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Histone H3 Lysine 4 Demethylation Is a Target of Nonselective Antidepressive Medications

Brief Communication

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Summary

Demethylation of histone H3 lysine 4 is carried out by BHC110/LSD1, an enzyme with close homology to monoamine oxidases (MAO). Monoamine oxidase A or B are frequent targets of selective and nonselective small molecular inhibitors used for treatment of depression. Here we show that in contrast to selective monoamine oxidase inhibitors such as pargyline, nonselective monoamine oxidase inhibitors potently inhibit nucleosomal demethylation of histone H3 lysine 4. Tranylcypromine (brand name Parnate) displayed the best inhibitory activity with an IC_{50} of less than 2 μM. Treatment of P19 embryonal carcinoma cells with tranylcypromine resulted in global increase in H3K4 methylation as well as transcriptional derepression of two BHC110 target genes, *Egr1* and the pluripotent stem cell marker *Oct4*. These results attest to the effectiveness of tranylcypromine as a small molecular inhibitor of histone demethylation.

Introduction

In eukaryotes, DNA is highly compacted within the nucleosome structure representing the in vivo target of multiple chromatin modifying enzymes [1]. Histones composing the nucleosome core particle undergo many different types of histone modifications including methylation, acetylation, phosphorylation, ubiquitination, and glycosylation [2]. Such histone modifications are hypothesized to signal changes in chromatin structure leading to gene expression changes and recruitment of regulatory transcription complexes [3].

A significant advance in the chromatin modification field was the discovery of enzymes (PAD4/PADI4, BHC110/LSD1 and JmjC domain-containing demethylases) capable of demethylating histones [4–9]. PAD4/PADI4 has been found to convert monomethyl-arginine to citrulline by demethylimination. The JmjC domain-containing demethylases demethylate mono-, di-, or trimethylated lysines by a hydroxylation-based mechanism [7–9]. BHC110/LSD1 has been shown to be a FAD-dependent polyamine oxidase responsible for demethylating mono- and dimethyl histone H3 lysine 4 (H3K4), which in turn repress gene transcription [6].

BHC110 has been found within a number of multiprotein complexes sharing the two enzymatic core subunits: histone deacetylase (HDAC1/2) and BHC110 [10–17]. Our earlier study has shown that repression of neuronal-specific genes by the neuronal silencer, REST, requires the recruitment of a BHC110-HDAC1/2-containing complex termed BHC (*B*RAF-*H*DAC *c*omplex) [12]. We and others have recently found that nucleosomal demethylation by BHC110 requires the participation of the corepressor of REST (RE1-silencing transcription factor) protein, CoREST [18, 19].

We have been interested in defining small molecules that inhibit BHC110 demethylation activity. Notably, the BHC110 active site shares sequence homology with monoamine oxidase (MAO) enzymes responsible for oxidizing arylalkylamine neurotransmitters such as dopamine and serotonin [18, 20]. Structural analysis of inhibitor-MAO complexes demonstrates that alkylation of the flavin cofactor of the enzyme is a mechanism of MAO inhibition [21]. Since BHC110 and MAO both contain a flavin ring as a cofactor, we asked whether MAO inhibitors may serve as inhibitors of BHC110. The results described below provide evidence that tranylcypromine, a nonselective MAO inhibitor, acts as a potent inhibitor of BHC110-mediated demethylation of H3K4.

Results and Discussion

Analysis of Monoamine Oxidase Inhibitors on Histone Demethylation

Since BHC110 contains an amino oxidase catalytic domain, we were interested to know whether monoamine oxidase inhibitors could act as histone demethylase inhibitors. We analyzed six monoamine oxidase inhibitors. Three monoamine oxidase inhibitors were specific for monoamine oxidase A or B and three were nonselective monoamine oxidase inhibitors (Figure 1A). We first examined high concentrations of each inhibitor (1 and 5 mM) on histone demethylation by recombinant BHC110 generated in insect cells (Figure 1B). While specific monoamine oxidase inhibitors, clorgyline and deprenyl, only displayed a small inhibition at 5 mM concentration, the nonselective inhibitors showed a robust inhibition at 1 mM concentration (Figure 1B). This was most evident with phenelzine and tranylcypromine (Figure 1B). We next analyzed phenelzine and tranylcypromine in more detail by lowering the concentration required for BHC110 inhibition. This analysis revealed that while 200 μM phenelzine could inhibit H3K4 demethylation by BHC110, tranylcypromine displayed an inhibitory activity even at 100 nM concentration (Figure 1C). These results demonstrate that the nonselective monoamine oxidase inhibitors phenelzine and tranylcypromine could serve as histone H3K4 demethylation inhibitors and tranylcypromine (brand name, Parnate) displays the best inhibitory activity.

