differentation status and/or proliferative capacity can significantly impact proteasome activity and selectively alter NF-κB responses in colon cancer cells. This information may be useful for the further development and targeting of HDAC inhibitors as anti-neoplastic and anti-inflammatory agents.

Keywords: NF-κB; IκBα; TNF-α; IL-1β; Butyrate; TSA; HDAC; Proteasome; β5; β1; β2; Caco-2

1. Introduction

Butyrate (BA) is a short chain fatty acid (SCFA) produced by bacterial fermentation of dietary fiber within the gastrointestinal tract. It is actively absorbed by intestinal epithelial cells and is the major luminal source of energy for colonocytes [1,2]. In addition to being a fuel source, butyrate has a number of other profound biological effects [3–6]. For instance, butyrate can readily induce apoptosis of transformed cell lines [7,8]. The ability for butyrate to induce apoptosis of cancer cells may contribute to the cancer preventive effects ascribed to dietary fiber [9–11]. Short chain fatty acids, particularly butyrate, can also suppress intestinal inflammation. Clinical trials have shown that topical butyrate applications alleviate symptoms in patients with mild and moderate ulcerative colitis [12]. Butyrate is also effective at treating other inflammatory conditions of the distal gastrointestinal tract [6,13,14]. In association, deficiencies in luminal butyrate production have been linked to colonic inflammation [9]. Such results suggest that butyrate may play an important role in regulating intestinal inflammation, as well as suppressing cellular transformation.

Biochemically, butyrate is a histone deacetylase (HDAC) inhibitor [15]. HDAC inhibitors in general have been noted for their ability to induce cell cycle arrest, differentiation and apoptosis of a wide spectrum of transformed cells. It has been proposed that HDAC inhibitors serve to normalize HDAC activity in transformed cells,

Abbreviations: SCFA, short chain fatty acid; HDAC, histone deacetylase; NF-κB, nuclear factor-κB; IκB, inhibitor-κB; IKK, IκB kinase; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; BA, butyrate; TSA, trichostatin A; CT-L, chymotrypsin-like; T-L, trypsyn-like; PGPH, post-glutamyl peptide hydrolyzing; E-MEM, minimal essential media with Earle's salts; EMSA, electrophoretic mobility shift assay; Suc-LLVY-AMC, *N*-succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin

* Corresponding author. Tel.: +1 860 486 0089; fax: +1 860 486 4331.
E-mail address: giardina@uconnvm.uconn.edu (C. Giardina).

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which often express elevated levels of certain HDAC proteins [16–19]. These findings have prompted clinical trials to assess the cancer therapeutic activity of HDAC inhibitors (such as SAHA and LAQ824) [20–22]. Gene expression profiles of cells treated with HDAC inhibitors have revealed that roughly 2% of the expressed genes are altered following HDAC inhibition [23]. Interestingly, within this population of genes, roughly half increase in expression, while the other half decreases. These selective changes in gene expression likely result from the enhanced acetylation of gene-regulatory transcription factors (e.g., GATA, p53, Sp1 and Sp3), in addition to the enhanced acetylation of histone proteins [24–27].

Recently, HDAC inhibitors have been reported to modulate the activity of the transcription factor NF-κB in a number of different cell types including colon cancer cell lines and macrophages isolated from the lamia propria of the colon [3,28–31]. NF-κB is a central mediator of the immune and inflammatory response and has been implicated in promoting tumorogenesis by protecting cancer cells from apoptosis [32]. Upon activation, NF-κB rapidly enhances the expression of proinflammatory genes such as cytokines and cell adhesion molecules, as well as genes involved in promoting proliferation, angiogenesis and cell survival [33–35]. The ability of butyrate and other HDAC inhibitors to modulate NF-κB activity coincides with its proposed cancer suppressing and anti-inflammatory activities.

