

Analysis of the Epigenetic Mechanism and Treatment of Huntington's Disease

Yi Shen^{1*}, Yuze Tang^{2*}

¹University of Alberta, Edmonton T6G 2R3, Canada

²Imperial College London, London SW7 2BX, United Kingdom

*Corresponding authors: Yi Shen, yshen9@ualberta.ca; Yuze Tang, yt2119@ic.ac.uk

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Abstract: Huntington's disease (HD) is an irreversible neurodegenerative disorder that is inherited in an autosomal dominant manner. In HD, many regions of the human brain are affected, including the striatum, thalamus, and cortex. The mechanism is by the expansion of CAG repeats, which encode glutamine (Q) in the Huntingtin gene on chromosome 4p16.3. Patients with more CAG repeats tend to have a younger age of onset and a higher risk. Mutant HTT protein, translated from mtHtt, would congregate or interact with other proteins, causing damage to the human body. Patients with HD show symptoms like chorea, which is an involuntary motor disability, cognitive deterioration, and psychiatric disturbances. Except for the genetic pathology of HD, the epigenetic mechanism of this disease has made a lot of progress in recent years. This paper primarily focuses on the alternation of deoxyribonucleic acid (DNA) methylation, histone modification, and non-coding ribonucleic acids (ncRNAs) in HD as well as the advancements of epigenetic therapy and healthcare in HD.

Keywords: Huntington's disease; Epigenetics; DNA methylation; ncRNAs; Healthcare

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1. Introduction

"Epigenetics" was first termed by Conrad Waddington in the 1940s whose great passion for the genetic field was evident ^[1]. He introduced epigenetics as the process of genotype expressed as phenotype ^[2]. Differing from genetics, epigenetics does not involve the change in deoxyribonucleic acid (DNA) sequence but studies the change in gene modification. The majority of epigenetics studies focus on DNA methylation, histone modification, and non-coding ribonucleic acids (RNAs) ^[3]. DNA methylation is a covalent modification of the DNA base, of which the most common is cytosine methylation and adenine and guanine methylation. DNA methyltransferase (DMNT) is responsible for DNA methylation that usually occurs in CpG islands ^[4]. Histone modification is a posttranslational modification of histone, including histone methylation, acetylation, ubiquitination, and phosphorylation. Different enzymes are expressed in the modification to regulate the structures of chromosomes and their functions ^[5]. Non-coding RNAs are RNAs that are unable to translate into proteins; they are found with other functions in gene expression ^[4]. Non-coding RNAs show alternations in different diseases. These modifications can regulate gene expression to cause diseases or symptoms in patients. Epigenetics can be changed by the environment, in which the change is known to be reversible. Therefore, epigenetic therapy has been extensively studied by scientists ^[4], especially in relation to cancer, aging, cardiovascular disease, neurodegenerative diseases, and other diseases.

Huntington's disease (HD) is a hereditary neurodegenerative disease that occurs worldwide. HD is still known as an incurable disease today. The aim of studying the epigenetic mechanism of HD is to make advances in HD treatment, targeting at the modification and alteration of the specific gene. This paper will discuss the DNA methylation, histone modification, and alternation of ncRNAs in HD as well as the epigenetic therapy and healthcare of HD, hoping to offer some references for future research.

2. Analysis of the epigenetic mechanism of Huntington's disease

2.1. DNA methylation

DNA methylation is the most well-studied epigenetic mechanism in mammals. It plays a key role in activating and inhibiting genes [6]. DNMT is the main enzyme that transfers the methyl group from the donor S-adenosylmethionine (SAM) to 5' cytosine on the CpG island [7]. While DNMT1 takes charge of maintenance methylation in DNA replication, DNMT3a and DNMT3b are responsible for *de novo* methylation [4]. 5' methylcytosine (mC) can oxidize to 5' hydroxymethylcytosine (hmC) [8]. Generally, the inhibition of transcription is caused by adding 5mC in the promoter region; the activation of transcription is linked to a greater 5hmC [9]. DNA methylation in a promoter directly disturbs the interaction between transcription factors and the gene sequence, thus transcription is disrupted. On the other hand, the methyl group binds with methylcytosine-binding domain (MBD) to recruit repressive protein complex and histone deacetylase (HDAC), which would modify it into inactive heterochromatin to inhibit transcription [10].

As shown in **Figure 1**, methyl groups can be transferred to 5' cytosine by DNMTs to make 5mC and further modified into 5hmC by ten-eleven translocation (TET) proteins. 5hmC can also be converted back into cytosine by oxidative removal.

