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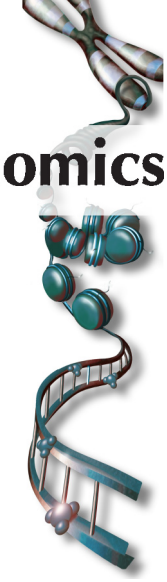
Epigenomics



Epigenetic profiles in polyglutamine disorders

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The dominant polyglutamine (polyQ) disorders are a group of progressive and incurable neurodegenerative disorders, which are caused by unstable expanded CAG trinucleotide repeats in the coding regions of their respective causative genes. The most prevalent polyQ disorders worldwide are Huntington's disease and spinocerebellar ataxia type 3. Epigenetic mechanisms, such as DNA methylation, histone modifications and chromatin remodeling and noncoding RNA regulation, regulate gene expression or genome function. Epigenetic dysregulation has been suggested to play a pivotal role in the pathogenesis of polyQ disorders. Here, we summarize the current knowledge of epigenetic changes present in several representative polyQ disorders and discuss the potentiality of miRNAs as therapeutic targets for the clinic therapy of these disorders.

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Keywords: epigenetic dysregulation • pathogenesis • polyglutamine disorders

Background

Polyglutamine disorders

Polyglutamine (PolyQ) disorders constitute a group of at least nine hereditary neurodegenerative disorders known to date, including Huntington's disease (HD), spinal and bulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, spinocerebellar ataxia (SCA) type 1, 2, 3, 6, 7 and 17 [1–3]. All polyQ disorders are autosomal dominantly inherited disorders except spinal and bulbar muscular atrophy, which is sex dependent. They are caused by unstable expanded CAG trinucleotide repeats in the coding regions of their respective causative genes, leading to mutant proteins with an abnormally long stretch of repeated glutamines (Table 1).

The most common type of polyQ disorders worldwide is HD, with a prevalence of 10.6–13.7/100,000 in the west [4]. It is caused by an expansion of CAG triplets in the *HTT* gene, and is characterized by progressive decline in motor function and cognition and the development of psychiatric symptoms. The SCA family comprises more than 35 subtypes of progressive neurodegenerative disorders so far, 6 of which are polyQ disorders. The worldwide prevalence of all SCAs combined is around 4/100,000, with geographical variability in some subtypes. SCA3, also known as Machado–Joseph disease (MJD), accounts for 15–45% of dominantly inherited ataxia in different countries and ethnic populations. Thus, although SCA3 is relatively rare globally, with a prevalence of 1–2/100,000, it is presently considered the most common subtype of SCA worldwide [5,6]. It is caused by a polyQ-coding expansion in the *ATXN3* gene, and is primarily characterized by neuronal dysfunction and degeneration in the cerebellum and functionally related brain regions. There are no effective treatments for these progressive and fatal disorders yet.

These polyQ disorders share many clinical and pathological features. One classic histopathological hallmark of all polyQ disorders is the insoluble intracellular aggregates or inclusion bodies formed by the various expanded polyQ-encoding proteins in different regions of the neuronal tissue, and the nucleus is thought to be the principal site of the majority polyQ disorder pathogenesis with the exception of SCA2 and SCA6 [7,8]. Intriguingly, the polyQ-containing proteins associated with each different disorder are expressed throughout the body, while the

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Table 1. Polyglutamine disorders summary.

PolyQ disorders	Prevalence	Gene	Chromosome location	Protein (length, MW)	PolyQ expansion		Typical onset (years)	Affected brain areas	Main clinical symptoms
					Normal	Pathological			
HD	5-10/100,000	*HTT*	4p16.3	Huntingtin (3142 aa, 348 kDa)	6–35	36–121	Third to fifth decades	Striatum, cortex, thalamus and subthalamic nucleus	Chorea, dystonia, hypometric saccades and catchy pursuit, disrupted fine motor movements, dysphagia, dysarthria, dysdiadokinesis, rigidity, ataxia, cachexia, progressive dementia and psychiatric symptoms
SBMA	1-2/100,000	*AR*	Xq11-q12	AR (920 aa, 100 kDa)	6–36	38–62	30–60	Lower motor neurons in the anterior horn, bulbar region and dorsal root ganglia	Slowly progressive muscle weakness and atrophy of bulbar, facial and limb muscles
DRPLA	<1/100,000	*ATN1*	12p13.31	Atrophin-1 (1191 aa, 125 kDa)	3–38	49–88	Vary from early childhood to late adulthood	Purkinje cells, cerebral cortex, globus palidus, striatum, dentate, subthalamic and red nuclei	Ataxia, chorea, myoclonic epilepsy and dementia
SCA1	1-2/100,000	*ATXN1*	6p23	Ataxin-1 (815 aa, 87 kDa)	6–44	49–91	Third or fourth decade	Cerebellar purkinje cells, inferior olive neurons and neurons within brainstem cranial nerve nuclei	Ataxia, dysarthria, ophthalmoparesis, muscle wasting, extrapyramidal and bulbar dysfunction
SCA2	1-2/100,000	*ATXN2*	12q23-24.1	Ataxin-2 (1313 aa, 140 kDa)	14–32	33–200	Third decade	Purkinje and granule neurons	Progressive gait and limb ataxia, dysarthria, tremor, nystagmus, slow saccadic eye movement and supranuclear ophtalmoplegia
SCA3	1-2/100,000	*ATXN3*	14q32.1	Ataxin-3 (364 aa, 42 kDa)	12–44	50–89	20–50	Cerebellum and brainstem, basal ganglia, thalamus, substantia nigra and spinal cord	Cerebellar ataxia, progressive external ophthalmoplegia, dysarthria, dysphagia, pyramidal signs, dystonia, rigidity and distal muscle atrophies, weight loss and restless legs syndrome
SCA6	<1/100,000	*CACNA1A*	19p13	CACNA1A (2505 aa, 282 kDa)	3–18	21–33	19–71	Cerebellar purkinje cells	Gait unsteadiness, stumbling and imbalance
SCA7	<1/100,000	*ATXN7*	3p21-p12	Ataxin-7 (892 aa, 95 kDa)	4–35	36–460	Third or fourth decade	Cerebellar cortex, deep cerebellar nuclei, inferior olive and anterior horns of the spinal cord, as well as axonal loss in spinocerebellar tracts	Cerebellar ataxia, uncoordinated movement, abnormal gait, dysarthria and dysphagia, degeneration of the retinal macula
SCA17	unknown	*TBP*	6q27	TBP (338 aa, 38 kDa)	25–44	45–63	Middle age	Small neurons in the caudate and putamen, purkinje cells and frontal and temporal cortex	Ataxia, dystonia and parkinsonism, dementia, psychiatric abnormalities and seizures