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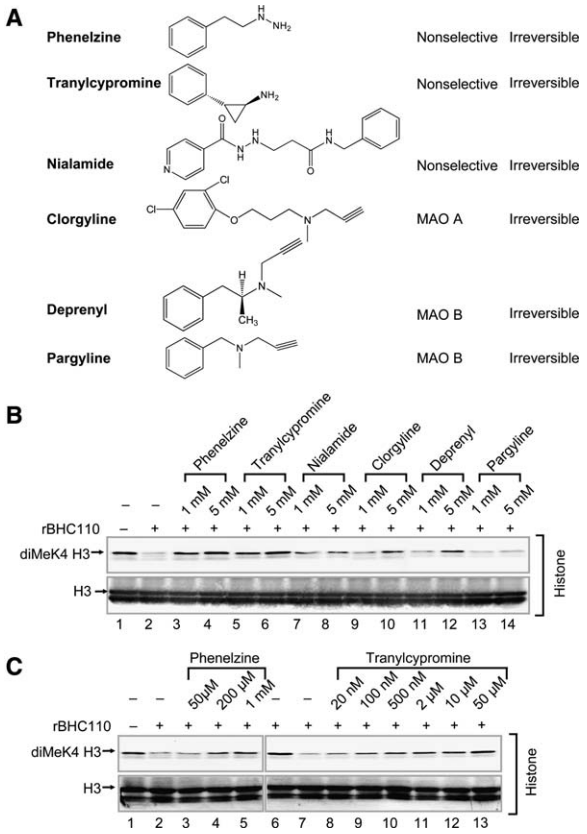

Figure 1. MAO Inhibitors Inactivate BHC110-Mediated H3K4 Demethylation

(A) Structures and properties of MAO inhibitors.
(B) Inhibitory effect of MAO inhibitors on histone demethylation by recombinant (r) BHC110 (1.5 μg).
(C) Effect of different concentrations of phenelzine and tranylcypromine on histone demethylation by recombinant BHC110 (1.5 μg).
In (B) and (C), bulk histones (4 μg) were used as substrate and the reaction mixture was analyzed by SDS-PAGE, followed by Western blotting.

