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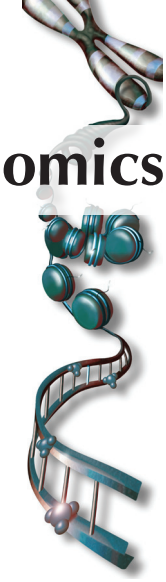
Epigenomics



Epigenetic modifications as novel therapeutic targets for Huntington's disease

Huntington's disease is a late-onset, autosomal dominant neurodegenerative disorder characterized by motor, cognitive and psychiatric symptomatology. The earliest stage of Huntington's disease is marked by alterations in gene expression, which partially results from dysregulated epigenetic modifications. In past decades, altered epigenetic markers including histone modifications (acetylation, methylation, ubiquitylation and phosphorylation) and DNA modifications (cytosine methylation and hydroxymethylation) have been reported as important epigenetic features in patients and multiple animal models of Huntington's disease. Drugs aimed to correct some of those alterations have shown promise in treating Huntington's disease. This article discusses the field of epigenetics for potential Huntington's disease interventions and presents the most recent findings in this area.

Keywords: 5-hydroxymethylcytosine • DNA methylation • epigenetic dysregulation • HDAC inhibitors • histone acetylation • histone methylation • histone ubiquitination • Huntington's disease • therapeutic target • transcriptional dysregulation

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Huntington's disease (HD) is a late-onset, autosomal dominant neurodegenerative disorder manifested by prominent motor, cognitive and psychiatric symptomatology [1,2]. HD is caused by a mutation in the *huntingtin* (*Htt*) gene, which contains an aberrant expansion of the trinucleotide sequence CAG (>36) at its exon 1 and produces a polyglutamine (polyQ) expansion on the N-terminus of the protein. Classical HD is characterized by personality changes, weight loss, cognitive decline, progressive motor dysfunction, including the hallmark feature of chorea (involuntary jerky movements of the face and limbs) and psychiatric disturbances. HD usually leads to progressive dementia and death approximately 15–20 years after onset. In HD, a typical morphological characteristic is the marked degeneration in medium spiny GABAergic neurons of the striatum, which is also accompanied by several other seriously affected neuronal types and brain areas as its pathology progresses [3]. So far, no effective therapeutic drugs are available to cure HD.

This CAG repeat expansion in *huntingtin* gene provokes two types of effects: first, the long polyQ in mutant *huntingtin* (*mHtt*) seems to induce an aberrant protein conformational change, which causes *mHtt* to form intracellular aggregates. These intranuclear inclusions are common in a transgenic mouse model of HD, but less so in a human HD brain, in which approximately 1–4% of striatal neurons have nuclear aggregates [4]. The long polyQ sequence can induce disease through interfering with the normal function of cellular proteins such as sequestration of important transcriptional factors. The polyQ expansion may also cause the loss of function of normal *huntingtin* gene (*Htt*). The *huntingtin* gene is expressed ubiquitously in various tissues and particularly in neurons of the brain [5]. It acts as an anti-apoptosis protein in neuronal cells in the CNS [6]. Wild-type *Htt* positively regulates expression of brain-derived neurotrophic factor (*BDNF*), which is necessary for survival of striatal neurons. However, *mHtt* abolishes

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this beneficial regulation, resulting in decreased BDNF production in HD, which may lead to striatal neuronal death [7]. Under the combined effect of these two forces, polyQ expansions of mHtt protein can lead to a number of cellular abnormalities, which include altered nucleosome dynamics and subsequent dysregulation of transcription [8].

Epigenetics includes a wide range of heritable changes in gene expression that do not result from an alteration in the DNA sequence itself. DNA modifications, histone modifications and ATP-dependent chromatin remodeling are three currently well-defined epigenetic landscapes [9–12]. Compelling studies have revealed the relevance between neurodegenerative diseases such as HD and epigenetic alterations, and proposed a therapeutic intervention strategy targeting epigenetic aberrations in neurodegeneration. A typical molecular characteristic of HD is mHtt-induced abnormal gene transcription, which is believed to be associated with aberrant epigenetic modifications.

