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Development of histone deacetylase inhibitors as therapeutics for neurological disease

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Abstract

Postsynthetic modifications of histone and other chromosomal proteins by reversible acetylation and/or methylation regulate many aspects of chromatin dynamics, such as transcription, replication and DNA repair. Aberrant modification states are associated with several neurological and neuromotor diseases. Thus, small molecules that inhibit or activate the enzymes responsible for these chromatin modifications have received considerable attention as potential human therapeutics. This paper summarizes the current state of development of histone deacetylase inhibitors in a variety of neurological diseases.

Keywords

acetylation; amyotrophic lateral sclerosis; chromatin; fragile X syndrome; Friedreich's ataxia; gene regulation; histone; histone deacetylase inhibitor; Huntington's disease; myotonic dystrophy; Rett's syndrome; Rubinstein–Taybi syndrome; spinal muscular atrophy; triplet repeat disease

Chromatin structure, histone postsynthetic modifications & gene expression

The basic subunit of chromatin in eukaryotic cells is the nucleosome, consisting of an octamer of two copies each of the four core histones, H2A, H2B, H3 and H4, plus 147 base pairs (bp) of DNA [1]. A variable linker region, spanning between 20 and 100 bp, depending upon the cell type and organism, connects nucleosomes to form higher order chromatin structures. The folding of nucleosome chains into higher order domains is dependent on members of the H1 family of linker histones [2]. While the nucleosome can be regarded as the primary subunit of chromatin, different specialized histone proteins are known to be associated with particular

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regions of the genome, such as histone H2AZ at the promoters of active genes or the H3 variant CENP-A at centromeric heterochromatin. The precise molecular architecture of higher order chromatin structure has been debated over the years, but it is clear that such folding plays an important role in regulating access of the genome to the transcription machinery. It is also clear that the histone proteins, and postsynthetic modifications of the histones, play pivotal roles in regulating chromatin dynamics. In particular, acetylation of particular lysine residues in the amino-termini of histone H4 has been shown to regulate higher order chromatin folding [3] and lysine methylation in histone H3 and H4 has been shown to regulate the association of chromatin-associated proteins with particular genes. Of interest to various neurological diseases (discussed later), trimethylation of lysine 9 of H3 (H3K9me3) recruits heterochromatin protein 1 (HP1) through its chromodomain, resulting in gene repression [4], while H3K4me3 is associated with active genes [5]. The pattern of histone acetylation and methylation in the core histones at any genetic locus has been proposed to form a histone code that specifies gene activity [6].

Histone acetyltransferase, histone deacetylases & histone deacetylase inhibitors

Acetylation and deacetylation of histone proteins, and of other proteins involved in transcriptional regulation, have critical roles in regulating gene expression. Protein acetylation and deacetylation is modulated by the interplay between histone acetyltransferases (HATs) and histone deacetylases (HDACs). Several HATs have been identified in the human genome, including various components of transcriptional regulatory complexes and the association of the various HAT enzymes with partner proteins to form multisubunit complexes provides a mechanism to both target and regulate HAT activity at gene promoters [7]. The association of HATs with histone chaperone proteins confers acetylation specificity between N-terminal lysines (H3K9 and H3K23) and those within the histone fold domain (H3K56) [7,8]. Typically, the increases in HAT activity lead to increased gene transcription by creating a more open conformation of chromatin, whereas HDAC activity leads to repression of gene expression via condensation of the chromatin structure [9]. While HAT inhibitors and activators have been identified [10,11], far more interest has focused on the development of HDAC inhibitors (HDACIs) for various human diseases (see later).

A total of 18 HDACs (more strictly, protein deacetylases) have been identified in the human genome, including the zinc-dependent HDACs (classes I, II and IV), and the NAD⁺-dependent protein deacetylase enzymes (class III, or sirtuins). HDAC 1, 2, 3 and 8 belong to class I, showing homology to the yeast enzyme RPD3. Class II is further divided into class IIa (HDACs 4, 5, 7 and 9) and IIb (HDAC 6 and 10), according to their sequence homology and domain organization. HDAC11 is the lone member of class IV. The sirtuins (class III and Sirt1–7) are related to the yeast Sir2 protein and are involved in the regulation of mitochondrial activity, metabolism and aging, and have received considerable attention as potential therapeutic targets in diabetes and other diseases.