AR: Androgen receptor; CACNA1A: Voltage-dependent P/Q-type calcium channel subunit alpha-1A; DRPLA: Dentatorubral-pallidoluysian atrophy; HD: Huntington's disease; MW: Molecular weight; PolyQ: Polyglutamine; SBMA: Spinal and bulbar muscular atrophy; SCA: Spinocerebellar ataxia; TBP: TATA-binding protein.

pathology is primarily restricted to neuronal tissue. These disorders further share a negative correlation between the polyQ expansion length and the age of disease onset [7,9,10]. Transcriptional dysregulation and defects in the ubiquitin proteosome system are other important common aspects to all of these disorders [11]. Moreover, many of the polyQ-encoding proteins have been shown to share interacting partners. For example, both Huntingtin (Htt) and ataxin-3 interact with p53, CBP and p300 [12–14]. Currently, the precise pathogenic mechanism in polyQ disorder patients remains elusive [1,2,12,15–17].

The shared features of these disorders indicate similarities in disease mechanisms relating to the expanded polyQ. As no cases of polyQ diseases with deletions or point mutations in their causative genes have been reported, the polyQ expansion is thought to confer a toxic gain-of-function to the affected proteins and trigger a pathogenic cascade, leading to distinct patterns of neuronal loss and clinical manifestation. The involvement of the polyQ expansion in the mechanism leading to the disease includes induction of conformational transition of the host protein, alteration of the normal protein function, generation of toxic polyQ-containing fragments, transcriptional disturbances, proteotoxic stress and mitochondrial dysfunction [3,18]. However, it is noteworthy that the polyQ tract within a protein is not always deleterious. For example, aggresomes formed by expanded polyQ protein, which is different from the small aggregates or oligomers formed by the self-association of the proteins, protect cells by enhancing the degradation of toxic polyQ-containing proteins [19,20]. The aggregates or intranuclear inclusions formed by misfolded and expanded disease proteins have been reported to be dissociated from the pathogenic process in HD and SCA1 [3,21–23].

Epigenetic mechanisms

Epigenetics is commonly defined as stable and heritable changes in gene expression or genome function without changes in the genotype. Modification of epigenetic processes, which can be influenced by a number of internal and external environmental risk factors, may alter chromosomal stability and gene expression and affect the phenotype of an organism. The role of epigenetics in human diseases is first recognized in oncology, but in the last decade the contribution of epigenetic modifications to neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD) and HD, has also been extensively investigated. Primary epigenetic mechanisms include DNA methylation and hydroxymethylation, histone modifications and chromatin remodeling and more recently noncoding RNA regulation [24].

DNA methylation & hydroxymethylation

DNA methylation is one of the most studied and best characterized epigenetic modifications. DNA methylation in mammals, catalyzed by DNA methyltransferases (DNMTs) using 5-adenosylmethionine (SAM) as the methyl donor [25], mainly occurs on the cytosine nucleotide in a CpG site, forming 5′-methylcytosine (5-mC) [26,27]. There are also reports of CpH methylation as well as guanine and adenine methylation [28,29]. How DNA methylation affects gene transcription is highly dependent on the location in or around the gene [30]. DNA methylation in promoter regions generally acts to repress gene transcription [25], while DNA methylation within gene bodies is reported to be associated with splicing-related regulation at introns [31,32]. 5-hydroxymethylcytosine (5-hmC) [33–37] and 5-formylmethylcytosine, two intermediate states of the DNA demethylation, are thought to act as newly functional epigenetic markers. They are present in high prevalence in the brain (~8% for 5-hmC and ~0.8% for 5-formylmethylcytosine, in contrast to ~80% for 5-mC), associating with relief of transcriptional silencing [38,39].