In this article, we will summarize the epigenetic alterations that have been documented in past decades in both cellular and animal HD models, and discuss the upstream mechanisms that potentially lead to such abnormalities. Aberrant histone modifications in the pathogenesis of HD and the development of chorea will be briefly introduced. Interested readers are referred to previously excellent reviews [13,14]. We will mainly focus on DNA modifications including DNA methylation and hydroxymethylation. Last, we will summarize the existing epigenetic targets for HD treatment and discuss the potential to develop therapeutic strategies targeting the aberrant histone and DNA modifications.

Histone modification alterations in HD

The important feature of chromatin is its dynamic response to various intracellular and extracellular signals. Chromatin plasticity is dependent on a complex and diverse molecular process referred to as epigenetics, which results in covalent modifications of its two components, DNA and histone. The fundamental unit of chromatin structure, the nucleosome, consists of 146 bp of DNA wrapped around an octamer of histones made up of two copies of each core histone: H2A, H2B, H3 and H4 [15]. These proteins not only provide a solid structure, but the N-terminal regions of histones extend from the nucleosome and can be easily modified. There are many types of post-translational modifications of the residues at histone tails, including phosphorylation at serine (Ser) and threonine (Thr), acetylation at lysine (Lys), methylation at Lys and Arginine (Arg), ubiquitination at Lys, ADP ribosylation at glutamic acid (Glu), carbonylation, SUMOylation at Lys, glycosylation and biotinylation [16].

mHtt can lead to epigenetic abnormalities including histone modifications and DNA methylation directly or indirectly in HD. In HD model striatal cell line cells, Bmi-1, a component of the hPRC1L E3 ubiquitin ligase complex, binds to the wild-type *Htt* more readily than *mHtt*, which facilitates free Bmi-1 to ubiquitylate H2A; thus, the level of ubiquitinated H2A increases in R6/2 transgenic HD mice brain [17]. Another example that *mHtt* can affect epigenetic modification is DNA methylation. In cells expressing the *mHtt* gene, significant changes in DNA methylation can be observed in a large fraction of genes [18].

Histone acetylation

The histone acetylation level is coordinated by two functional antagonistic enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs and HDACs catalyze the acetylation and deacetylation of histone proteins at Lys residues, respectively. Histone acetylation is associated with loose chromatin conformation, which facilitates the recruitment of transcription factors and other chromatin-association proteins, and eventually promotes gene transcription [19,20]. Histone acetylation, which is generally believed to promote gene transcription, is overall reduced in HD models [21,22]. However, hyperacetylation at selected gene loci is increased slightly [23].

So far, altered histone acetylation has been demonstrated in a vast array of HD models and in neuronal populations from patients by independent research groups [13]. The expression of HDACs and acetylated core histones (AcHs) was investigated by immunohistochemistry in HD patients. Specific and significant losses of AcH2A, AcH2B, AcH3 and AcH4 expression were found in the caudate nucleus and Purkinje cells of the cerebellum in HD patients compared with control cases [24]. Despite no change in the overall acetylated histone level, a significant decrease in acetylation of histone H3 can be observed at promoters of downregulated genes in 4-week-old R6/2 mice compared with controls. This decrease in H3 acetylation can be rescued with HDAC inhibitor treatment, resulting in correction of the abnormal expressions of those downregulated genes [25]. McFarland and colleagues have investigated the correlation between histone modifications and transcriptional abnormalities in the striatum of 12-week-old R6/2 HD model mice in the whole genome. They found that the total number of binding sites occupied by acetylated histone H3 decreased significantly in a HD mouse model. The acetylation of histone H3K9 and H3K14 was observed to be localized at genes strongly expressed in both wild-type mice and a HD model mice. However, AcH3 binding at select gene loci increased only slightly [23].