A diverse class of compounds that inhibit HDAC enzymes has been developed and several studies have focused on the use of these HDACIs as therapy for modifying histone acetylation and transcriptional activation in cell culture studies and *in vivo* mouse models of neurodegeneration [12]. Chemically, the HDACIs can be classified into six structural groups, including the small carboxylates, such as sodium butyrate, valproic acid and sodium phenylbutyrate; the hydroxamic acids, such as trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), and their derivatives (the benzamides CI-994 and MS-275); the epoxyketones such as the trapoxins; cyclic peptides such as apicidin and depsipeptide; and hybrid molecules containing cyclic peptide motifs and hydroxamic acid moieties [13]. As previously mentioned, the dynamic interplay between histone/protein acetylation and

deacetylation serves as a central regulatory mechanism governing gene expression, which in turn guides cellular differentiation and development. It is thus quite surprising that small molecule HDACI therapeutics that inhibit this basic molecular pathway are well tolerated in humans [14] and show therapeutic promise in a wide range of human diseases including cancer, metabolic and neurological diseases.

Triplet-repeat sequences affecting chromatin structure & gene expression: fragile X & Friedreich's ataxia

In fragile X syndrome and Friedreich's ataxia, the pathological hyperexpansion of repeated sequences triggers epigenetic changes, leading to gene silencing and consequent loss of function. In both cases, the repeat expansions are in noncoding regions of the affected gene; thus, reactivation of silenced genes is an attractive therapeutic approach. In Fragile X syndrome, expansion of a CGG•CCG-repeat in the 5'-UTR of the *FMR1* gene to more than 200 repeats results in gene silencing, and a concomitant loss of FMR1 protein. This expansion triggers methylation of a CpG island upstream of the *FMR1* gene, hypoacetylation of associated histones and chromatin condensation. In cell lines from fragile X patients, there is a decrease in H3K4me3 with a large increase in H3K9me3 – changes that are consistent with a switch from euchromatin to heterochromatin at the FMR1 locus in the disease state [15,16]. Several reports have documented increases in *FMR1* gene expression by decreasing DNA methylation with 5-azadeoxycytidine, and a synergistic reactivation of the silenced gene has been reported by combination treatment with 5-azadC and the HDACIs 4-phenylbutyrate, sodium butyrate and TSA [17]. Recently, it was reported that a class III histone deacetylase, SIRT1, plays an important role in *FMR1* gene silencing, and the inhibition of this enzyme with splitomicin produces significant gene reactivation [16]. The authors of this study argue that SIRT1 inhibitors may be more useful for *FMR1* gene reactivation in postmitotic cells, such as neurons, since DNA methylation inhibitors require DNA replication in order to be effective [16].

The related neurodegenerative disease Friedreich's ataxia (FRDA) is caused by expansion of the simple triplet repeat DNA sequence GAA•TTC within intron 1 of the *FXN* gene, encoding the essential mitochondrial protein frataxin. The GAA•TTC repeat expansion was originally believed to alter the DNA structure of the *FXN* gene [18] and thereby block transcription elongation by RNA polymerase II. Indeed, the loss of *FXN* mRNA and frataxin protein is well established as the cause of FRDA [19]. However, more recent studies indicate GAA•TTC repeats in the *FXN* gene caused gene silencing via an altered chromatin structure [20–23]. Histone H3/H4 hypoacetylation and H3K9 methylation have been observed on FRDA *FXN* genes, both of which are hallmarks of heterochromatin [22]. These results have now been extended to FRDA mouse models [20,24] and to human FRDA autopsy material [20], and the consensus in the field is that FRDA is a heterochromatin gene-silencing disease. Based on these observations, we speculated that HDACIs might reverse FXN silencing by directly increasing histone acetylation on the *FXN* gene, leading to chromatin decondensation and active transcription. We found that a commercially available HDACI (BML-210), and derivatives we have synthesized (pimelic diphenylamides), relieve repression of the *FXN* gene in lymphoid cell lines derived from FRDA patients, in primary lymphocytes from donor FRDA patient blood and in the brain and heart of a mouse model for FRDA [22,24]. The HDACIs directly affect the histones associated with the expanded *FXN* gene in patient cells and in the mouse model, increasing acetylation at particular lysine residues on histones H3 and H4, providing evidence for the chromatin model. Unexpectedly, we found that only members of the pimelic diphenylamide family of HDACIs increase *FXN* gene expression, and many common and highly active HDACI, including the hydroxamates TSA and SAHA are inactive. The pimelic diphenylamides are currently under development by Repligen Corporation (MA, USA) and clinical trials in FRDA are expected in the near future.