Histone modifications & chromatin remodeling

Gene expression is generally regulated by the binding of transcriptional coregulators and the chromatin structure alterations, with the latter being primarily regulated by histone modifications. Nucleosome, the fundamental unit of chromatin, is a histone octamer consisting of two molecules of each core histone (H) H2A, H2B, H3 and H4 around which 147 bp of DNA is wrapped. The histone tail, which is the flexible N-terminals of histones protruding from the nucleosome, is subjected to numerous post-translational modifications on multiple residues (known as the so called 'histone code'), such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, carbonylation, glycosylation, biotinylation and hydroxylation, among which lysine acetylation and lysine methylations are particularly important [40]. The histone code plays important roles in gene expression regulation by altering chromatin dynamics and influencing histone-DNA interactions, as well as recruiting regulatory proteins and enzymes to chromatin.

Acetylation of histones, catalyzed by histone acetyltransferases (HATs), is generally believed to lead to the decondensation of the chromatin and promote transcriptional activity, whereas histone deacetylases (HDACs) exert the opposite effect [41]. In addition to remodeling chromatin, acetylated-lysine residues may act as transcriptional activators, thus indirectly accelerating transcriptional initiation.

In contrast to histone acetylation, histone methylation, which is regulated by the counteracting activity of histone-methyltransferases and histone-demethylases, appears to be more specific. Lysine residues of histone proteins are able to be mono-, di- or tri-methylated by specific enzymes while arginine is able to be mono- or di-methylated.

Histone methylation can either increase or decrease the gene expression, depending on which amino acids are methylated and the number of methyl groups that are added to these residues. For example, hypermethylation of histone H3K9 and H4K20 residues frequently represses transcription while di- and tri-methylation of H3K4, H3K36 and H3K79 are generally correlated with active chromatin [42]. The interplay between different histone modifiers determines chromatin structure and function. In addition, chromatin remodelers, including SWI/SNF, ISWI, CHD and INO80 family, can regulate the chromatin accessibility by modifying the presence, composition and nucleosome positioning [43].

Noncoding RNAs

The best characterized group of ncRNAs is miRNAs. miRNAs are small, 18–22 nt long ncRNAs that represent a major system of post-transcriptional regulation. They have been shown to regulate gene expression by promoting either degradation or translational repression of target mRNAs [44]. The expression of miRNAs varies with developmental processes, suggesting their involvement in various developmental processes such as cell fate determination, cell division and programmed cell death. In mammals, the majority of miRNAs are expressed abundantly in the CNS in specific spatial and/or temporal patterns, suggesting their potential roles in neurodevelopment, nervous system morphogenesis, synaptic plasticity and neurodegeneration.

Epigenetic crosstalk

Proteins that regulate DNA methylation are associated with proteins that regulate histone modifications, thus linking these two epigenetic processes. For example, MeCP2 is associated with HDACs, thus linking DNA methylation and histone deacetylation [45]. Generally, DNA methylation could exert great impact on interaction between histone and DNA, changing chromosome structure and gene expression [46]. In addition, DNA methylation and histone modification can regulate miRNA expression and vice versa. For example, treatment by DNA demethylation agent and/or HDAC inhibitors causes miRNA expression changes in cells [47,48], and enzymes involved in DNA methylation and histone modification, such as members of DNMTs [49,50] and DNA methyl-CpG-binding proteins (MBDs) as well as HDACs, are targeted by miRNAs [51,52]. For a more comprehensive view of different epigenetic marks crosstalk see [53].

Epigenetic alterations in polyQ disorders

HD, as well as other polyQ diseases, is a single gene disorder. However, there is enormous variability in disease at onset and severity, suggesting that other genetic and/or environmental factors may influence disease phenotypes. Accumulating evidences show that the alteration of epigenetic processes, such as DNA methylation and post-translational modifications of histone proteins, is linked pathologically with polyQ disorders in many aspects, such as modification of the disease progression and regulation of the instability of CAG repeats expansion, thus providing an epigenetic mechanism of gene regulation in these disorders. In this review, we first focus on the roles of DNA methylation and histone modifications in the pathological process of several polyQ disorders. We then summarize recent findings that highlight the association of miRNAs with polyQ disorders and discuss the potential therapeutic applications of miRNAs as targets to treat polyQ disorders, as well as their utilization as biomarkers.

Methylation-based epigenetic regulation in polyQ disorders

Alteration of methylation in HD

Mounting evidence in the literature supports the notion that aberrant DNA methylation is potentially linked with HD-related transcriptional dysregulation and neuronal dysfunction, as summarized below [54].

Global DNA (de)methylation changes in HD

Global levels of DNA methylation have been studied in cell lines and different mouse models of HD as well as HD patient samples. An immortalized mouse striatal cell line carrying polyQ-expanded HTT (STHdhQ111/Q111) shows a general tendency toward hypomethylation as measured by reduced representation bisulfite sequencing [55]. Similar results have been obtained in fibroblasts from HD patients [56]. In human HD brain and animal models, global levels of 7-methylguanosine are significantly reduced in the motor cortex [28]. A previous study from our lab reports the genome-wide DNA hydroxymethylation in HD mouse brain, with global reduced 5-hmC being observed in the striatum and cortex of YAC128 transgenic mice [57]. These studies indicate that HD is generally associated with lower levels of DNA methylation independently of the sample used. Dysregulation of

other methylated marks, such as 7-methylguanosine and 5-hmC, may also contribute to the known transcriptional pathology of HD.