The observation of abnormal histone acetylation in HD correlate with the loss of a well-known Lys acetyltransferase CREB-binding protein (CBP) and E1A-associated protein p300 (CBP-P300). In HD patients, HD model mice and HD model mice-derived cells, CBP is depleted from its normal nuclear location and present in the inclusion body formed by mHtt protein aggregation and other proteins, which become swept up in the mHtt aggregate, indicating a reduction of soluble CBP. The recruitment of CBP to mHtt induces aggregation, which enhances the degradation of CBP through the ubiquitin proteasome. This eventually results in decreased CBP activity and dysregulation of transcription of downstream target genes [26]. In addition, mHtt can physically interact with CBP, which inhibits CBP-mediated gene expression [27]. Accordingly, the transcriptional dysregulation induced by polyQ-mediated disruption of CBP in HD may partially contribute to the loss of neurons [27–29]. Although there is the similarity of structure and function between CBP and p300, p300 behaves differently from CBP in HD. Unlike CBP, it cannot be sequestered in mHtt-induced inclusions to facilitate degradation by the ubiquitin proteasome [27]. Overexpression of p300 is not capable of rescuing the cell from mHtt toxicity [30].

In a cell-free system, the protein encoded by Htt exon 1 can inhibit the acetyltransferase activity of three proteins: p300, P/CAF and CBP. Expression of Htt exon 1 in cultured cells reduces the level of acetylated histone H3 and H4, and this reduction can be reversed by administering inhibitors of HDAC [31]. In PC12 cell line, expression of an expanded polyQ (148Q) N-terminal truncation of *Htt* gene provokes a significant reduction of HAT activity and global histone acetylation compared with expression of normal *Htt* [32].

In human HD brain studies, Anderson *et al.* [33] uncovered interesting evidence for the potential role of histone hyperacetylation that was in contrast to the hypoacetylation observed in transgenic *Drosophila* and rodent models. They saw significant increases in *HAT1* and *H3F3B* mRNA expression in the striatum and cortex of the HD brain. They also observed increased gene repression in specific gene clusters, such as Chr1p34, Chr17q21 and ChrXp11.2, all of which encode HDAC genes (*HDAC 1, 5* and *6*, respectively). These results point to possible species differences among transgenic *Drosophila* models, rodent models and the human disease state.

Histone methylation

Histone H3 can be methylated at five Lys residues in its N-terminal tail – K4, K9, K27, K36 and K79 [34]. Moreover, each of these residues can have three (me3), two (me2), one (me1) or no methyl groups. Di- and tri-methylation of K4, K36 and K79 represent 'open' chromatin structure and positively correlates with transcriptional activity, and are frequently referred to as 'active' marks, while the di- and tri-methylation of K9 and K27 associated with gene repression are frequently referred to 'repressive' marks [35]. Methylations of histone Lys residues are catalyzed by a family of SET domain-containing proteins, with the exception of H3 Lys79 methylation [36].

Currently, histone methylation alterations in HD mainly occur at H3K4me3 and H3K9me3. H3K4me3 is one of the most clearly interpreted histone post-translational modifications in HD. In most studies, this mark has been considered to positively correlate with transcriptional activity and is thought to represent a form of cellular memory for previous gene activation [37–40].

Brains from the HD mouse model R6/2 and from human HD patients have shown gene-specific changes in H3K4 trimethylation (H3K4me3) at downregulated genes. The H3K4me3 deficit is likely due to the upregulation of the specific histone demethylase Lys-specific demethylase 5C (KDM5C), also known as SMCX or Jarid1c, given the fact that reducing the level of the H3K4 demethylase SMCX/Jarid1c in primary neurons can rescue the downregulation of key neuronal genes caused by mHtt expression. Accordingly, knocking down SMCX/Jarid1c in primary neurons from HD model mice or *Drosophila* has proven to be neuronal protective [41].

By contrast, the methylation mark associated with repressive genes, dimethylation (me2) and trimethylation (me3) of H3K9 are elevated in brain tissues of R6/2 and N171–82Q mice and in HD patients [14,42]. The histone H3K9 hypermethylation is primarily caused by the alteration of upstream chromatin regulatory factors. Ferrante *et al.* [42] showed that the level of H3K9me3 is positively correlated with its specific histone Lys methyltransferase SETDB1. Meanwhile, GC-box-binding transcription factors Sp1 and Sp3 are proposed to be responsible for the SETDB1 transcriptional upregulation. This inference is consistent with the observation that Sp1 activity is elevated in HD [43,44].