Master epigenetic regulators: MECP2 & Rett syndrome, CBP & Rubinstein–Taybi syndrome

These diseases are caused by mutations affecting factors that mediate general epigenetic mechanisms, leading to widespread gene-expression dysregulation. Methyl-CpG-binding protein (MECP2) couples DNA methylation, an important epigenetic mark, to chromatin remodeling and changes in gene expression. Cyclic AMP response element-binding protein (CREB)-binding protein (CBP) is a major histone acetyltransferase. Rett syndrome (RTT) is caused by loss-of-function mutations in the *MECP2* gene on the X chromosome (Xq28) [25]. The gene encodes the two isoforms of the methyl-CpG-binding protein (MECP2 and MECP2B). Almost all mutations occur *de novo* and act as X-linked dominant, meaning most affected individuals are girls. RTT is a neurodevelopmental disorder that becomes apparent after 6–18 months of apparently normal development. In its typical form, it is characterized by loss of communication skills and of the purposeful use of hands, which become engaged in stereotypic movements, progressive slowing of head growth, motor disturbances, irritability, seizures and breathing abnormalities [26]. However, *MECP2* mutations cause a wide spectrum of phenotypes in addition to classical RTT, in girls as well as in boys. Girls may have a milder disease or even be asymptomatic [26]. Skewed X chromosome inactivation may explain some, but not all phenotypic variability in girls [26]. Male germline *MECP2* mutations may cause a severe encephalopathy with death at birth, as seen in brothers of RTT girls, or X-linked recessive mental retardation (XLMR) [27]. MECP2 is thought to act primarily by recruiting a protein complex composed by Sin3A, RbAp46, RbAp48, and HDAC1 and HDAC2 to methylated CpG nucleotides [28]. This protein complex then carries out histone modifications and chromatin remodeling leading to silencing of the target genes. MECP2 carries out this function in the brain, while the protein MBD is active in the periphery, explaining the specific vulnerability of the brain to *MECP2* mutations. Recent studies on MECP2 function revealed that it has additional roles in chromatin remodeling, RNA splicing and control of gene expression [28]. MECP2 appears to be a multifunctional protein that regulates gene expression in the brain, both repressing and activating sets of target genes [29]. Therapeutics that can affect chromatin structure and modulate gene expression may eventually be treatments for this devastating disorder, but further studies are necessary to identify potentially useful compounds or classes of compounds. HDACIs may not appear as obvious therapeutic candidates for a disease where gene de-repression is thought to play a major role. In some experimental paradigms, HDACIs have even been shown to mimic some RTT features, such as behavioral abnormalities linked to basolateral amygdala dysfunction [30]. However, the complexity of MECP2 function and in particular the discovery that some genes are activated by this protein [29] suggest that a role of these compounds in future treatments cannot be excluded.