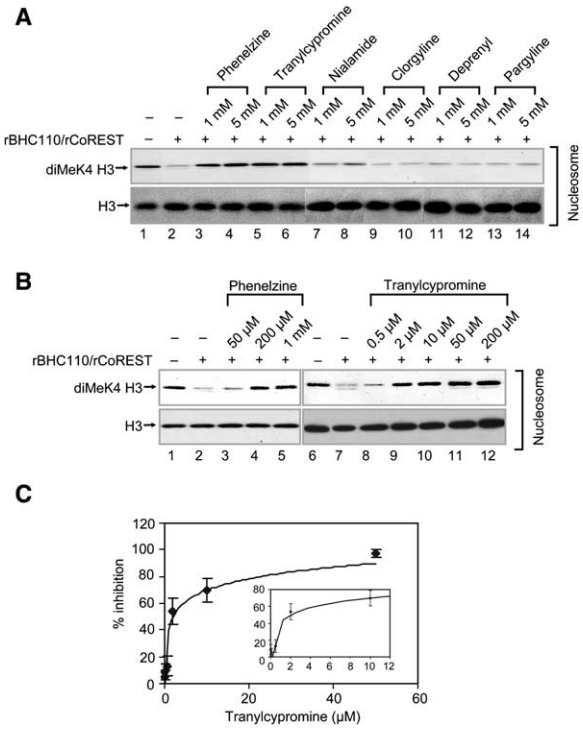

Figure 2. MAO Inhibitors Inactivate Nucleosomal H3K4 Demethylation

(A) Inhibitory effect of MAO inhibitors on nucleosomal demethylation by recombinant BHC110 (1.5 μg) and CoREST (0.75 μg).
(B) Effect of different concentrations of phenelzine and tranylcypromine on nucleosomal demethylation by recombinant BHC110 (1.5 μg) and CoREST (0.75 μg). In (A) and (B), nucleosomes were used as substrate and the reaction mixture was analyzed by SDS-PAGE, followed by Western blotting.
(C) A representative plot for inhibition of rBHC110 demethylation activity by tranylcypromine. Bulk histones (4 μg) were used as substrate. Followed by Western blot analysis, dimethyl K4 levels were quantified. Data are presented as the mean ± SEM of at least three experiments. The inset represents a smaller range of titration between zero to 12 μM tranylcypromine.

Analysis of Monoamine Oxidase Inhibitors on Nucleosomal Demethylation

We next examined the monoamine oxidase inhibitors for inhibition of nucleosomal demethylation by recombinant BHC110 and CoREST generated in insect cells. Although BHC110 alone catalyzes demethylation of lysine 4 in free H3, the SANT domain-containing protein CoREST is a necessary cofactor for nucleosomal demethylation by BHC110 [18, 19]. Interestingly, while nialamide, clorgyline, deprenyl, and pargyline were devoid of any inhibitory activity, phenelzine and tranylcypromine displayed potent inhibitory activity at 1 mM (Figure 2A). Titration of lower concentrations of phenelzine and tranylcypromine revealed that, similar to the effect of inhibitors on recombinant BHC110, tranylcypromine displayed a greater inhibitory effect on nucleosomal demethylation by recombinant BHC110/CoREST (Figure 2B). Multiple titrations of decreasing concentrations of tranylcypromine revealed an IC_{50} of less than 2 μM for both histone and nucleosomal demethylation (Figure 2C and data not shown). It is noteworthy that the reported IC_{50} of tranylcypromine for inhibiting either monoamine oxidase A or B is 20 μM [22], reflecting a greater efficacy for tranylcypromine in inhibiting H3K4 demethylation by BHC110.

Tranylcypromine Inhibits the Demethylase Activity without Affecting the Deacetylase Activity

Since BHC110 is a component of complexes containing HDAC1/2, we assessed the effect of tranylcypromine on H3K4 demethylation and deacetylation of H3K9 and H3K14. BHC110-containing complexes were isolated using stable cell lines expressing Flag-BHC110 or Flag-CoREST (Figure 3A). Titration of increasing concentrations of tranylcypromine revealed a specific inhibition of H3K4 demethylation of nucleosomes by BHC110-containing complexes while deacetylation of nucleosomal Histone H3 by HDAC1/2 was not affected (Figures 3B and 3C). These results point to a specific role for tranylcypromine in inhibition of histone H3K4 demethylation.

Analysis of Tranylcypromine on Histone Demethylation In Vivo

We were interested to know whether OCT4/POU5F1, an important regulator of early development and ES cell

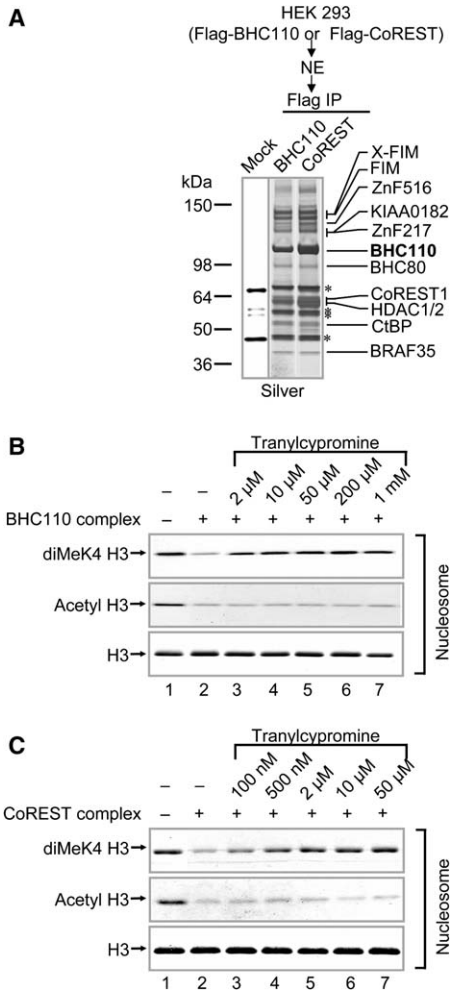

Figure 3. Tranylcypromine Inhibits Nucleosomal Demethylation but Not Deacetylation