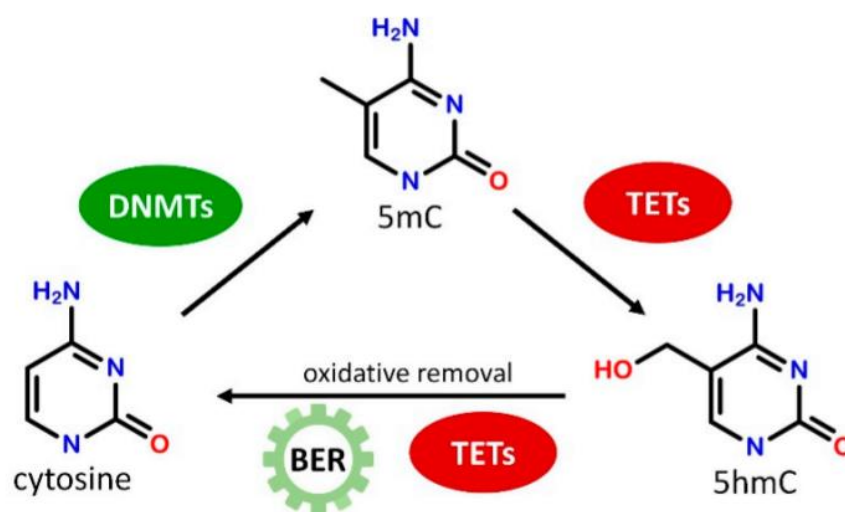


Figure 1. Methylation and hydroxymethylation of cytosine [11]

In a study, Ng *et al.* [12] used reduced representation bisulfite sequencing (RRBS) to monitor the change in DNA methylation between wildtype (STHdhQ7/Q7) and mutant HTT cell (STHdhQ111/Q111). DNA methylation can be divided into those that occur in CpG rich regions and CpG poor regions. CpG rich region often refers to promoter sequence, and in this region, the change of DNA methylation happens more frequently. Genome-wide chromatin immunoprecipitation sequencing (ChIP-Seq) was used to study the transcription level. The DNA methylation in CpG islands has a negative relationship with gene expression. It has been demonstrated that the relatively higher level of methylation in the promoter region of Ap-1, Sox 2, Pax 6, and Nes gene but lower expression in STHdhQ111/Q111 than that in wild type STHdhQ7/Q7. Meanwhile, the linkage between gene expression and the DNA methylation level in CpG poor regions

would be more complicated. The change of DNA methylation may be caused by obtaining and losing DNA-binding proteins. It has also been confirmed that HTT protein is able to bind with DNA sequence, which may directly influence the epigenetic mechanism. Some of these genes with lower expression are related to neurons and may also play a role in other neurodegenerative diseases; therefore, DNA methylation is one of the reasons to explain the cognitive deterioration in HD patients ^[13].

In addition, the expression of adenosine A2a receptor has been observed to be reduced in HD. Experiments have been performed to test the 5mC and 5hmC content in A2a gene in a mice model and a human brain sample. It has been shown that the reduced expression is accompanied by less 5hmC in the 5'UTR region in the R6/1 mice model, whereas 5mC was more with less 5hmC in HD patients ^[14].

In recent years, DNA methylation other than on 5' cytosine has also been studied. 7-methylguanine is a newly found epigenetic mechanism which might be related to the pathology of HD. Unlike 5mC, higher gene expression is shown with more methylation on guanine, and it would occur both on DNA and RNA. Through the experiment, the level of 7-methylguanine has been observed to have had changed in HD patients and mice models ^[15].

2.2. Histone modification

The role of histone proteins is to package DNA, which wraps around eight histone proteins into nucleosome, chromatin, and chromosomes. Histone modification refers to the posttranslational modification (PTM) of histone proteins, including methylation, phosphorylation, acetylation, ubiquitylation, and SUMOylation ^[16]. The modification of histone proteins may impact various cellular processes, such as transcription activation and repression, DNA repair, and chromosome packaging ^[17]. Generally, acetylation of lysine residue leads to transcription activation, while methylation of lysine and arginine residues leads to transcription repression ^[16]. The combination of core histone modifications creates a precise pattern that turns on or off specific genes. This is referred to as histone code ^[18]. Transcriptional dysregulation is a well-known pathogenic feature of HD, but its underlying epigenetic mechanism remains unclear.

Histone acetylation is a well-studied mechanism of histone modification, which have been found to be associated with numerous diseases. Histone acetylation and deacetylation are regulated by two enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC), working in corporation with each other to regulate chromatin structure and gene transcription. HAT activity leads to a more opened chromatin structure that increases gene transcription, whereas HDAC activity condenses chromatin and leads to decreased gene transcription ^[19].