Gene-specific DNA methylation changes in HD

Gene-specific DNA methylation changes have focused on genes related to HD [56,58]. For example, adenosine A2A receptor (A2AR) gene (*ADORA2A*) is a gene that shows severely decreased expression in HD patients (7). An increase in 5-mC levels and a reduction in 5-hmC levels in the striatal 5′UTR of *ADORA2A* of HD patients have been observed, with both mechanisms being related to the pathological decrease of *ADORA2A* in HD [58]. However, this differential methylation of the *ADORA2A* gene may be driven by different cell-type proportions in brain instead of disease status [59]. Another example is *hairy and enhancer of split 4* (*HES4*), a notch signaling gene which is recently suggested to be linked with HD pathogenesis. Hypermethylation of the *HES4* promoter has been observed in HD cortical neurons [60]. Therefore, these studies show that some HD-associated genes are epigenetically dysregulated at the DNA methylation level in HD, but they require further validations using cell-type-specific studies.

DNA methylation changes at the HTT locus

There are also studies to investigate how the HD mutation impact local DNA methylation patterns at the *HTT* locus itself [59,61,62]. A more than 28-fold of variability of methylation in DNA from peripheral blood has been detected at a locus (D4S95) closely linked to the *HTT* gene [61]. No HD-associated DNA methylation changes at the *HTT* locus has been detected in HD cortex samples [59], and 38 sites within the *HTT* gene locus are differentially methylated between matched cortex and liver samples [59]. Therefore, no conclusive evidences have emerged from these studies. However, it should be noted that due to technique limitations, these studies fail to assess DNA methylation levels within the CAG tract of mutant *HTT* loci directly.

Alteration of DNA methylation-related gene expression in HD

Recent studies have also explored the possible causes for DNA methylation difference observed in HD. As mentioned above, DNA methylation in mammals is mainly catalyzed by DNMTs. Decreased expression of DNMT genes has been found in HD models by independent groups [55,63–65]. Moreover, several other DNA (de)methylation-related genes, such as *Gadd45a* [56,65], *Gadd45g* [55] and *Rnf4* [56,63], have also been found to be differentially expressed in HD models. These studies would partly explain the correspondingly lowered levels of DNA methylation in HD.

Alteration of methylation in SCAs

Two large CpG islands are found in the promoter region (from -1089 to +1) of the *ATXN3* gene [66], with the methylation levels in the first CpG island being significantly increased in SCA3/MJD patients and the second CpG island being hypomethylated in both patients and controls, suggesting an essential role of the first but not the second island DNA methylation in the SCA3 pathogenesis [67]. Genome-wide methylation analysis shows that *ATXN2* is significantly methylated in a case series of coronary artery disease [68]. The *ATXN2* promoter contains a CpG island rich segment without a TATA box. Both hyper- and hypomethylation states are found in the *ATXN2* promoter in SCA2 patients [69,70]. In SCA1, local DNA methylation patterns have been detected at *ATXN1* locus. Dnmt1 deficiency leads to opposite effects on local Sca1 DNA methylation in testes and ovaries of Dmnt1+/-SCA1 mice, with elevated levels in testes and reduced levels in ovaries using a mouse model for SCA1 [71]. In the case of SCA7, it is reported that CpG methylation of CTCF-binding site adjacent to the expanded CAG tracts of *ATXN7* could enhance triplet repeat instability [72].

Taken together with data from these polyQ disease models, although the changes of DNA methylation or hydroxymethylation are relatively small in these studies, and the interpretation of these results can be influenced by many factors, such as the sample size, tissue difference, data analysis method and different measure techniques, they illustrate that aberrant DNA methylation is a key feature in the transcriptional dysregulation observed in polyQ diseases, which may, at least in part, contribute to altered gene expression and neuronal dysfunction, thus playing an important role in the transcriptional pathology of these diseases. Future studies aiming at assessing specific forms of DNA methylation using cell-type-specific studies will advance our understanding of the role of DNA methylation in the pathogenesis of these diseases.

Histone modifications & chromatin remodeling in polyQ disorders

Histone modifications, such as hypo/hyperacetylation and hypermethylation, have been identified in cell models of HD, HD animal models and patients [64,73–76]. Global levels of histone acetylation are reduced in HD models [76,77] and in HD patients [78–81], although hyperacetylation at certain gene loci is shown to be increased [82,83]. Different truncated Htt with an expanded polyQ domain has been found to inhibit the HAT function and reduce histone acetylation in cell culture by independent groups [76,84]. Similarly, in a transgenic SCA3 mouse model, mutant ataxin-3 impairs HAT activity, leading to hypoacetylation of H3 or H4 histone and further the transcriptional repression of cerebellar genes required for long-term depression (LTD) [85,86]. A reduction in acetylation induced by expanded polyQ proteins is also observed in yeast and in a cell model of Kennedy's disease [76]. Therefore, these studies strongly implicate that histone hypoacetylation caused by a reduced HAT activity may be an important component of polyQ pathogenesis, and HDAC inhibitors may represent a relevant therapeutic strategy for polyQ diseases.