NF-κB is regulated through the binding of inhibitory molecules collectively referred to as the IκB proteins [36,37]. IκB family members include IκBα, IκBε, IκBγ, IκBζ, Bcl-3, p105, p100, splicing variants IκBβ1 and IκBβ2 [37–42]. Perhaps the most important and well-characterized inhibitor of the IκB family is IκBα. It is the most abundant inhibitor overall, and is responsible for the rapid activation of NF-κB [43–47]. During NF-κB activation, IκBα is phophorylated by the IκB kinase (IKK) complex and subsequently ubiquitinated by the multisubunit E3 ubiquitin-ligation enzyme, $SCF^{\beta TrCP}$ [48,49]. Ubiquitinated IκBα is then rapidly degraded by the proteasome, releasing NF-κB to influence target gene expression [50–52]. It has been reported that butyrate's ability to suppress NF-κB activity depends in part on its ability to suppress cellular proteasome activity [30,53].

Here, we provide evidence that HDAC inhibitors butyrate and trichostatin A (TSA) suppress proteasome activity by down-regulating the expression of select proteasome subunits. This ultimately prevents the ubiquitin-mediated, proteasome-dependent degradation of IκBα limiting NF-κB activation. Furthermore, we find that HDAC inhibitors selectively interfere with the proteasome-dependent activation of NF-κB, while having little effect on the proteasome-independent pathway. These findings further classify the cellular activities of a promising new class of anti-inflammatory/anti-neoplastic agents.

2. Materials and methods

2.1. Cell culture and treatments

All cell lines were purchased from American Type Culture Collection (Manassas, VA). Caco-2 cells were propagated in minimal essential media containing 2 mM L-glutamine and Earle's salts (E-MEM) supplemented with 10% fetal bovine serum, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, streptomycin (50 mg/ml) and penicillin (50 U/ml). HT-29 and SW480 cells were propagated in McCoy's 5A medium supplemented with 10% fetal bovine serum, 0.1 mM non-essential amino acids and antibiotics. All medium components were purchased from Invitrogen Life Technologies (Carlsbad, CA). All cell types were treated with a number of agents in this study. The concentration and source of these agents are as follows: TNF-α, 50 ng/ml, (R&D Systems, Minneapolis, MN); IL-1β, 4 ng/ml, (Promega, Madison, WI); sodium butyrate, 4 mM, (Sigma–Aldrich, St. Louis, MO); TSA, 2 μM, (Calbiochem, San Diego, CA); MG-132, 60 μM, (Calbiochem). The TNF-α and IL-1β concentrations used were selected because they generated a reproducibly high level of NF-κB activity in the cell lines examined. These conditions were selected to ensure maximal sensitivity for the detection of cellular pathways that down-regulate the NF-κB response.

2.2. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared based on a previously reported protocol with minor modifications as described in Inan et al. [3]. For the DNA binding assays, double-strand NF-κB DNA oligonucleotide (Promega) were end-labeled with [γ-^{32}] adenosine triphosphate (3000 Ci/mmol at 10 mCi/ml; Amersham Pharmacia, NJ) using T4 polynucleotide kinase. Binding reactions were performed by mixing 7.5 μg of nuclear extract (in 7.5 μl) with 2.5 μg poly-dIdC and 1 μg bovine serum albumin to give a final volume off 14 μl. After 15 min incubation on ice, 40 fmol of labeled oligonucleotide (1 μl) was added to each reaction. Reactions were then transferred to room temperature for an additional 15 min. Reaction products were separated on a 4% polyacrylamide/Tris borate EDTA gel and analyzed by autoradiography. Antibody supershift and oligonucleotide competition experiments were performed to demonstrate the specificity of the EMSA results [3].

2.3. Immunoblotting

Cytosolic extracts were prepared as described in Inan et al. [3]. For immunoblotting studies, 25 μg of cytoplasmic protein (quantified by the Bio-Rad protein assay) was denatured under reducing conditions, separated on 10% sodium dodecyl sulfate (SDS) polyacrylamide gels, and transferred to nitrocellulose by voltage gradient transfer.