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Implications of small molecule activators and inhibitors of histone acetyltransferases in chromatin therapy

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Abstract

Histone acetylation is a diagnostic feature of transcriptionally active chromatin. The group of enzymes, histone acetyltransferases (HATs), involved in this crucial step of gene regulation, covalently modifies the N-terminal lysine residues of histones by the addition of an acetyl group from acetyl coenzyme A. Dysfunction of these enzymes is often associated with several diseases, ranging from neurodegenerative disorders to cancer. These enzymes thus are potential new targets for therapeutics. We have discovered few small molecule compounds, which target HATs and either activate or inhibit the enzyme potently. These compounds would be useful as biological switching molecules for probing into the role of HATs in gene regulation and cell cycle and may be useful as new chemical entities for the development of new drugs.
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The eukaryotic genome is maintained as a nucleoprotein complex called chromatin, which consists, mainly of positively charged proteins called histones. The repeating unit of chromatin is the nucleosome, consisting of 146 base pairs of DNA wrapped twice around an octamer comprising of two copies each of the histones H2A, H2B, H3 and H4. The DNA is kept wound around the core by histone H1, also called the linker histone. The complex of the nucleosome along with H1 constitutes the chromatosome. The nucleosomes are spaced into a regular array, which can be viewed as the 'beads-on-a-string', under the electron microscope [1,2]. Higher order packaging of chromatin involves interactions among the nucleosomes and with other non-histone chromosomal proteins [3,4]. The packaging of the eukaryotic DNA into chromatin solves the problem of accommodating the enormous length into the limited confines of the nucleus. Though apparently repressive, the precise organization of chromatin is essential for replication, repair, recombination and chromosomal segregation. Alteration in the chromatin organization modulates the expression of underlying genes. The dynamic changes in the chromatin structure are brought about by post-translational modifications of the amino terminal tails of the histones and the ATP dependent chromatin remodeling. Specific amino acids within the histone tails are the sites of a variety of modifications including phosphorylation, acetylation, methylation, ADP-ribosylation and ubiquitination [5,6]. Of these modifications, acetylation and more recently methylation have been most widely studied in the context of gene expression. It is a long-standing observation that the transcriptionally active genes are associated with hyperacetylated histones, whereas the silent genes are associated with hypoacetylated ones. More recently, the idea that histone acetylation is causally related to transcriptional activation has received solid support from the discovery that a number of transcriptional coactivators have histone acetyltransferase activity. These include GCN5 and PCAF, p300 and CBP, nuclear hormone coactivators SRC1 and ACTR and the TATA box binding protein-associated factor TAFII250 [5,7]. The remodeling factors are recruited onto a gene through interactions with

Abbreviations: CBP, CREB binding protein; PCAF, p300/CBP associated factor; HAT, histone acetyltransferase; HDAC, histone deacetylase; CTPB, *N*-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzimidine; AIB1, amplified in breast cancer 1

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the activator. Mechanistically, the order of recruitment of histone modifications and ATP dependent chromatin remodeling cannot be generalized. Presumably, the variations would be gene specific or time specific relative to cell cycle [8,9]. Thus the whole concept of chromatin remodeling plays an important role in turning 'on' and 'off' genes at specific times, in a co-ordinated and highly organized manner. Any aberration that occurs in this process may have severe repercussions like cancer, neurodegenerative diseases, asthma and may also activate latent HIV infections (Fig. 1).