The wild-type Htt protein interacts with various histone modifiers such as CBP, TBP, p300 and REST/NRSF [87,88]. The abnormal histone acetylation in HD is related to the loss of CBP, a transcriptional cofactor with HAT activity. The stronger binding of CBP with mHtt leads to its sequestration in mHtt-induced inclusions and to the hypermethylation and hypoacetylation of histones, and the subsequent neuronal transcriptional dysfunction in brains of HD mice [74,77,82,89–92]. Moreover, a loss of CBP function results in an increase in H3K9me3, which is linked to upstream transcriptional dysregulation [79,89,93]. In agreement, upregulation of CBP rescues HD phenotypes in a *Drosophila* model of HD, which is associated with recovery of histone acetylation and normalization of the transcription profile [94]. However, partial deletion of CBP in HD transgenic mice fails to affect the global levels of histone H3 or H4 acetylation in the brain [95]. Further investigation is required to study the role of CBP in HD.

Additionally, mHtt can alter histone methylation [89,90,93] and ubiquitination [96–98]. For example, increased levels of ubiquitinated H2A has been observed in a cell model of HD and in HD mice, which is due to a disruption of the interaction between mHtt and Bmi1, a component of the hPRC1L E3 ubiquitin ligase complex [97].

Similar to Htt, some polyQ-encoding proteins in SCAs can also interact with various histone modifiers, thus linking them directly with chromatin regulation. For example, ataxin-1 interacts with Tip60, which is involved in transcriptional activation by acetylating H4 and H2A [99], whereas ataxin-3 can interact with CBP, p300, HDAC6 and PCAF [100–102]. SCA7 is caused by polyQ expansion of the ataxin-7, which is a component of the mammalian SAGA and SLIK HAT complexes [103]. Mutation of ataxin-7 leads to the disruption of the structural integrity of the SAGA complex and aberrant chromatin acetylation patterns at the promoters of genes [104].

Taken together, these studies implicate the important roles of histone modification and chromatin remodeling in the pathogenesis of polyQ diseases as well as in the regulation of the polyQ-encoding protein function, and chromatin-directed compounds such as HDAC inhibitors could be developed as potential drugs for polyQ diseases in the future.

Dysregulation of miRNA in the pathophysiology of polyQ disorders

miRNA dysregulation is emerging as a critical factor contributing to neurodegeneration [105–112]. Major alterations in brain mRNA levels have been observed in various neurodegenerative disorders, and as mentioned above, altered neuronal transcriptional activity is a persistent feature of most polyQ disorders. More and more miRNAs have been identified to play potential roles in polyQ disorders, either by post-transcriptionally regulating the expression of polyQ disease causing proteins in mammals or by modulating toxicity through miRNA-mediated mechanisms. Bilen *et al.* provided the first evidence that the miRNA pathways play protective roles in the polyQ neurodegeneration in both flies and human cells [113,114]. They have identified the anti-apoptotic miRNA *bantam (ban)* to prevent degeneration caused by the mutant ataxin-3 in *Drosophila* by modulating polyQ toxicity [113,114]. This finding has been supported by other studies [115,116]. For example, Liu *et al.* report that upregulation of a markedly conserved miRNA, miR-34, dramatically mitigate neurodegeneration induced by polyQ expanded mutant ataxin-3 in flies, thus supporting the protective role of miRNAs in SCA3 [117]. In the section below, we will discuss several brain-enriched miRNAs as well as circulating miRNAs (cmiRNAs) implicated in HD and other polyQ disorders.

Brain-enriched miRNAs dysregulation in polyQ disorders

Many transcription factors have been reported to interact with Htt and are recruited to the mHtt aggregates in the brain. Importantly, mHtt can interact with Ago1 and Ago2, which are involved in miRNA biogenesis, resulting in the inhibition of the formation of processing bodies [118]. Therefore, miRNA dysregulation is expected in HD. Widespread neuronal-specific miRNA dysregulation has been detected in the brains of HD animal models and HD patients [119–121], with some miRNAs being downregulated, such as miR-29b, miR-125b, miR-146a and miR-150 [122–124], and some miRNAs being significantly upregulated, such as miR-10b-5p, miR-196a-5p, miR-196b-5p, miR-200a and miR-200c (Table 2) [124–127]. However, it should be noted that these studies show considerable heterogeneity between different species of disease samples, and only a small fraction of miRNAs have been confirmed by different groups. Some of the studies appear compelling. For example, miR-132, a brain-enriched miRNA, is first found significantly depressed in both human and mouse HD samples [128,129], which has been confirmed later by Lee *et al.* [130] in two different HD mouse models. However, Packer *et al.* observe no change in miR-132 at stages 1 and 2, but significant upregulation later in human brain samples of HD grades 1–4 [124]. These seemingly contradictory results are probably because different miRNAs (precursor vs mature miRNA) are tested.

miRNA dysregulation in HD has been reported to be partly due to aberrant increased expression and the nuclear localization of REST [129]. REST and CoREST, two components of the REST complex, are known to suppress the expression of neuronal genes in non-neuronal cells. In healthy neurons, REST is primarily sequestered in the cytoplasm partly through binding to Htt [131]. However, in HD patients, the polyQ expansion abrogates REST-Htt binding, enabling its nuclear translocation, occupies RE1 repressor sequences and decreases neuronal gene expression such as BDNF [132,133]. Therefore, REST is thought to be one of the downstream effectors of HTT in HD pathology. A set of REST-target miRNAs including miR-29a, miR-124a, miR-132 and miR-330 have been found to be decreased in a mouse model of HD, among which only miR-132 downregulation has been confirmed in human samples [129].