Recent studies have revealed that histone acetylation plays an important role in HD pathogenesis ^[20]. Researchers have observed reduced histone acetylation level in several HD models in the early 2000s. Furthermore, CREB-binding protein (CBP), a HAT, has been detected in Htt aggregates in HD mice models and brains from individuals with HD ^[18]. These findings have garnered widespread attention as they could potentially explain the cause of transcriptional dysregulation in HD.

As shown in **Figure 2**, CBP is recruited by phosphorylated CREB in normal conditions, leading to histone acetylation. In HD, abnormal Htt expression would bind to CREB and CBP, thus inhibiting the functions of HAT and reducing the acetylation level. Low acetylation level leads to a more condensed structure with less gene expression.

Further studies have provided a deeper insight into the cause of hypoacetylation seen in HD. Mutant Htt has been shown to interact with both HAT and HDAC. Due to CAG repeats in HD genes, mutant huntingtin proteins are produced, which are then cleaved by caspases to form huntingtin fragments ^[18]. These fragments can be transported into the nucleus of neurons and aggregated into neuronal inclusions (NIs). NIs has been shown to trap transcription factors, thus disrupting transcription of genes that are critical to cell survival. Researchers have identified CBP as one of the molecules that is associated with NIs. When

trapped by NIs, CBP will be rendered incapable of opening the chromatin structure and would fail to allow the binding of transcription factors, thereby disrupting transcription [20]. Sequestered CBP in HD affects the transcription of tumor suppressor protein p53, which leads to abnormalities in gene transcription and potential cell death.

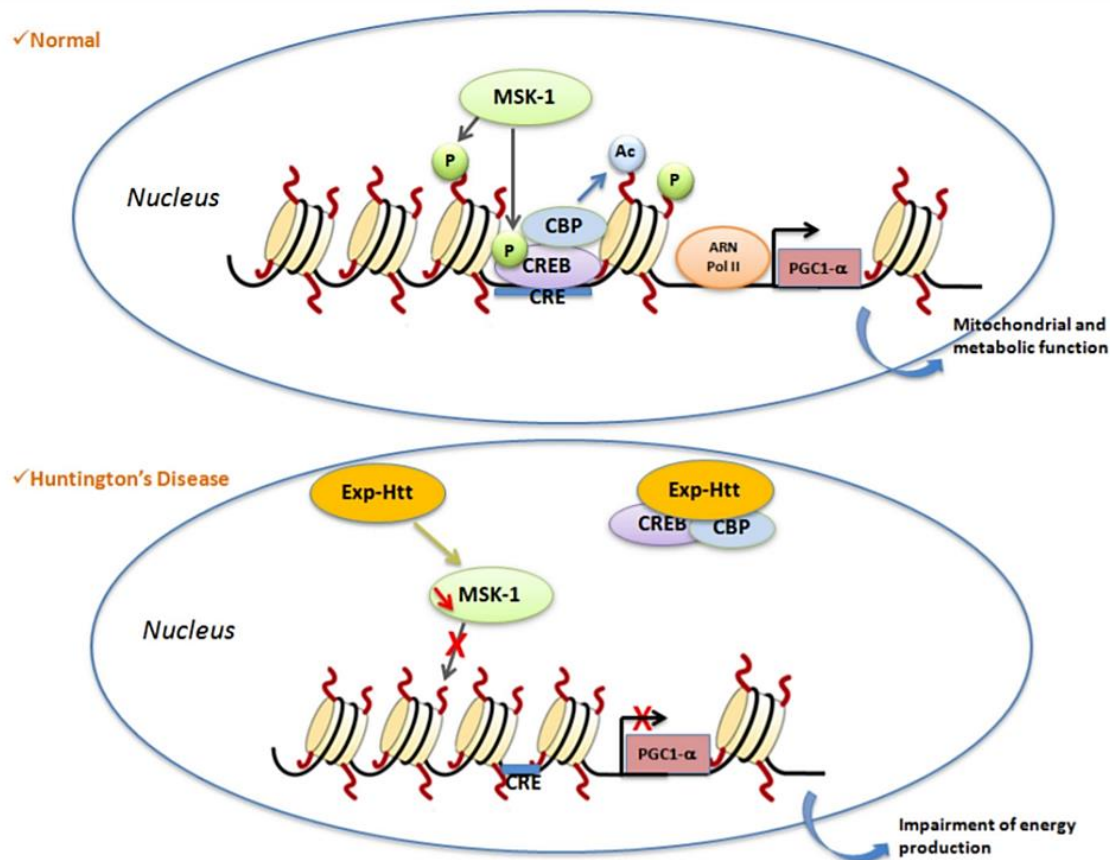


Figure 2. Histone acetylation in normal and pathologic conditions [21]

2.3. Alternation of non-coding RNAs

Non-coding RNAs are considered a group of RNA molecules that are not translated into proteins. They include transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), long ncRNAs (lncRNA), microRNAs (miRNAs), and so on, with each having a different function. However, many of them work on gene silencing [22].