Approximately 50% of the cases of Rubinstein–Taybi syndrome (RTS) result from heterozygous mutations in the transcriptional coactivator CBP [31] or its homolog p300 [32, 33]. RTS has a complex phenotype including short stature, microcephaly, abnormal facial features (beaked nose, slightly malformed ears, highly arched palate, antimongoloid slant of eyes, and heavy or highly arched eyebrows), broad thumbs and/or great toes that may be angulated. Mental retardation is a feature of RTS, it is usually moderate, but may vary from mild to severe [34]. CBP and p300 are involved in transcriptional activation of a large number of target genes via HAT activity [35] and chromatin remodeling [36]. Several mouse models with *CPB* and *p300* mutations have been generated. These mice have cognitive abnormalities, particularly in long-term memory [37]. Treatment with HDACIs, such as SAHA and TSA, can correct these cognitive deficits [37], suggesting that loss of HAT activity has a primary role in their pathogenesis, and by extension in the pathogenesis of mental retardation in RTS. Therefore, HDACIs may be a promising approach for the human condition, at least for its neurocognitive aspects. Interestingly, loss of CBP and p300 activity is observed in

polyglutamine disorders, where these proteins interact with polyglutamine protein aggregates and are sequestered in inclusions [38]. The consequent loss of HAT activity may underlie the sensitivity of polyglutamine diseases to HDACI treatment (see later sections).

Modulation of gene expression & potential therapies for spinal muscular atrophy & myotonic dystrophy

In spinal muscular atrophy (SMA) and myotonic dystrophy, increasing the expression of the *SMN2* and muscleblind genes, respectively, may compensate for the primary genetic defects. Such induction may be obtained through drugs acting upon epigenetic mechanisms. SMA is an autosomal recessive disease of motor neurons that causes muscle weakness and wasting in children [39]. In the most severe form (SMA type I), weakness and hypotonia are evident at birth, affected children never sit or walk and they die within 2 years. Children with the intermediate form (SMA type II) become able to sit, but never walk and also have a severely shortened life expectancy. SMA type III is the less severe form, affected children start to walk, but eventually develop proximal muscle weakness and lose deambulation. Individuals with SMA type III survive into adult age. In some SMA type III cases (sometimes known as SMA IV), the disease onset occurs after childhood, in the third decade of life. SMA is caused by severe deficiency of a protein involved in RNA processing termed SMN, encoded by a duplicated gene on chromosome 5 [40]. The two copies, termed *SMN1* and *SMN2*, only differ by a single nucleotide in exon 7. The CAGACAA sequence in *SMN1* exon 7 is a binding site for the splicing factor SF2/ASF and acts as an exonic splicing enhancer (ESE) promoting the inclusion of exon 7 in the mature mRNA. In *SMN2*, this sequence has changed to TAGACAA (UAGACAA in the corresponding RNA) and does not bind SF2/ASF anymore, with loss of ESE function and frequent (>80%) skipping of exon 7 in the mature *SMN2* mRNA [41]. SMA is caused by mutations that inactivate *SMN1*, most frequently deletions, so that SMN synthesis entirely depends on *SMN2*. Motor neurons are exquisitely sensitive to the consequently reduced SMN levels and undergo degeneration. Complete absence of SMN is lethal for all cell types, as shown in knockout animals [42]. The different severities of SMA type I, II and III is partly explained by a variation in *SMN2* copy number and therefore in the residual amount of SMN [43]. A recent study demonstrated that the *SMN2* gene is subject to silencing by DNA methylation involving MeCP2, while DNA methylation levels inversely correlate with disease severity [44]. Even though silencing of the *SMN2* gene involves DNA methylation, HDACIs have been identified that boost *SMN2* expression and thus increase SMN protein levels. Early studies showed that valproic acid increases SMN protein in patient-derived cell lines [45,46] and has been beneficial in mouse models of the disease [47], leading to several small, open-label trials in SMA patients. So far, improvement has been modest and limited to a subset of patients [48–51]. However, other HDACIs are active in patient-derived cells, like sodium butyrate [52], sodium phenylbutyrate [53] and in particular, SAHA. The latter not only appears to be much more potent than valproic acid in increasing *SMN2* expression, it also significantly increases the proportion of full-length (with exon 7 included) *SMN2*-derived mRNA [54]. Other benzamide HDACIs may also be effective in raising SMN levels [55]. Very recently, LBH589 (panobinostat), a pan-HDACI, which is approved by the US FDA for the treatment of cutaneous T-cell lymphoma, was shown to increase full-length *SMN2*-derived SMN protein levels two- to three-fold. Such an increase resulted from the combined effect of the drug at both RNA and protein levels. In particular, LBH589 increased H3K9 acetylation at the *SMN2* promoter, facilitated exon 7 splicing through an effect on the splicing factor hTRA2-b1, stabilized SMN by reducing its ubiquitinylation and facilitated its incorporation into the SMN complex [56]. Therefore, despite some initial disappointment with valproic acid, HDACIs remain a promising therapeutic approach for SMA.