Similar to the case in HD, alteration of numerous mRNA transcripts as well as several miRNA levels have been observed in brains of SCA sufferers as well as animal models [134,135]. Interestingly, ataxin-2, the protein involved in SCA2 disease, might be required for miRNA function, as lack of ataxin-2 impairs the repressive activity of several miRNAs [136]. In SCA3, miR-181a and miR-494, which interact with the *ATXN3*–3′-UTR, are found dysregulated in human MJD neurons as well as other MJD cell and animal models, and overexpression of these miRNAs alleviated MJD neuropathology *in vivo* [137]. In SCA17, downregulation of miR-29a and miR-29b is observed in a cellular model of SCA17, which was inversely correlated with BACE1 expression [138]. In addition, TBP gene was reported to be one of the targets of miR-146a, which was downregulated in cell and animal models of HD [122]. Studies of the dysregulation of miRNA expression in different SCA1 animal models as well as SCA1 patients show that most of miRNAs were downregulated, and only a small fraction of miRNAs were upregulated, such as miR-19a, miR-101, miR-130a, miR-144 and miR-150 [139–141].

It is important to mention that some miRNAs have been found to be involved in more than one neurodegenerative disease conditions. Here, we take miR-9 for example. miR-9 is one of the most abundant miRNAs in the brain, which has RE1 binding sites, and is a direct target of REST. Levels of mature miR-9/miR-9* are decreased in cell models of HD and HD mouse models as well as HD patients [122,124,128]. Decreased miR-9/9* in HD would increase REST transcription, amplifying its accumulation in the presence of mHtt. This phenomenon is further magnified because miR-9/9* transcription depends on REST. Aberrant expression of miR-9 has also been observed in the cerebella of mouse model of SCA3 [137] and SCA1 [140], being downregulated in SCA3 and upregulated in SCA1. The miR-9 dysregulation in SCA3 is proposed to be related with an impairment in miRNA biogenesis. In contrast, both upregulation [142,143] and downregulation [144,145] of miR-9 have also been reported in AD models. These dysregulations are thought to be associated with one of its target BACE1 as well as Aβ accumulation [146].

Another miRNA, miR-29a, has also been found to be dysregulated in three polyQ disorders, including HD [129], SCA3 [147] and SCA17 [138], due in large part, to the disrupted miRNA transcriptome. miR-29a has also been found to be decreased in AD patients and AD mice models [144,145], which is correlated with the increase of BACE1, a confirmed target of miR-29.

Taken together, the involvement of miRNA dysregulation in different neurodegenerative diseases, either causally or as part of positive feedback loops, suggests that each miRNA can regulate numerous targets and play different roles in the brain with different mechanisms.

Table 2. Aberrant expression of miRNAs in polyglutamine disorders.