Another protein that modulates H3K9me3 level is ATRX, a DNA-dependent ATPase/helicase that specifically recognizes H3K9me2/3 [45,46]. ATRX expression is elevated in both a HD cell line model and a transgenic model, via cdx-2 activation, which increases the level of H3K9me3 and condensation of pericentromeric heterochromatin. The upregulation of ATRX can be attributable to the increased expression of the

upstream transcription factor caudal type homeobox and its binding occupancy at the ATRX promoter in R6/2 striatum [47]. Moreover, neurotoxicity induced by mHtt is influenced by genetic manipulation of ATRX levels in *Drosophila*.

In mammalian cells, histone H4 can be methylated at R3, K20 and acetylated at K5, K12, K8 and K16 [48]. Overall, the decreased level of H4 acetylation was observed in cells expressing *mHtt* exon 1, and this reduction can be rescued by a HDAC inhibitor. Another study identified many fragments with hypoacetylated H4K12 in HD 82Q mice [49].

Several studies have suggested that both acetylation and methylation of histones are altered in HD. Histone acetylation and methylation are considered to be mutually antagonistic and their impact on the level of gene expression is a combined effect. A good example is H3K9, of which methylation and acetylation are mutually exclusive [50]. Therefore, it is not surprising that their levels become imbalanced in murine models [14] and negatively affect the expression of genes with neuronal functions.

Histone ubiquitylation

Histone ubiquitylation mainly occurs at histone H2A and H2B and takes the form of monoubiquitylation [51]; it is the least understood modification in HD. H2A is monoubiquitylated at Lys 119, which correlates with gene silencing. However, H2B is monoubiquitylated at Lys 120, which is associated with actively transcribed DNA [52]. In HD transgenic R6/2 mice, repressed gene expression showed increased H2A-Ub and decreased H2B-Ub in promoter regions, while the positively regulated gene demonstrated a different pattern [17]. The increased H2A-Ub in the brains of R6/2 transgenic mice could be explained by the reduction of Ubc expression, because Ubc-knockout mice prevent increased levels of H2A-Ub [53].

DNA modifications

DNA methylation in HD

Epigenetic DNA modification encompasses three different base modifications: cytosine methylation, cytosine hydroxymethylation, N-7 guanine methylation and DNA phosphorothioation. DNA methylation at the fifth carbon position of cytosine is the most abundant DNA modification and highly represented in vertebrates [54]. In mammals, it is found almost exclusively in CpG dinucleotides. CpG islands (CGIs) are genomic sequences enriched in CpG dinucleotides, and are represented with a very limited frequency throughout the human genome, but are concentrated at promoter regions of some genes involved in gene regulation [55]. A CpGI is defined as a 200-bp region of DNA where the GC content is greater than 60%. This modification is catalyzed by three DNA methyltransferase enzymes, DNMT1, DNMT3a and DNMT3b, which create 5-methylcytosines (5mCs). DNMT1 is responsible for copying maternal DNA methylation status to daughter DNA during DNA replication and thus is referred to as 'maintenance DNA methyltransferase'. DNMT3a and DNMT3b can transfer the methyl to the cytosine coordinating with associated proteins to generate new methylation during embryogenesis, and is referred to as '*de novo* synthesis methyltransferase' [56,57].

DNA methylation is often associated with a gene repressive environment, and maintaining proper DNA methylation status is essential for normal development. Aberrant DNA methylation patterns are frequently linked to the pathogenesis of numerous diseases, including neurological disease and cancer [10,58–60]. In the mammalian CNS, 5mC plays a significant role in the temporal control of neural differentiation and neurodevelopment [61,62]. Alternation in the 5mC profile of genomic DNA has recently been linked to some neurological disorders such as hereditary sensory and autonomic neuropathy type-1 [63], autosomal-dominant cerebellar ataxia deafness and narcolepsy [64], Rett syndrome-like phenotypes [65], HD [18] and Alzheimer's disease [66].