The myotonic dystrophies (DM1 and DM2) are autosomal dominant systemic diseases resulting from expansion of noncoding, but transcribed repeat sequences (a CTG repeat in the

3'-UTR of the *DMPK* gene in DM1 [57] and a CCTG repeat in an intron of the *ZNF9* gene in DM2 [58]). RNAs containing these sequences adopt a hairpin secondary structure and accumulate in nuclear foci [58,59]. The *Drosophila* muscleblind proteins (MBNL1–3) are the human homologs of the *Drosophila* muscleblind RNA-binding protein that regulates the splicing and maturation of a set of pre-mRNAs [60]. MBNLs bind the CUG/CCUG-containing hairpin RNA structures that accumulate in DM1 and DM2 and are sequestered in nuclear RNA foci [61]. The resulting MBNL depletion leads to misregulated maturation of multiple pre-mRNAs, which is a major contributor to DM1 and DM2 pathophysiology [62]. The important role of the loss of MBNL activity in DM pathogenesis is confirmed by the development of a DM-like phenotype by *mbnl*-knockout mice [63] and by the ability of Mbnl overexpression to correct the phenotype of CAG repeat expansion-based DM animal models [64]. Therefore, enhancing MBNL expression may be an interesting approach to treat the myotonic dystrophies. Accordingly, the possibility that some HDACIs may induce MBNLs and be potential therapeutics for DM1 and DM2, although as yet untested, is worth exploring.

Amyotrophic lateral sclerosis

Histone deacetylase inhibition has also been proposed as a therapeutic approach for amyotrophic lateral sclerosis (ALS) on the basis that disequilibrium between HAT and HDAC activities, possibly related to CBP loss-of-function, may contribute to the pathogenesis of this lethal degenerative disease of motor neurons. Phenylbutyrate increased survival in the SOD1 G93A transgenic mouse model of ALS when used alone or in combination with an antioxidant [65]. In the same mouse model, valproic acid was also effective in extending survival and in delaying onset, particularly when used in association with the glycogen synthase kinase-3 inhibitor lithium [66]. These promising preclinical findings led to a recently published Phase IIA open-label trial of sodium phenylbutyrate in ALS patients. A total of 26 patients completed the 20-week treatment with 9–21 g/day of the medication reporting good tolerability, with no mortality and no clinically relevant laboratory changes. Histone acetylation in peripheral blood cells increased even with the lowest dose of 9 g/day [67]. Placebo-controlled Phase IIB trials with this molecule in ALS will now be needed to show whether this HDACI promises to have any clinical efficacy.

HDACIs in polyglutamine repeat disorders

The CAG repeat/polyglutamine (polyQ) expansion disorders include Huntington's disease (HD), the spinocerebellar ataxias (SCAs) 1, 2, 3, 6, 7, 8 and 17, spinobulbar muscular atrophy (SBMA) and dentatorubral pallidoluysian atrophy (DRPLA). All of these disorders are progressive in nature and result in severe neurological disturbances. A general mechanism of gene expression dysregulation may be common to these diseases, owing to the effect of nuclear protein microaggregates or inclusions on transcription factors or other components of the transcriptional machinery. Furthermore, some of the mutated proteins are transcriptional regulators themselves (Ataxin 7 in SCA7 and TBP in SCA17), whose activity is affected by the presence of the expanded polyglutamine tract. While the nature of these intranuclear inclusions remains controversial (pathogenic or protective), they represent a pathological hallmark for HD, as well as for many other polyQ diseases [68]. Studies in cell culture, yeast, *Drosophila* and, more recently, mouse models of polyQ disease, have indicated that HDACIs might provide a useful class of agents to ameliorate the transcriptional deficits in HD and some of the SCAs [69–72]. Four studies have examined the potential therapeutic effects of the HDACIs SAHA [70], sodium butyrate [71], phenylbutyrate [73] and a pimelic diphenylamide [74] in HD mouse models. Two studies using R6/2 transgenic mice found that sodium butyrate and SAHA treatment improved motor performance and demonstrated neuroprotective effects. No effects on Htt aggregation or HD transgene expression were detected by either compound [70,71]. In both studies, treatment with these HDACIs increased global acetylation of histones.