1. Alteration of HAT function leads to different diseases

Being one of the key group of enzymes involved in chromatin modifications, the histone acetyltransferases CBP or p300 exemplify the causal relationship of HATs with diseases. Analysis of colorectal, gastric and epithelial cancer samples show that in several instances there is a misssense mutation as well as deletion mutations in the p300 gene [10]. Furthermore loss of heterozygosity of p300 gene has been found to be associated with atleast 80% of glioblastoma [11]. By far, the best-known association between mutated acetylase and cancer is between the steroid receptor coactivator, AIB1 (a homolog of SRC1) and breast cancer [12]. Amplification and over-expression of the AIB1 gene in the breast cancer samples cause a deregulation of the AIB1 controlled genes. However the role of AIB1 mediated histone acetylation in the cancer manifestation has not yet been elucidated. In acute myeloid leukemia (AML), the gene for CBP is translocated and fused to either the Monocytic Leukemia Zinc finger (MOZ) gene, another transcriptional coactivator with intrinsic HAT activity [13] or to MLL (A homeotic regulator, mixed lineage leukemia) [14–16]. In both cases, the HAT activity of CBP remains intact. The translocation-derived fusion proteins cause aberrant gene expression through improper targeting to genes that are not the normal targets of either protein. Retention of partial acetylase function by the fusion protein may also result in the improper regulation of the target gene, while a loss of function of the fusion protein may result in the normal gene not being transcribed at all. In either case, this results in aberrant cell cycle regulation leading to cancer. In yet another case, two inversions within chromosome 8 that are also associated with leukemia cause the fusion of MOZ to another HAT, TIF2 (transcriptional mediator/intermediary factor 2) [17]. The acetylase TIF2 interacts with both CBP and p300 [18]. Indirect evidences for the involvement of acetylases in cancer come from the observation that p300 and PCAF play an important role in MyoD dependent cell cycle arrest [19]. p300/CBP is also required for the regulation of transcription factors, which in turn are involved in the differentiation of erythrocytes, B cells, and melanocytes [20]. Therefore, a mutation in the p300/CBP acetylase domain would result in the deregulation of the transcription factors involved in the differentiation of the cells. This may lead to excessive proliferation of cells, resulting in cancer. The binding of the onco-protein E1A to p300/CBP is necessary for the former to regulate transcription, suppress differentiation and to induce the immortalization of the cell cultures [21]. In this context, p300/CBP can be viewed as a tumor suppressor, analogous to the retinoblastoma protein.

Mutations in HATs cause several other disorders apart from cancer. Mutations in CBP result in the Rubinstein–Taybi syndrome (RTS) [22]. It was found that a single mutation at the plant homeodomain (PHD)-type zinc finger in the HAT domain of CBP, which alters a conserved finger amino acid (E1278K), causes this syndrome. Interestingly, this mutation (G to C at 4951) in CBP also abolishes its HAT activity [22,23]. Furthermore, degradation of CBP/p300 is found to be associated with certain neurodegenerative diseases [24]. Functional HATs are also essential for the proper replication of HIV. It was elegantly demonstrated that treatment of latent HIV containing cells with HDAC inhibitors activates HIV, presumably by inducing acetylation of Tat transactivator and the nucleosomes on the LTR [25,26]. These examples clearly highlight the necessity to target histone acetyltransferases for therapy.

2. Small molecule modulators of histone acetyltransferases

During the last decade, a number of HDAC inhibitors have been identified that induce apoptosis in cultured tumor cells [27]. These inhibitors were also found to be potent anticancer agents in vivo. Furthermore, some of these inhibitors (e.g. SAHA) are already in human trial as antineoplastic drugs [28]. Although substantial progress has been made in the study of HDAC inhibitors, very little has been done in the area of HAT inhibitors. Long before the discovery of HATs, polyamine–CoA conjugates were found to inhibit the acetyltransferase activity of cell extracts [29]. Availability of recombinant HATs (p300 and PCAF) made it possible to synthesize and test more targeted, specific inhibitors, Lys–CoA for p300 and H3–CoA-20 for PCAF [30]. Lys–CoA, a conjugate of the aminoacid lysine and coenzyme A, has proved useful for blocking the HAT activity of p300 specifically. Though it has been extensively employed for in vitro transcription studies [31] and in vivo via microinjection or with the use of cell permeabilizing agents [32], Lys–CoA is not easily taken up by cells from the surrounding medium in cell culture conditions. The cells were also found to be impermeable to the PCAF-specific inhibitor of the same class, H3–CoA-20, which contain CoA conjugated with twenty amino acid residue peptide from the N-terminus of histone H3. Recently, we have isolated the first naturally occurring HAT inhibitor anacardic acid (AA), from cash-