Among the studies of DNA methylation in HD, two aspects can be cataloged based on the research scope. The first of these is study on the genome-wide distribution of DNA methylation; the second is focused on the DNA methylation status in promoter regions of the genes playing vital roles in nervous system function, especially those that are downregulated in HD. The first study on genome-wide DNA methylation alteration was reported in 2013 by Ernest Fraenkel and his colleagues [18]. They carried out reduced representation bisulfite sequencing to map sites of DNA methylation in cells expressing either wild-type *Htt* or *mHtt*. They measured the degree of methylation at 97,006 cytosines in STHdhQ111 and STHdhQ7 cell lines. Overall, 61,940 bases decreased in methylation in STHdhQ111 relative to STHdhQ7, and 33,974 bases increased in methylation. A large fraction of the genes, when differentially expressed in mHtt-expressing cells, exhibit significant changes in DNA methylation at their promoter regions. Regions with low CpG content are disproportionately affected, and those regions have previously been shown to undergo changes of methylation in response to neuronal activity, Genome-wide chromatin immunoprecipitation sequencing revealed that transcriptional regulators AP-1 and SOX2 are associated with DNA methylation changes of regions. These results also raise important questions about the potential effects of DNA methylation changes on

neurogenesis and cognitive decline in HD patients. The examples of altered DNA methylation status in specific gene promoter regions are *BDNF* and adenosine A2A receptor (*Adora2a)* gene. *BDNF* is an essential neurotrophin and its expression regulation is complex owing to multiple 5′-untranslated exons, which are separately spliced to a common coding exon to form unique mRNA transcripts. Disruption of *BDNF* gene expression correlates with a change in the DNA methylation profile in R6/1 transgenic HD mice [67]. There is an overall greater level of methylation in two specific CpG islands in HD mice when compared with wild-type mice. The methylcytosine content in the 5′-untranslated region of *ADOR2A* was tested in HD patients, and increased 5mC levels and reduced 5-hydroxymethylcytosine (5hmC) were found in the 5′-untranslated region of the *ADORA2A* gene compared with age-matched controls. Therefore, an altered DNA methylation pattern in *ADORA2A* seems to play a role in the pathologically decreased A2AR expression levels found in HD.

It has been reported that increased N-7 guanine methylation in CpGIs led to protein dissociation and chromatin remodeling, which results in increased gene expression [68]. Both methylation and oxidation of guanine at positions 7 and 8 have been shown to cause decreased interactions with methylated cytosine DNA binding proteins and associated proteins [69]. Significant differences in guanine methylation in the nuclear fractions of brain samples has been observed from two murine HD models compared with the control samples [70], suggesting that changes in guanine methylation status may play a role in HD pathology.

5hmC in HD

DNA methylation can be followed by an additional step of 5mC oxidization to create hydroxymethylcytosines (5hmCs), which are catalyzed by the ten-eleven translocation (TET) proteins [71]. The 5hmC epigenetic mark was first identified in the T-even bacteriophage in 1953 [72], and it was later found in the vertebrate brain and in several other tissues [73–77]. Although 5hmC exists in mouse embryonic stem cells at relatively high levels, it decreases significantly after embryonic stem cell differentiation [78,79]. Interestingly, the level of 5hmC rises again in terminally differentiated cells, such as Purkinje neurons [74], which suggests a potential biological function of 5hmC and a role in the regulation of DNA methylation dynamics in early development. Human TET1, a member of the Tet family of proteins, is identified as a 5mC dioxygenase responsible for catalyzing the conversion of 5mC to 5hmC [79]. The mammalian Tet family contains three members, Tet1, Tet2 and Tet3, all of which share a high degree of homology within their C-terminal catalytic domain [80,81]. The discovery of this family of enzymes suggests a potentially novel mechanism for the regulation of DNA methylation, with 5hmC acting as an intermediate during DNA demethylation, although the biology and regulation of 5hmC and Tet family enzymes during development remain elusive.