In a third study, HD-N171-82Q mice were treated with phenylbutyrate after the onset of symptoms, in order to determine whether HDACIs could reverse deficits already in progress [73]. While no effects on motor performance were noted, phenylbutyrate treatment did increase survival, which was associated with increased acetylation of histones H3 and H4. In addition, phenylbutyrate displayed neuroprotective effects of decreasing striatal atrophy and reducing ventricular enlargement [73]. A recent study indicated that one of the pimelic diphenylamides developed for FRDA (HDACI 4b) also shows clinical efficacy in the R6/2 transgenic mouse model for HD [74]. This HDACI ameliorated the motor and behavioral symptoms associated with disease progression in these mice and corrected transcriptional abnormalities associated with polyQ-expanded Htt protein in the mouse brain. Importantly, unlike the other HDACIs tested in HD mouse models, this compound partially prevented the weight loss that is associated with disease progression in the mouse model, a feature that is also present in human HD [74].

Future perspective

The HDACI SAHA (and vorinostat) has been approved by the FDA for the treatment of cutaneous T-cell lymphoma [14], the first HDACI specifically approved as a human therapeutic. Earlier drugs, such as 4-phenylbutyrate and valproic acid, are indeed HDACIs, but were approved based on efficacy as anticonvulsant and mood-stabilizing drugs (valproic acid) and for patients with urea cycle disorders (4-PBA). Some of the recent clinical trials with these compounds have been mentioned previously. A search using an online database [101] reveals that 70 trials are currently underway or planned to test the efficacy of various HDACI as monotherapies or combination therapies, with most of these trials testing HDACI as cancer therapeutics. The majority of current HDACI relatively nonspecifically, targeting each of the members of a particular class of HDACs with comparable IC₅₀ values [75]. Although current HDACI are relatively well tolerated in humans, they have been associated with numerous side effects, such as cardiac arrhythmia, bone marrow depression, clotting disorders, diarrhea, fatigue and electrolyte disturbances in Phase I and II studies [76]. We expect that compounds that specifically inhibit a subset of the HDACs, targeted to obtain a desired clinical outcome, will be less toxic and will substantially broaden the therapeutic uses of HDACI in neurological diseases. In this regard, we recently reported that the HDACI 06, which is active in a mouse model of FRDA [24], specifically targeted HDAC3, with at least a tenfold specificity toward this enzyme over other class I HDACs, and essentially no activity against class II HDACs [77]. Derivatives of this compound are under development as therapeutics for FRDA and HD. Similarly, other isoform-selective HDACIs have been shown to be neuroprotective in oxidative stress-induced cellular models for neurodegeneration, without the cytotoxic effects of hydroxamate-based inhibitors. These include cysteine-based moieties for zinc chelation [78] and HDAC6-selective mercaptoacet-amines [79]. In contrast to the HDAC3-selective compounds that show efficacy in the FRDA and HD models previously mentioned, these latter studies demonstrated that similar benzamides, as well as hydroxamates and other thiol-containing compounds, were ineffective in protecting primary cortical neurons from homocysteine-induced stress [79]. Thus, inhibition of class II HDACs might be more beneficial than inhibition of class I HDACs in neurological diseases such as ALS, while inhibition of specific class I HDACs, such as HDAC3, may well be the best therapeutic approach in diseases where transcription defects are the core pathology of the disease. To avoid the potential toxic effects of HDACI treatment, a recent study demonstrated that pulsed inhibition of HDACs in cortical neurons induce neuroprotection without apparent toxicity [80]. Thus, pulsed treatment regimens might be effective in humans without the side effects found in cancer clinical trials with hydroxamate-based HDACIs [76]. The combination of new inhibitors that show improved selectivity for different members of the various HDAC classes along with innovative treatment regimens will likely yield therapeutic benefit for the diseases described in this paper. Researchers in the field hope that within the next few years, human clinical trials will be

initiated for these diseases, and there is reasonable hope that approved therapeutics should appear by the middle of the next decade.