(A) Analysis of BHC110-containing complexes isolated from nuclear extracts by silver staining (right panel). Left panel depicts silver staining of the Flag affinity eluate from untagged HEK 293 cell (mock). Asterisks denote nonspecific polypeptides.

(B and C) Effect of different concentrations of tranylcypromine on nucleosomal demethylation/deacetylation by BHC110 complex (B) or CoREST complex (C). Nucleosomes were used as substrate. The reaction mixture was analyzed by SDS-PAGE, followed by Western blotting.

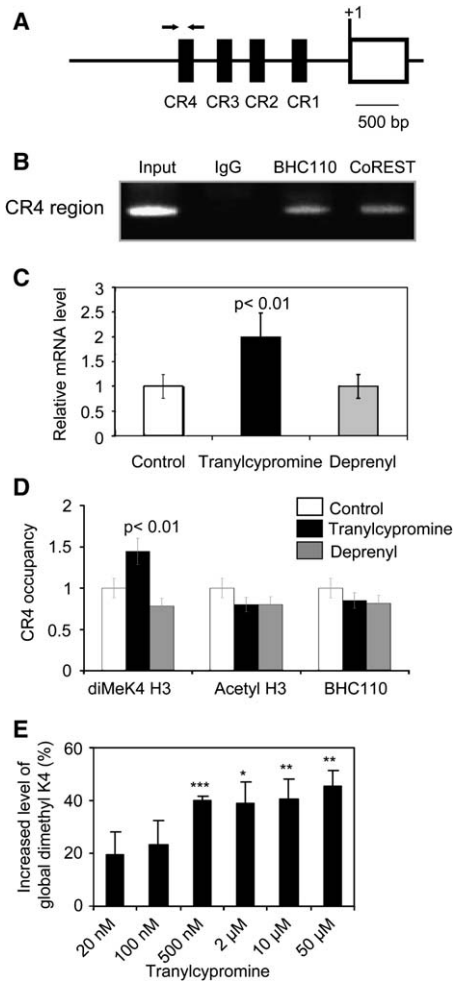

Figure 4. Tranylcypromine Increases Pou5f1/Oct4 Expression and Inhibits Demethylation in P19 EC Cells In Vivo

(A) A schematic representation of the mouse Oct4 promoter showing a cluster of DNA response elements. Arrows above CR4 element denote primers.

(B) Analysis of binding of CoREST and BHC110 to the CR4 regulatory region of Pou5f1/Oct4 promoter using ChIP assay.

(C) Analysis of Oct4 mRNA levels measured by qRT-PCR after treatment of P19 EC cells with tranylcypromine (2 μM) and deprenyl (2 μM).

(D) Analysis of BHC110 and dimethyl H3K4 occupancy on Oct4 promoter by qChIP after treatment of P19 EC cells with tranylcypromine (2 μM) and deprenyl (2 μM).

(E) Analysis of dimethyl H3K4 levels after treatment of P19 EC cells for 21 to 26 hr with various concentrations of tranylcypromine (20 nM to 50 μM). Whole-cell lysates were analyzed by SDS-PAGE, followed by Western blotting. Dimethyl K4 levels were quantified. Data are presented as the mean ± SEM of at least three experiments. *p < 0.02, **p < 0.01, ***p < 0.001.

identity [23, 24], is repressed through a demethylation mechanism. Chromatin immunoprecipitation (ChIP) experiments using antibodies against BHC110 and CoREST demonstrated the localization of the BHC complex to the CR4 region responsible for stem cell maintenance in the *OCT4* promoter in pluripotent P19 embryonal carcinoma cells (Figures 4A and 4B). We next treated P19 cells with 2 μM tranylcypromine (Parnate) or 2 μM deprenyl as a control for three hours. Analysis of transcription by quantitative RT-PCR revealed a specific derepression of *OCT4* transcription by 2 μM tranylcypromine (Figure 4C). Moreover, analysis of histone H3 dimethyl K4 levels at *Oct4* promoter showed a specific enhancement of histone methylation following treatment of P19 cells with tranylcypromine consistent with the inhibition of BHC110 histone demethylation activity (Figure 4D).