The genome-wide DNA 5hmC level is precisely regulated during development and its abnormal genome-wide level and aberrant distribution pattern is correlated with a variety of diseases. In neuronal cells, the level of 5hmC in the whole genome is programmed acquired during different development stages [82], which is consistent with the observation of a substantial increase in 5hmC in both the striatum and cortex regions, with the highest levels being observed at 3 months of age. The high 5hmC levels are followed by reduced levels at 8 months of age. Together, these results suggest that continuous remodeling of the 5hmC epigenetic architecture takes place in the striatum and cortex [83].

We previously presented the first report on genome-wide DNA hydroxymethylation in HD mouse brain [83]. A 5hmC-specific chemical labeling and enrichment technology coupled with high-throughput deep sequencing of DNA fragments was employed to investigate distribution patterns in the whole genome. A dynamic change in DNA 5hmC in mouse brain striatum and cortex regions during development was observed [83]. A genome-wide study on 5hmC in mouse cerebellum and hippocampus during neurodevelopment and aging showed a significant increase in 5hmC signal from P7 to 6 weeks in both the cerebellum and hippocampus [82]. More importantly, the authors identified stable and dynamically 5hmC-regulated regions during development and aging, which indicates that the change of DNA hydroxymethylcytosine during development is a programmed modification process. The global level of 5hmC was reduced significantly in the striatum and cortex of YAC128 mice in pre-symptomatic stages, suggesting a deficiency in 5hmC reconstruction in HD brains during postnatal development. In this study, the 5hmC level was found to be positively associated with gene expression, which was in agreement with a recent report that 5hmC is associated with developmentally activated genes and actively transcribed genes in the cerebellum and hippocampus of mouse brain [82].

The level of DNA hydroxymethylation can be affected by disease states such as cancer and neurological disorders, environmental factors, aging and chemicals. This suggests that DNA hydroxymethylation is an important mechanism to integrate extracellular signals to better adapt to the environment. In addition to the function of 5hmC in HD, its dysregulation can be

detected in other diseases such as cancer and neurological diseases. Global 5hmC content was increased during development with no detectable decrease of 5mC level in mouse hippocampus, suggesting that 5hmC could act as an stable epigenetic marker other than an intermediary in DNA demethylation [82,84].

Noncoding RNAs in HD

A decreased expression level of neuronal-specific miRNAs in murine models and brains of HD patients was observed. This dysregulation was probably caused by increased repression by REST. Decreased miRNAs expression in HD neurons can result in a secondary effect; that is, increased levels of the expression of target genes regulated by miRNAs [85]. Lee *et al.* have identified nine miRNAs (miR-22, miR-29c, miR-128, miR-132, miR-138, miR-218, miR-222, miR-344 and miR-674*) that were downregulated in YAC128 and R6/2 HD transgenic mice [86]. Long noncoding RNAs that have been found in the human genome are considered to contribute to neurodegenerative disease including HD [87]. Seven long noncoding RNAs (TUG1, LINC00341, RPS20P22, NEAT1, MEG3, DGCR5 and LINC00342) were found to be dysregulated in HD brain, several of which contain transcriptional repressor REST binding sites, a key mediator of transcriptional changes in HD [88].

Therapeutic strategies

In last three decades, studies on epigenetic therapies have focused on cancers and related conditions [89]. Because of the potential reversibility of epigenetic modifications, the epigenetic alterations observed in HD models and patients in recent years promote the concept of using epigenetic intervention as a new therapeutic strategy to treat HD patients. Researchers in the HD field have been searching for therapeutic agents targeting the dysregulated epigenetic modification seen in HD models and patients. The HDAC inhibitor turns out to be a good candidate. HDACs play a crucial role in homeostasis of protein acetylation in histones and other proteins, which in turn regulate fundamental cellular activities such as transcription. A wide range of neurological disorders have been shown to be associated with imbalances in protein acetylation levels and hence transcriptional dysfunctions. HD transgenic models generally exhibit reduced histone acetylation, correlating well with the decreased gene expression pattern. In this context, the therapeutic potential of HDAC inhibitors (e.g., butyrates) is based on the inference that the improvement of HAT activity can increase and restore histone acetylation levels. So far, butyrates are the most clinically studied HDAC inhibitors because they can readily cross the blood–brain barrier [90]. Butyrate (or sodium butyrate) is one of most widely studied HDAC inhibitors, which also include phenylbutyrate, trichostatin A, and suberoylanilide hydroxamic acid [20]. Pharmacological treatment of sodium butyrate to the R6/2 transgenic mouse can correct the global hypoacetylation of histones, significantly modulate transcription dysregulation, and eventually extend survival in a dose-dependent manner [21,91]. In a *Drosophila* HD model, butyrates can also arrest polyglutamine-dependent neurodegeneration [31].