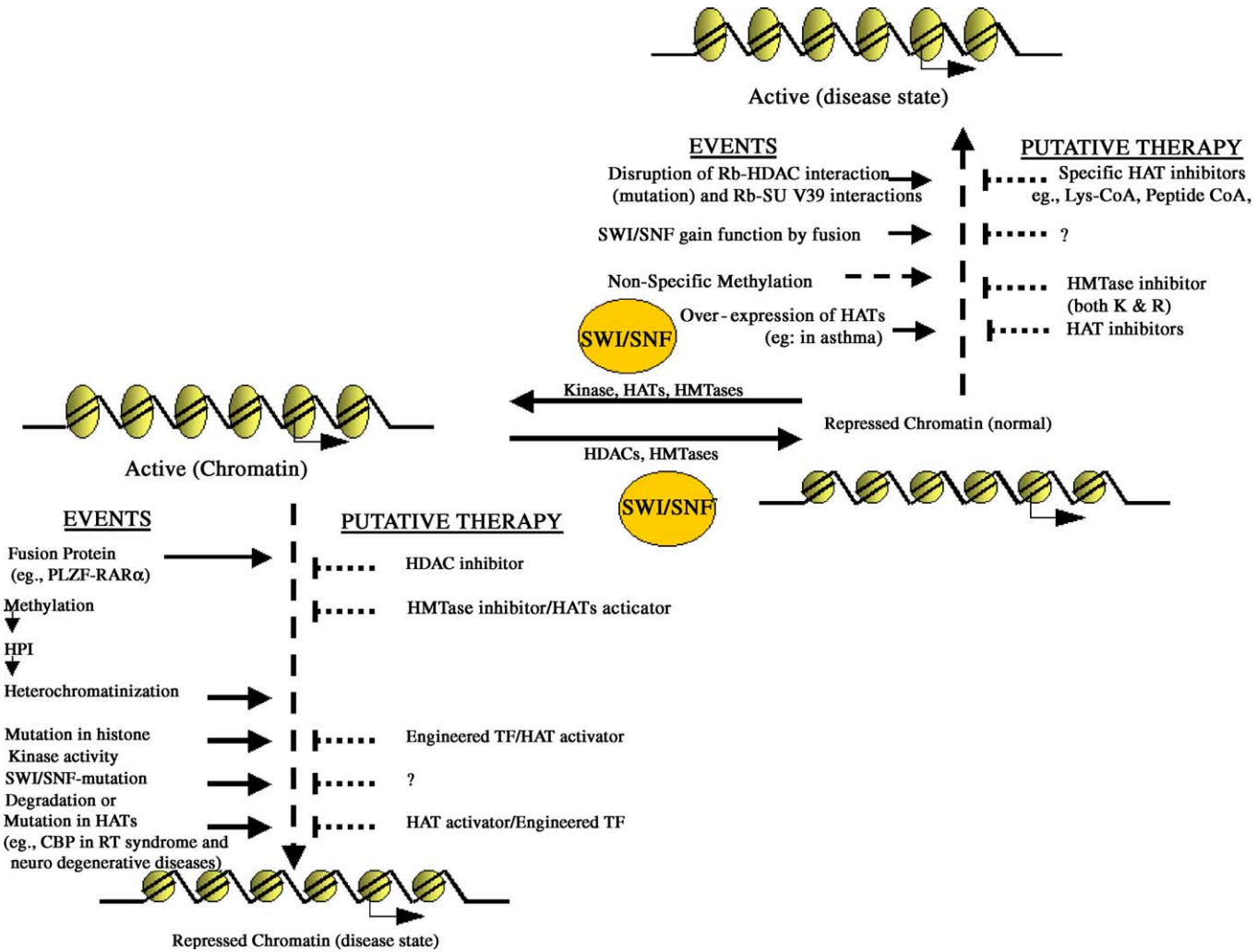

Fig. 1. Chromatin therapy-targeted to dynamic structure-function of chromatin: The active and inactive chromatin dynamically changes from one form to another with the help of chromatin modifying enzymes. Dysfunction of these enzymes alters the regulation of gene expression (as well as cell cycle) causing several diseases. These enzymes thus are new targets for new generation therapeutics.