PolyQ disorders	miRNAs	Changes	Sources	Species	Ref.
HD	miR-9/9*, miR-124, miR-132	Downregulated	Cortex	Human and mice	[128]
	miR-29a, miR-124a, miR-132, miR-135b, miR-204	Downregulated	Cortex and/or hippocampus	Mice	[129]
	miR-132	Downregulated	Cortices	Human	
	miR-29a, miR-330	Upregulated			
	miR-9/9*, mir-29b, miR-124a, miR-132	Downregulated	Cortex (Brodmann's area 4)	Human	[124]
	miR-128	Upregulated			
	miR-22, miR-29c, miR-128, miR-132, miR-138, miR-218, miR-222, miR-344, miR-674*, miR-28*, miR-466h	Downregulated	Striata	Mice	[130]
	miR-34b-3p, miR-207, miR-448, miR-669c, miR-18a*	Upregulated	Striata	Mice	
	miR-183, miR-96	Downregulated	Striata	Rat	
	miR-200a, miR-200b, miR-429	Upregulated	Striata	Rat	
	miR-9/9*, miR-100, miR-125b, miR-135a, miR-135b, miR-138, miR-146a, miR-150, miR-181c, miR-190, miR-218, miR-221, miR-222, miR-338-3p	Downregulated	ST*Hdh*^{Q111}/*Hdh*^{Q111} *cells*	Mice	[122,123,165]
	miR-145, miR-199-5p, miR-199-3p, miR-148a, miR-127-3p, miR-200a, miR-205, miR-214, miR-335-5p, miR-299-5p, miR-323-3p, miR-154	Upregulated			
	miR-185, miR-194, miR-128a, miR-33a, miR-320, miR-17-3p, miR-181c, miR-220b, miR-940	Downregulated	Frontal cortex	Monkey	[121]
	miR-451, miR-133c	Upregulated			
	miR-124, miR-127-3p, miR-128, miR-139-3p, miR-181d, miR-221, miR-222, miR-382, miR-383, miR-409-5p, miR-432, miR-433, miR-485-3p, miR-485-5p, miR-95	Downregulated	Frontal cortex and the striatum	Human	[120]
	miR-100, miR-106b, miR-148b, miR-151-3p, miR-151-5p, miR-15b, miR-16, miR-17, miR-193b, miR-19b, miR-20a, miR-219-2-3p, miR-219-5p, miR-27b, miR-33b, miR-363, miR-451, miR-486-5p, miR-887, miR-92a	Upregulated			
	miR-432, miR-146a, miR-19a	Downregulated	ST*Hdh*^{Q111}/*Hdh*^{Q111} *cells*	Mice	[166]
	miR-10b-5p, miR-196a-5p, miR-196b-5p, miR-615-3p, miR-10b-3p, miR-1247-5p	Upregulated	Prefrontal cortex (Brodmann's area 9)	Human	[125,126,150]
	miR-674-5p, miR-221, miR-24*, miR-693, miR-674-3p, miR-34a, miR-711, miR-143, miR-138, miR-222, miR-326,miR-216a, miR-221, miR-448, miR-199b	Downregulated	Cerebral cortex	Mice	[127]
	miR-141, miR-182, miR-429, miR-200c, miR-183, miR-200a, miR-96, miR-152, miR-190, miR-496, miR-181d, miR-805, miR-369-3p, miR-384, miR-361, miR-135a, miR-136, miR335, miR-706, miR-365, miR-744, miR-703, miR-694, miR-761	Upregulated			
	miR-10b-5p, miR-486-5p	Upregulated	Plasma	Human	[148]
	miR-34b	Upregulated	Plasma	Human	[149]
	miR-877-5p, miR-223-3p, miR-223-5p, miR-30d-5p, miR-128, miR-22-5p, miR-222-3p, miR-338-3p, miR-130b-3p, miR-425-5p, miR-628-3p, miR-361-5p, miR-942	Upregulated	Plasma	Human	[151]
SCA3	miR-25, miR-125b, miR-29a	Downregulated	Serum	Human	[147,167]
	miR-34b	Upregulated			
	miR-33-5p, miR-92a, miR-100-5p	Upregulated	Head	*Drosophila*	[168]
	miR-1-3p	Downregulated			
	miR-9, miR-181a, miR-494	Downregulated	Cerebella	Mice	[137]
SCA1	miR-144, miR-101, miR-130a, miR-19a, miR-302	Upregulated	Cortex	Human	[139,141]
	miR-381, miR-203, miR-34c, miR-489, miR-224, miR-484, miR-329, miR-133b, miR-423, miR-138, miR-487b, miR-206	Downregulated	Cerebella	Mice	[140]
	miR-22, miR-125b, miR-194, miR-24, miR-30c, miR-16, miR-191, miR-143, miR-376b, miR-376a, miR-26a, miR-218, miR-195, miR-361, miR-150, miR-100, miR-7, miR-146b, miR-335, miR-26b, miR-96, miR-379, miR-9*, miR-30b, miR-126-3p, miR-128b, miR-9, miR-31, miR-30d, miR-23a, miR-27a, miR-350, miR-129-3p, miR-99a	Upregulated			

HD: Huntington's disease; PolyQ: Polyglutamine; SCA: Spinocerebellar ataxia.

Table 2. Aberrant expression of miRNAs in polyglutamine disorders (cont.).

PolyQ disorders	miRNAs	Changes	Sources	Species	Ref.
	miR-33-5p, miR-92a-5p, miR-34-5p	Upregulated	Head	*Drosophila*	[168]
SCA7	miR-375-3p	Downregulated	Head	*Drosophila*	[168]
	miR-33-5p, miR-92a-5p	Upregulated			
SCA17	miR-29a, miR-29b	Downregulated	Cell model		[138]

HD: Huntington's disease; PolyQ: Polyglutamine; SCA: Spinocerebellar ataxia.

cmiRNAs dysregulation in polyQ disorders

Outside of the CNS, significant alterations in miRNA expression have also been detected in extracellular fluids in HD and other polyQ disorders [148–151]. For example, miR-10b-5p [148,150] and miR-34b [149] were shown to be significantly elevated in the plasma of HD patients and HD asymptomatic gene carriers, respectively. Most recently, cmiRNAs analysis of plasma samples from 15 HD symptomatic patients shows that 168 cmiRNAs are altered. Most of upregulated miRNAs in HD patients are involved in metabolism regulation [151]. In SCA3, four miRNAs, including miR-25, miR-125b, miR-29a and miR-34b, are dysregulated in the serums of SCA3/MJD patients, with the first three being decreased and the last being dramatically elevated [147]. These results indicate that polyQ patients show markedly altered cmiRNA expression pattern, although the levels of some of the dysregulated miRNAs may be different in brain and plasma. These findings implicate that serum miRNAs, due to their stability and specificity, have the potential to be useful biomarkers for these disorders, thus being clinically beneficial for the diagnosis of polyQ patients.

Taken together, these studies provide evidences for a direct or indirect role of miRNAs in the pathogenesis of polyQ disorders and open the possibility for miRNA-based diagnosis, prognosis and therapeutic developments. However, it is quite possible that not all the identified miRNAs are directly associated with neurodegeneration in polyQ diseases although they are dysregulated. The precise and detailed roles of individual miRNAs demand further studies in future.

Potential therapeutic roles of miRNAs to treat polyQ disorders

The gene regulation role of miRNAs suggests a potential therapeutic approach of post-transcriptional silencing that targets the underlying disease etiology rather than the downstream pathological consequences. Besides, as mentioned above, certain miRNAs target multiple genes related to the diseases in a pathway simultaneously, making them more effective mediators. Therefore, those miRNAs whose expression are dysregulated and are directly associated with neurodegeneration can be biomarkers for the diagnosis and prognostication of these diseases. cmiRNAs, due to their high stability and specificity, may potentially serve as novel noninvasive biomarkers for neurological diseases. Moreover, some miRNAs are common in more than one polyQ disease conditions, making them global targets for novel therapeutic strategies.