HDAC inhibitors can prevent polyQ-induced toxicity and neurodegeneration in a *Drosophila* HD model [31,92]. The HDAC inhibitor phenylbutyrate could improve the survival rate of HD mice in a dose-dependent manner through increasing histone H3 and H4 acetylation [22]. Sodium butyrate treatment induced hyperacetylation and reduced neural and brain atrophy and improved motor performance [21]. Furthermore, other HDAC inhibitors such as suberoylanilide hydroxamic acid [93] and the HDAC inhibitor 4b [94] could also improve motor deficits and neuronal atrophy in several mouse models of HD. Preclinical investigations of HDAC inhibitors in various HD models have provided important information for drug development. The HDAC inhibitors are currently under investigation and being optimized for HD therapeutic agents.

To date, no DNA methyltransferase inhibitor has been tested in a HD model. It is possible to manipulate histone methylation indirectly, by using GC-binding anthracyclines, such as mithramycin A and chromomycin, a group of bacterial compounds with anticancer and antibiotic properties [95]. Mithramycin and chromomycin are shown to interfere with the binding of transcriptional activators to the CpG-rich promoter region, and thus inhibit the expression of genes that activate oxidative stress and apoptotic pathways [96]. In the HD model mouse R6/2, pharmacological treatment with mithramycin extends the survival rate by 29.1%, with improved motor performance and markedly delayed neuropathological performance. Mithramycin treatment prevents the increase in H3 methylation observed in HD mice, suggesting that the enhanced survival rate and neuroprotective effects seen in mithramycin-treated HD models might be attributable to the relief of repressed gene expression vital to neuronal function and survival [97].

The potential beneficial effect of a combined pharmacological treatment with mithramycin and cystamine (a transglutaminase inhibitor) in R6/2 mice has also been recently evaluated [42]. This combined pharmacological strategy was shown to decrease hypertrimethylation of histone H3 and to extend R6/2 mice survival over 40%, well beyond any other treatment

that has been reported in R6/2 mice to date. In addition, the combined treatment was able to significantly improve rotarod performance, delay gross brain atrophy, ventricular hypertrophy, striatal neuronal atrophy and the striatal neuronal intranuclear inclusion (NII) formation. A reduced number of NIIs is likely due to cystamine treatment, as mitramycin alone had no effect on NII formation. CpG-rich regions are prime targets for DNA methylation. However, the action of GC-binding anthracyclines seems to be wider, as they can also reverse the hypoacetylation of histones H3 and H4 [14].