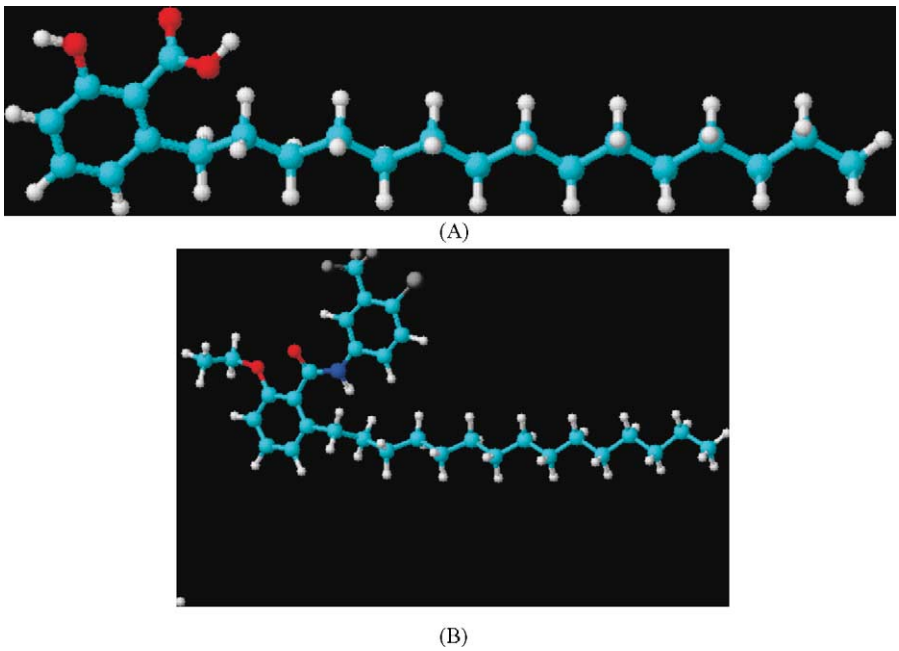

Fig. 2. Ball and stick model of (A) anacardic acid and (B) CTPB.

ewnut shell liquid (CNSL), which inhibits the HAT activity of both p300 and PCAF very effectively [33]. By using AA as a synthon, we have synthesized an amide derivative of anacardic acid, CTPB, which is the only known small molecule activator of any histone acetyltransferase, in our case, p300 (Fig. 2). Significantly, CTPB shows an exclusive specificity for p300 HAT activity. However, cells are impermeable or poorly permeable to both anacardic acid and CTPB (Varier and Kundu, unpublished work). More recently, we have discovered that garcinol, a polyisoprenylated benzophenone derivative from *Garcinia indica* fruit rind, is a potent inhibitor of histone acetyltransferases p300 ($IC_{50} \geqslant 7$ μm) and PCAF ($IC_{50} \geqslant 5$ μm) both in vitro and in vivo. Thus garcinol is the first reported cell permeable HAT inhibitor.

The reaction mechanism for p300 and PCAF to acetylate the lysine residues is contrastingly different. The GNAT family member, PCAF, employs ternary complex mechanism that involves the ordered binding and release of substrates and products. On the other hand, p300/CBP family follow the double displacement (ping-pong) mechanisms. However, kinetic analysis suggests that all the modulators described above bind to the respective enzyme and regulate (inhibit or activate) the activity [34]. Detailed analysis of the p300 specific inhibitor, lysyl CoA showed that it exhibits slow, tight-binding kinetics suggesting that a slow conformational change or solvent reorganization is necessary to achieve the high affinity complex. The PCAF selective bisubstrate analog inhibitor H3–CoA-20 was found to be competitive versus acetyl CoA and non-competitive versus the peptide substrate H3-20 [35]. The reasons for HAT selectivity of these inhibitors are yet to be elucidated. Presumably, formation of unique catalytic intermediates by p300 and PCAF/GCN5 are at least partly responsible for the specificity [36]. Functional mechanisms of natural HAT inhibitors, anacardic acid and garcinol are poorly understood. The garcinol-mediated inhibition kinetics shows that with changing concentration of acetyl CoA it behaves like an uncompetitive type of inhibitor whereas for core histones as a competitive inhibitor [37]. Anacardic acid was found to be a non-competitive type of inhibitor [33]. CTPB, on the other hand is the only known activator of any histone acetyltransferase and the mechanism of activation is yet to be addressed. Presumably it binds to some specific domains of p300 and enhances the recruitment of acetyl CoA.

We have conclusively demonstrated that treatment of HeLa cells with garcinol inhibits the activated histone acetylation (Fig. 3B and C, compare lanes 5 and 6). HAT activity dependent chromatin transcription was strongly inhibited by garcinol, whereas transcription from DNA template remained unaffected. Furthermore, it was found to be a potent inducer of apoptosis, and it alters (predominantly down regulates) the global gene expression in HeLa cells [37]. Acetylation is a diagonostic feature of active genes. Thus inhibition of acetylation in vivo would repress majority of the genes. Microarray analysis of garcinol treated HeLa cell gene expression indeed showed that more than 72% of the genes tested were down regulated. Interestingly, the microarray data revealed that several proto-oncogenes are down regulated in presence of garcinol, indicating that garcinol may function as an anticancer compound. However, a systematic investigation of the effect of garcinol on normal (untransformed) and several cancerous cell lines is essential to elucidate the specific role of garcinol in cancer prevention.