A recent study on ncRNAs in HD has demonstrated lower levels of miRNA, such as miR-22, miR-29c, miR-125b, miR-128, and others, in HD patients and models [4]. However, the impact of the regulation of each ncRNA in HD has yet to be established. The study has also shown an increase in their target mRNAs. Both miR-125b and miR-150 target p53, which increases the level of p53 in the nucleus. p53 is an important tumor suppressor gene, which is also found in the central nervous system. Therefore, p53 is able to interact with mtHtt and may cause neuronal damage in HD patients [23].

Figure 3 shows that the interaction between mutant Htt and p53 can cause mitochondrial dysfunction in both transcription-dependent and transcription-independent pathways.

The protein REST/NRSE interacts with HTT, but the expansion of repeats in mtHtt inhibits the binding, causing accumulation of REST in the nucleus. The interaction between REST and RE1 may cause damage in transcription. REST can also regulate certain miRNAs, like miR-132, which are lower in HD patients. This might explain the alternation of miRNAs [25].

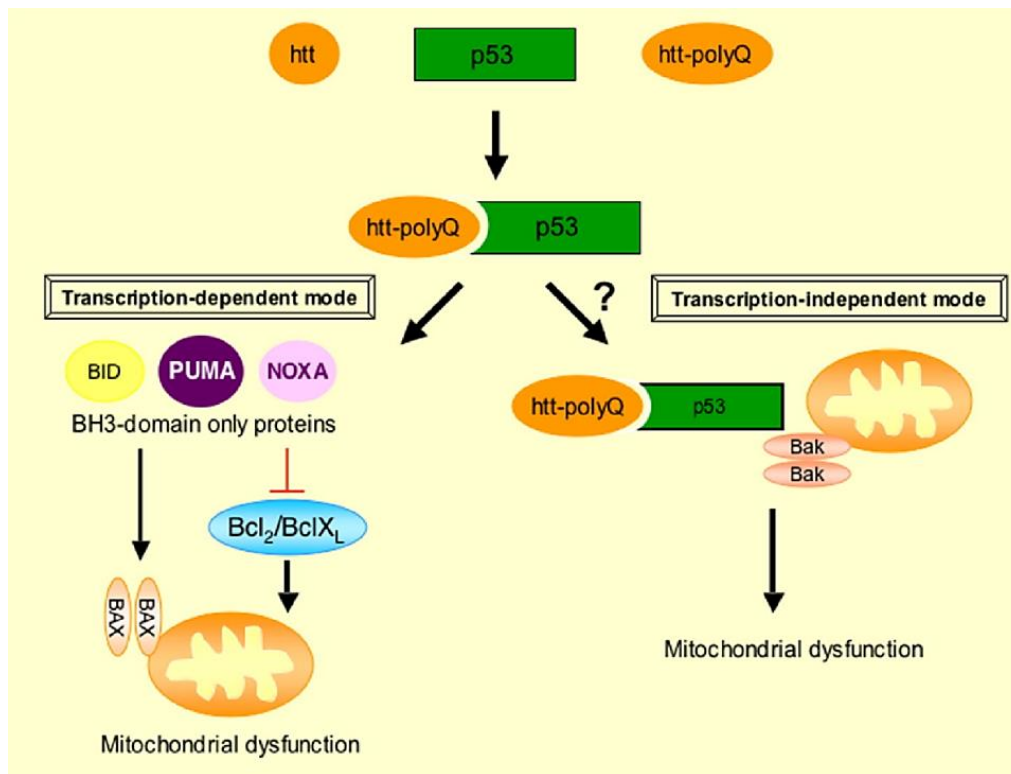


Figure 3. Mitochondrial dysfunction as a result from the interaction between p53 and mtHtt [24]

3. Potential treatment

Epidemiological studies have shown that HD has much higher prevalence in western regions (10.6–13.7 individuals per 100,000) compared with that in Asia (1–7 per 1,000,000). This is attributed to genetic distinctions among populations. Data have shown that western ancestry has greater average CAG repeats with 18.4–18.7 in *HTT* genes, and less in Asian ancestry (16.9–17.4) [26].