Another target of HD treatment is DNA hydroxymethylation modulation. In the HD model mouse YAC128, the overall level of the DNA hydroxymethylation is significantly decreased in both the striatum and cortex regions compared with wild-type mice, which provides the possibility that increased DNA hydroxymethylation may ease HD symptoms and thus provide a viable new drug target to cure HD [83]. Some chemicals that can affect the genome-wide DNA hydroxymethylation have been reported recently. Dimethyl sulfoxide can increase global and gene-specific DNA hydroxymethylation levels in preosteoblastic MC3T3-E1 cells, which may do this by increasing the expression of genes involved in DNA hydroxymethylation (e.g., TET) and nucleotide excision repair (GADD45), or through decreasing the expression of genes related to DNA methylation (e.g., Dnmt1, Dnmt3b and Hells) [98]. Ascorbic acid (AA) or vitamin C has been shown to directly enhance the catalytic activity of TET dioxygenases for the oxidation of 5mC, suggesting that AA may also acts as a cofactor of TET enzymes. In mouse embryonic stem cells, AA significantly increases the levels of all 5mC oxidation products (by more than an order of magnitude), particularly 5-formylcytosine and 5-carboxylcytosine, and thus leads to a global loss of 5mC (~40%) [99]. Given that the overall level of the DNA hydroxymethylation is significantly reduced in both the striatum and cortex regions of HD mice brains, the chemicals that are capable of increasing DNA hydroxymethylation may have therapeutic potential. However, we should point out here that targeting DNA hydroxymethylation is a complete speculation. Further studies are needed to evaluate the therapeutic potential of these chemicals.

Executive summary

Histone modification alterations in Huntington's disease

- Despite no change in the overall acetylated histone level, a significant decrease of histone H3 acetylation can be observed at the promoters of downregulated genes in HD transgenic mice compared with controls.
- The brains from Huntington's disease (HD) mouse model R6/2 and human HD patients have shown gene-specific changes in H3K4 trimethylation at downregulated genes.
- The increased H2A-Ub in the brains of R6/2 transgenic mice could be explained by the reduction of Ubc expression, because Ubc-knockout mice prevent the increased level of H2A-Ub.

DNA modifications in HD

- A large fraction of the genes that are differentially expressed in mutant huntingtin-expressing cells exhibit significant changes in DNA methylation at their promoter regions.
- The dynamic change in DNA 5-hydroxymethylcytosine (5hmC) in mouse brain striatum and cortex regions during development can be observed. The global level of 5hmC was reduced significantly in the striatum and cortex of YAC128 mice in presymptomatic stages, suggesting a deficiency of 5hmC reconstruction in HD brains during postnatal development.

Noncoding RNAs in HD

- The decreased expression level of neuronal-specific miRNAs in murine models and brains of HD patients was observed. Decreased miRNAs expression in HD neurons can result in secondary effect, that is the increased levels of the expression of targets genes regulated by miRNAs.

Therapeutic strategies

- Researchers in HD field have been searching for therapeutic agents targeting the dysregulated epigenetic modification seen in HD models and patients. The histone deacetylase inhibitor turns out to be a good candidate.
- The potential beneficial effect of a combined pharmacological treatment with mithramycin and cystamine (a transglutaminase inhibitor) has also been recently evaluated [42]. This combined therapeutic strategy was shown to reduce hypertrimethylation of histone H3 and to extend survival over 40%.

Future perspective

- Targeting epigenetics could be beneficial especially in early stages of the disease to prevent further transcriptional problem and accumulation of pathological factors.
- Promoting gene expression by epigenetic manipulation could still be a novel therapeutic approach for preventing or at least delaying the development of motor deficits in HD, given the fact that significant alterations of histone and DNA modification occur in HD.

Conclusion & future perspective

Dysregulated epigenetic modification is an important and early molecular event during HD pathology, preceding much earlier than the aberrant gene transcription, thus targeting epigenetics could be beneficial especially in early stages of disease to prevent further transcriptional problems and accumulation of pathological factors. However, our knowledge about epigenetics is still limited, especially when it comes to neurodegenerative diseases such as HD. The detailed, exact alternations of epigenome (histone modification and DNA modification) in HD remain to be revealed. It is also unclear to what extent altered epigenetic modifications contribute to the aberrant transcription observed in HD. The currently available chemicals inducing epigenetic modification lack the necessary specificity for solely correcting the altered epigenome regions, which has the potential to cause averse side effects. More research is necessary for the refinement of epigenetic targets and development of agents that can specifically reconstruct the altered epigenome in the diseased state. Nevertheless, promoting gene expression by epigenetic manipulation could still be a novel therapeutic approach for preventing or at least delaying the development of motor deficits in HD, given the fact that significant alterations of histone and DNA modification occur in HD. Development of effective epigenetic therapies suitable for HD still requires much future investigation.

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