In addition, we analyzed another gene *Egr1* that acts as an important regulator of many cellular processes such as growth control, transformation, and apoptosis [25]. Similar to the *Oct 4* promoter, BHC110 and CoREST are recruited to the proximal region of the *Egr1* promoter (Figures S1A and S1B in the Supplemental Data available with this article online). After Parnate treatment, levels of dimethyl H3K4 of *Egr1* promoter are significantly increased, resulting in concomitant derepression of *Egr1* gene expression (Figures S1C and S1D). Finally, global analysis of dimethyl H3K4 levels following

treatment of P19 cells with increasing amounts of tranylcypromine revealed enhanced levels of methylation (Figure 4E). Taken together, our results identify tranylcypromine as a small molecular inhibitor of histone H3K4 demethylation in vitro and in vivo.

In this study we demonstrate the utility of nonselective monoamine oxidase inhibitors as small molecular inhibitors of histone H3K4 demethylation by BHC110. Importantly, only the nonselective monoamine oxidase inhibitors displayed inhibitory activity toward BHC110, consistent with a previous report that selective inhibitors including pargyline failed to inhibit BHC110 activity toward demethylation of H3K4 [26]. Pargyline was previously reported to inhibit the androgen receptor-induced altered activity of BHC110 toward demethylation of H3K9 at a high concentration of 1 mM [27]. At the present it is not clear how an irreversible monoamine oxidase inhibitor, pargyline, could inhibit H3K9 demethylation without affecting H3K4 demethylation.

Tranylcypromine displayed the best activity with an IC_{50} of less than 2 μM. The efficacy of tranylcypromine in inhibiting H3K4 is remarkable because the IC_{50} for inhibition of monoamine oxidase A or B by tranylcypromine is 20 μM [22], reflecting a better activity for the drug in inhibiting BHC110. Importantly, tranylcypromine has been used for the symptomatic treatment of moderate to severe depression. It has also been used to treat psychotic depressive states such as depressive phases of manic-depressive psychosis, involutional melancholia, reactive depressions and psychoneurotic depressions of moderate to severe intensity. Since the efficacy of tranylcypromine for inhibiting BHC110 is nearly 10-fold greater than for inhibiting monoamine oxidases, it will be interesting to know whether lower doses of tranylcypromine selectively targeting BHC110 will have beneficial effects on treatment of depression. Moreover, small molecular inhibitors such as trichostatin A and 5-azacytidine have not only been utilized for research applications to probe genomic deacetylation and DNA demethylation, respectively, but also have potential clinical applications [28–30]. It is likely that tranylcypromine would be a valuable tool in the future to probe the role of histone demethylation in gene regulation and other cellular processes such as differentiation and oncogenesis.

Significance

Dimethyl H3K4 is a histone mark associated with euchromatin and actively transcribed genes. The histone demethylase (BHC110/LSD1), a component of multiprotein corepressor complexes, was recently shown to target dimethyl H3K4 for demethylation. Since the catalytic domain of BHC110 has extensive sequence homology with monoamine oxidases, we reasoned that monoamine oxidase inhibitors displaying antidepressive effects may target BHC110. Our results demonstrate that nonselective monoamine oxidase inhibitors are potent inhibitors of demethylation by BHC110 with tranylcypromine displaying an IC_{50} of less than 2 μM. In addition, treatment of cells with tranylcypromine increases global levels of dimethyl H3K4 and derepresses the transcriptional activity of BHC110 target genes. These findings not only illuminate histone demethylation as a novel target of antidepressive medication, but also identify small molecular inhibitors that would prove beneficial for dissecting the cellular processes involving histone demethylation.