HDAC inhibitors are considered a group of molecules that can inhibit the enzymatic activity of HDACs. The action of HDAC inhibitors causes an overall increase in histone acetylation levels, and thus activates the transcription of genes that have been previously silenced through histone deacetylation. HDAC inhibitors are commonly used in cancer therapy, as they can compensate the silencing of tumor suppressor genes [18]. These findings, together with the epigenetic mechanism of transcriptional dysregulation in HD, motivates researchers to explore the potential therapeutic effect of HDAC inhibitors in HD [27].

Several recent studies using cellular, drosophila, and mouse models have revealed some promising therapeutic effects of HDAC inhibitors on HD. Steffan *et al.* used a transgenic *Drosophila* model that expresses mutant Htt [28]. These Htt mutant fruit flies showed neuronal degeneration and reduced survival rate, of which both characteristics have also been observed in HD patients. Steffan *et al.* administered HDAC inhibitors including sodium butyrate and suberoylanilide hydroxamic acid (SAHA) to the HD flies, and they observed increased global histone acetylation level, reduced neural degeneration, and increased survival rates in HDAC-treated flies. Hockly *et al.* performed similar experiments using a HD mice model. The results showed that SAHA-treated mice had better motor coordination compared to the placebo group [28].

The therapeutic effect of SAHA has also been studied at the molecular level. Mielcarek *et al.* [28] have also performed experiments that showed a reduction in HDAC4 levels in the brain stem and cortex of HD model mice with the use of SAHA. SAHA has also been proven to reduce mutant Htt aggregation and partially restore the level of brain-derived neurotrophic factor (BDNF), a key protein for cell survival and growth, which is inhibited in HD [29].

Although HDAC inhibitors such as SAHA have shown promising therapeutic effects, significant weight loss has been found to be a potentially dangerous side effect, which hinders the development and clinical applications of SAHA-based HD treatment. Fortunately, Thomas *et al.* have demonstrated that HDAC inhibitor (HDACi) 4b shows excellent therapeutic effects when administered to HD mice [30]. HDACi 4b-treated HD mice displayed improved motor coordination with less weight loss. Furthermore, HDACi 4b also compensated for other negative effects caused by HD. Normally, the overall brain size is reduced in HD mice; however, when HD mice were treated with HDACi 4b, normal-sized brains were observed in these mice. The low toxicity of HDACi 4b makes it a promising candidate for potential HD treatment, which can be used in further phases of clinical trials.

Like most neurodegenerative diseases, HD lacks specific treatment. At present, HD is treated symptomatically. Although there is no effective drug to delay the progress of HD, reasonable drug treatment can be used to improve symptoms, such as chorea and mental disorders, to varying degrees as well as the quality of life of patients, while preventing complications. Therefore, even in the absence of effective treatment at this stage, we should pay attention to symptomatic treatment, which focuses on relieving psychological and neurological symptoms, in addition to the necessary supportive treatment, so that these patients and those who may be ill would be able to build self-confidence, assist one another, and create an optimistic family.

4. Conclusion

This paper describes the lower gene expression in HD patients with higher 5mC level and puts forward the discoveries of alternating 5hmC and 7-methylguanine, and their impact on HD mice models and human samples; the transcriptional dysregulation in HD patients caused by aggregation of CBP as HAT, and the interaction between mutant Htt fragments and neuronal inclusions; as well as the decreasing levels of ncRNAs followed with higher levels of targets like p53, which is the reason for neuronal damage, and REST protein in the nucleus, which can regulate certain miRNAs that are influenced by mtHtt. Htt protein itself interacts with several transcription factors and epigenetic regulators. Therefore, it has been hypothesized that the reversal of epigenetic marks associated with HD may restore, at least partially, the normal transcriptional program and ameliorate the pathological phenotype. Potential treatments like sodium butyrate and SAHA, focusing on HDAC inhibitors, have shown optimistic results through experiments. Using various HD models and next-generation sequencing technologies, researchers have uncovered more insights about the possible causes of transcription dysregulation in HD. Although these studies have shown strong correlation between altered epigenome and HD phenotype, our understanding of the HD epigenome is still far from satisfactory. It is still unclear to us to what extent epigenetic alteration plays a causal role in transcriptional dysregulation.

Currently, only a few epigenetic marks have been analyzed using genome-wide approaches, in which these findings highlight the complexity of HD epigenome. A comprehensive analysis of HD epigenome using genome-wide techniques is crucial to widen our understanding of HD pathology.

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Disclosure statement

The authors declare no conflict of interest.

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