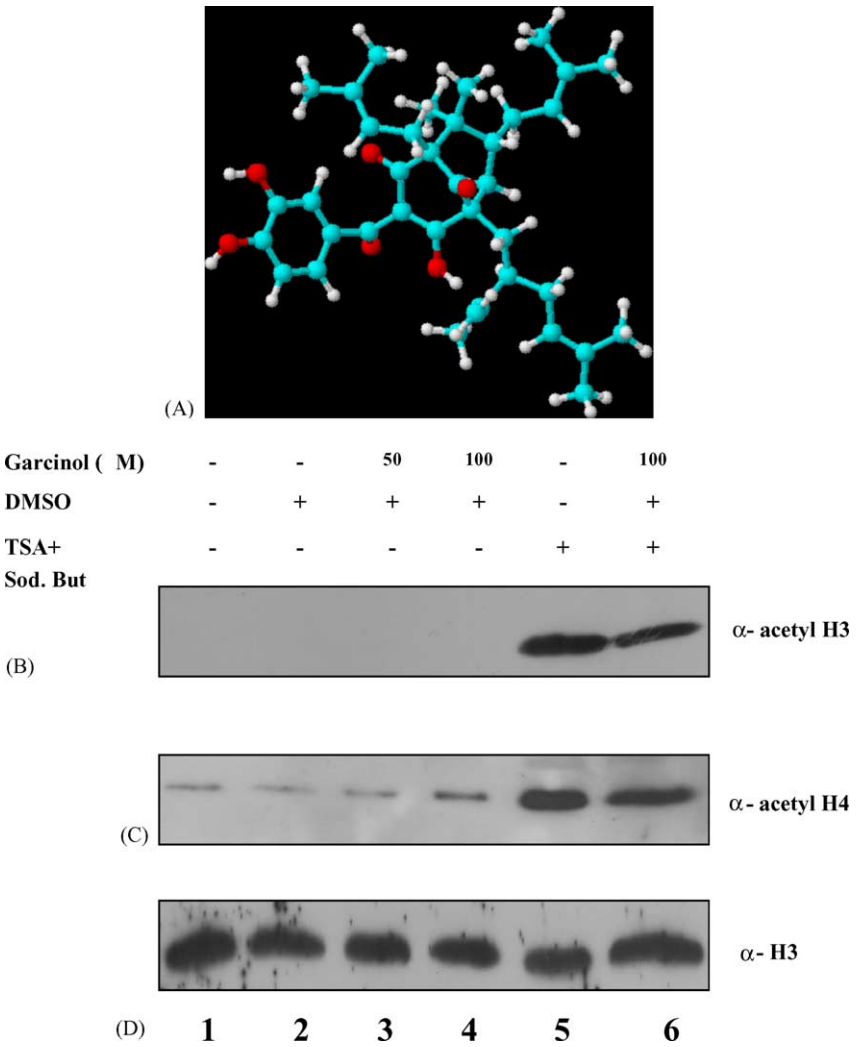

Fig. 3. (A) Ball and stick model of garcinol. (B, C) Garcinol inhibits HAT activity in vivo: HeLa cells were treated with various concentrations of garcinol as indicated, for 24 h, histones were acid extracted and then subjected to western blotting analysis using the indicated antibodies. Histones extracted from untreated cells (lane 1), DMSO (solvent control) treated cells (lane 2), garcinol (50 and 100 μM, respectively) treated cells (lanes 3–4), trichostatin A(2 μM) and sodium butyrate (10 mM) treated cells (lane 5) and trichostatin A (2 μM), sodium butyrate (10 mM) and garcinol (100 μM) treated cells (lane 6) were probed with anti-acetylated H3 and α-acetylated H4, respectively. The equal loading of histones were ensured by western blotting with anti-histone H3 antibodies (D).

3. Future prospects

As suggested in a recent review [5], the development of small molecular weight HAT inhibitors and activators as therapeutic agents is the next step, following the HDAC inhibitors. Our recent findings of anacardic acid, CTPB and garcinol, are successful efforts in that direction. However, the preference would be for a more specific and cell permeable inhibitor. Further modifications of these modulators in conjunction with continued search for new molecules could lead to the development of potential drugs from HAT modulators. Since histone methylation and acetylation are highly correlated and functionally dependent, effect of HAT modulators should also be studied in the context of methylation-dependent functional pathways. This information would be very useful to design combinatorial therapeutics, targeted towards histone acetyltransferases and methyltransferases. The HAT inhibitors may also be used with existent HDAC inhibitors for anti-HIV therapy. The HAT activator molecule could be an excellent alternative to HDAC inhibitor, considering their analogous function, in causing hyperacetylation. The small molecule modulator of HATs thus should open up new possibilities to design better therapeutics in future.

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