Executive summary

Chromatin structure, histone postsynthetic modifications & gene expression

- The higher order structure of chromatin is controlled by postsynthetic modifications of the histone proteins, such as acetylation and methylation of specific lysine residues. These modification states of the histones dictate whether or not a region of the genome is accessible to the transcription machinery of the cell.

Histone acetyltransferases, histone deacetylases & histone deacetylation inhibitors

- The human genome encodes numerous enzymes responsible for both histone acetylation (histone acetyltransferases [HATs]) and histone deacetylation (histone deacetylases [HDACs]). Modulation of the activities of these enzymes with small molecules has been shown to regulate gene expression.
- Various small molecule HDAC inhibitors have been identified and considerable effort has been expended to derive molecules that are selective for various members of the four classes of HDAC enzymes.

Triplet-repeat sequences affecting chromatin structure & gene expression: fragile X & Friedreich's ataxia

- These diseases are caused by chromatin-mediated silencing of particular genes: *FMR1* in the case of fragile X syndrome and *frataxin* in the case of Friedreich's ataxia.
- Small molecules, such as DNA methyltransferase inhibitors and HDAC inhibitors have been shown to reactivate these genes and offer potential therapeutic approaches.

Master epigenetic regulators: methyl-CpG-binding protein & Rett syndrome, CBP & Rubinstein–Taybi syndrome

- These diseases arise from mutations in two central epigenetic regulators, the methyl-CpG-binding protein 2 in Rett syndrome and the histone acetyltransferase, cyclic AMP response element-binding protein (CREB)-binding protein, in Rubinstein–Taybi syndrome.
- Thus, HDAC inhibitors offer promise for therapy in Rett syndrome and Rubinstein–Taybi syndrome.

Modulation of gene expression & potential therapies for spinal muscular atrophy & myotonic dystrophy

- Spinal muscular atrophy is caused by a mutation in the survival of motor neuron (*SMN1*) gene, and activation of the related *SMN2* gene with HDAC inhibitors has been shown to increase SMN protein levels in both cellular and animal models of this disease.
- Myotonic dystrophy type 1 is caused by a toxic RNA gain-of-function, where long CUG repeat RNA sequesters essential proteins involved in RNA splicing, such as the human homolog of the *Drosophila* protein muscleblind (MBNL1). Thus, activation of *MBNL1* gene expression with small molecules has been proposed as a potential therapy.

Amyotrophic lateral sclerosis

- Histone deacetylase inhibition has also been proposed as a therapeutic approach for amyotrophic lateral sclerosis (ALS), based on the hypothesis that a disequilibrium between HAT and HDAC activities may contribute to the pathogenesis of this lethal degenerative disease of motor neurons.
- Clinical trials of sodium phenylbutyrate in ALS patients have begun.

Histone deacetylase inhibitors in polyglutamine repeat disorders

- Dysregulation of gene expression may be a common element among the polyQ repeat disorders such as Huntington's disease and the spinocerebellar ataxias. Polyglutamine repeats cause protein aggregation or inclusions in the nucleus, and result in sequestration of transcription factors or other components of the transcriptional machinery.
- Histone deacetylase inhibitors have proved beneficial in mouse models for HD.

Conclusion & future perspective

- Clinical trials of several nonselective first-generation HDAC inhibitors, such as valproic acid and 4-phenylbutyrate, have been performed in neurodegenerative and neuromuscular diseases; however, these molecules have met with limited success.
- Histone deacetylase inhibitors that are selective for specific members of the various classes of HDAC enzymes are expected to be less toxic and would show a better therapeutic outcome.
- Pulsed treatment regimens might also be more beneficial and would likely show lower levels of unwanted side effects, such as those observed with the US FDA approved HDAC inhibitor, suberoylanilide hydroxamic acid (and vorinostat).

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