Experimental Procedures

Histones, Nucleosomes, and Other Reagents

Bulk histones were purchased from Sigma (H9250). Analysis of histone H3 in bulk histone showed a second band that has been described as a cleaved form of histone H3 spanning amino acid residues 28–135 in a previous report [31]. Nucleosomes were purified from HeLa nuclear pellet as previously described [32, 33]. Mammalian expression plasmid encoding FLAG-CoREST was generated using HindIII/SalI sites in pFLAG-CMV™2 (Sigma). Anti-BHC110 antibodies were generated as previously described. Anti-dimethyl K4 H3 (12–460) and anti-acetyl (K9/K14) H3 (06–599) antibodies were purchased from Upstate. The anti-H3 (ab1791) antibody was from Abcam Ltd (Cambridge, United Kingdom) and recognizes C-terminal of histone H3. Tranylcypromine hydrochloride and clorgyline hydrochloride were from BIOMOL research laboratories and MP biomedical, respectively. Nialamide and pargyline hydrochloride were from Sigma. Phenelzine sulfate and selegiline (deprenyl) were from Spectrum Chemicals.

Affinity Purification of the BHC110 Complex and Recombinant Proteins

To generate a stable cell line expressing FLAG-CoREST, HEK (human embryonic kidney) 293 cells were cotransfected with the mammalian expression plasmid encoding FLAG-*CoREST* and a selectable marker for puromycin resistance. Transfected cells were grown in the presence of 5 μg/ml puromycin, and individual colonies were isolated and analyzed for stable expression of FLAG-CoREST. The BHC110 and CoREST complexes were purified from 150–200 mg nuclear extracts isolated from the stable cell lines using anti-FLAG M2 affinity resin as previously described [18]. Baculoviral recombinant proteins (FLAG-BHC110 and FLAG-CoREST-$(His)_6$) were purified from Sf21 insect cells infected by recombinant viruses using anti-FLAG M2 affinity resin (Sigma) as previously described [18]. BHC110-associated proteins have been identified and described [12, 17, 18]. The amount of BHC110 in complexes was determined by silver staining, and recombinant proteins were determined by colloidal staining compared with known amounts of BSA.

Demethylation and Deacetylation Assays

Demethylation and deacetylation assays were performed as previously described [18]. To measure IC_{50}, the assays were performed for 7 min.

Quantitative RT-PCR and Chromatin Immunoprecipitation

Cells were treated with either vehicle (0.02% DMSO), 2 μM deprenyl or 2 μM tranylcypromine for 3 hr in DMEM media supplemented with 15% FBS, 0.1 mM nonessential amino acid solution (Invirogen), 0.1 mM β-mercaptoethanol, antibiotics and antimycotics. RNA was prepared using the Qiagen Rneasy kit and reverse-transcribed using Invitrogen's First Strand Synthesis kit. Quantitative PCR was performed using the Opticon2 (MJ research) with Finnzymes DyNAmo HS SYBR Green qPCR kit. Each sample was analyzed in triplicate for both GAPDH and *Pou5f1*/*Oct4* (with primers: sense 5′-TTGGGCT AGAGAAGGATGTGGTT-3′ and antisense 5′-GGAAAAGGGACTGAG TAGAGTGTGG-3′), and quantified using opticon software. Messenger RNA levels were normalized by GAPDH levels. Relative mRNA levels represent fold increase over control. Data are presented as mean ± SEM. Chromatin immunoprecipitation assays were performed as described [12]. Anti-BHC110 (10 μl) or anti-dimethyl K4 (5 μg) was used for each assay. Each sample value in qPCR was normalized by input value. PCR was performed using primers (sense 5′-GGAACTGGGTGTGGGGAGGTTGTA and antisense AGCAGATTAA GGAAGGGCTAGGACGAGAG) spanning the response element CR4 located in mouse Pou5f1/Oct4 promoter (−2098 to −1928) [34]. Promoter occupancy levels of BHC110 and dimethyl K4 H3 were measured with the DyNAmo HS SYBR Green qPCR kit using Opticon2 software, and are expressed as fold change over control (vehicle). Data are presented as the mean ± SEM.

Supplemental Data

Supplemental Data include one figure and are available at http://www.chembiol.com/cgi/content/full/13/6/563/DC1/.

Acknowledgments

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