Two main miRNA-based therapeutic strategies have been developed *in vivo*, which are replacing these miRNAs with their synthetic mimics and blocking the miRNAs of interest by anti-miRNA molecules. Modified oligonucleotides as well as peptide nucleic acids oligonucleotides and morpholinos (mainly used in zebrafish system) have been shown to be effective inhibitors of miRNA activity (for reviews, see [152]). In recent years, synthetic sponge miRNAs, which have complementary binding sites to a miRNA of interest, have been developed to inactivate the target miRNAs [153].

Most cases of AD and PD, the most prevalent neurodegenerative diseases worldwide, are sporadic, meaning no significant associations with certain genes are detected in these cases. However, progress has been made in RNA-based silencing of targets linked to common sporadic forms of AD and PD despite of the difficulty of defining the appropriate therapeutic targets. For instance, the α-synuclein pathway has been successfully targeted in PD [154]. In contrast to AD and PD, each of the polyQ disorder is caused by a defined genetic mutation. Therefore, it is therapeutically possible that reducing the mutant gene expression may slow or even prevent disease progression. Most of the progress in the development of RNA-based therapies has been made in these hereditary neurodegenerative diseases. We specifically highlight the implications of miRNA mimics in polyQ disorders in this review.

miRNA mimics have been applied to silence the corresponding mutant genes in different polyQ disorder models, either non-allele specifically or allele specifically [40,155–158]. For example, viral-mediated expression of mi2.4, an artificial miRNA silencing of both mutant and wild-type *HTT* mRNAs, has been shown to improve HD-related behavioral abnormalities dramatically in HD-N171–82Q mice [157,158]. miR-196a is previously identified as a possible candidate for involvement in the pathogenesis of HD. A double transgenic mouse carrying mutant *HTT* and miR-196a reveals the suppression of mutant HTT in the brain and also shows improvements in neuropathological progression [159]. Reducing expression of mutant ataxin-1 using virally delivered artificial miRNAs improves disease phenotypes in SCA1 transgenic mice [160] and knock-in mice [161]. Artificial anti-*ATXN3* miRNA mimics based on the human miRNA-124 primary sequence have been shown to reduce mutant *ATXN3* expression, enhance the clearance of mutant ataxin-3 and partially restore specific miRNA steady-state levels in SCA3/MJD84.2 mice [162]. However, long-term expression of this miRNA mimic fails to alter the progression of motor deficits significantly in homozygote SCA3/84.2 mice [163]. Although miRNA-based therapeutic applications for polyQ disorders are promising, several hurdles should be overcome in the future, such as the mutant allele selectivity, stability, target specificity, efficacy and safety, as well as optimization of the synthetic and delivery techniques. Among these challenges, off-target effects of delivering miRNA targets are particularly important, as they may cause severe unanticipated responses. This is because, as summarized in this review, some miRNAs, such as miR-9 and miR-29a, are involved in more than one polyQ disease conditions. Moreover, these miRNAs have many different downstream targets, but only minor of them have been identified.

Future perspective

The importance of epigenetic variation in the neuronal development and activity is increasingly recognized [164]. A growing body of evidence reveals that epigenetic modifications contribute to the pathology of different kinds of polyQ disorders. However, to date, the extent to which epigenetic modification may modulate the onset, progression or severity of a specific polyQ disorder remains largely unknown. Here, we summarize epigenetic changes in several representative polyQ disorders. There remain a number of future challenges that need to be addressed before we can fully understand the mechanisms and consequences of the epigenetic regulation in the neuronal development and activity. There is a need to identify the specific DNA methylation maintenance factors that are involved in each polyQ disorder in the future. How miRNAs exactly mediate the pathological mechanisms in polyQ disorders also await further investigation. In addition, it is crucial to understand to what extent these epigenetic variation contribute to the pathogenesis of polyQ disorders.

Executive summary

Epigenetic alterations in polyglutamine disorders

- Aberrant DNA methylation is a key feature in the transcriptional dysregulation observed in polyglutamine (polyQ) diseases.
- Histone modification and chromatin remodeling are involved in the pathogenesis of polyQ diseases as well as in the regulation of the polyQ-encoding protein function, and chromatin-directed compounds such as histone deacetylase inhibitors could be developed as potential drugs for polyQ diseases in the future.
- Dysregulation of miRNAs is involved in different neurodegenerative disease processes, either causally or as part of positive feedback loops.

Potential therapeutic roles of miRNAs to treat polyQ disorders

- Diagnosis and prognosis: circulating miRNAs, whose expression are dysregulated and are directly associated with neurodegeneration, may potentially serve as novel noninvasive biomarkers for polyQ diseases.
- Treatment: replacing miRNAs with their synthetic mimics as a miRNA-based therapeutic application for polyQ disorders is promising.

Future perspective

- There is a need to identify the specific DNA methylation maintenance factors that are involved in each polyQ disorder in the future.
- How miRNAs exactly mediate the pathological mechanisms in polyQ disorders await further investigation.
- It is crucial to understand to what extent these epigenetic variation contribute to the pathogenesis of polyQ disorders.

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