Current understanding on the pathogenesis of polyglutamine diseases

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Abstract: Polyglutamine (polyQ) diseases are a family of neurodegenerative disorders including Huntington's disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy and several spinocerebellar ataxias. polyQ diseases are caused by abnormal expansion of CAG repeats in certain genes. The expanded CAG repeats are then translated into a series of abnormally expanded polyQ tracts. Such polyQ tracts may induce misfolding of the disease-causing proteins. The present review mainly focuses on the common characteristics of the pathogenesis of these polyQ diseases, including conformational transition of proteins and its influence on the function of these proteins, the correlation between decreased ability of proteolysis and late-onset polyQ diseases, and the relationship between wide expression of disease-causing proteins and selective neuronal death.

Keywords: polyglutamine; the central nervous system; neurodegenerative diseases; late-onset disorders; ubiquitin; autophagy

1 Introduction

Polyglutamine (polyQ) diseases are a family of late-onset, inherited neurodegenerative disorders arising from the expansion of CAG repeats encoding glutamine within the coding region in certain genes^[1]. There are 9 known polyQ diseases, including spinobulbar muscular atrophy (SBMA), Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), and 6 spinocerebellar ataxias (SCAs) (Table 1). A common feature of polyQ diseases is the expansion of the length of polyQ repeat, resulting in the formation of intracellular inclusions containing the disease proteins that are ubiquitinated and often associated with various transcription factors, chaperones, and proteasome components. Sev-

eral lines of experimental evidence and data from computational simulation indicate that the expanded polyQ tract within mutant proteins can induce abnormal changes in protein conformation, which triggers misfolding and aggregation of mutant proteins, leading to protein aggregation and inclusion formation. This review highlights the common features of the pathogenesis of these polyQ diseases, including the mechanism of mutant protein misfolding, its influence on the functions of these disease-causing proteins, the organelle abnormalities triggered by accumulation of misfolded proteins, and the selectivity of neuronal loss.

2 Protein misfolding

As a common pathological feature of polyQ diseases, protein aggregate or inclusion body is detected not only in the neural tissues of patients, but also in animal models of polyQ diseases. It has been speculated that the expanded polyQ tract can cause protein misfolding, leading to the for-

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Table1. An overview of polyQ diseases

Disease	Gene	Protein	Localization	Putative normal function	Most affected regions
SCA1	<i>ATXN1</i>	ataxin-1	Nucleus	Transcription (interaction with corepressor SMRT)	Cerebellar cortex, dentate nucleus, brainstem, cerebral cortex
SCA2	<i>ATXN2</i>	ataxin-2	Nucleus	RNA metabolism	Cerebellar cortex, brainstem, cerebral cortex
SCA3	<i>ATXN3</i>	ataxin-3	Nucleus	Deubiquitylating enzyme	Cerebellum, basal ganglia, brainstem, spinal cord
SCA6	<i>CACNA1A</i>	$\alpha 1A$ voltage-dependent calcium channel subunit	Cytoplasm	P/Q type $\alpha 1A$ calcium channel subunit	Cerebellum
SCA7	<i>ATXN7</i>	ataxin-7	Nucleus	Subunit of TFTC/STAGA coactivator complexes	Cerebellum, retina, brainstem, visual cortex
SCA17	<i>TBP</i>	TATA box binding protein	Nucleus	General transcription factor	Cerebellum, striatum
HD	<i>HD</i>	huntingtin	Cytoplasm and nucleus	Vesicular transport, signaling, transcription	Striatum, cerebral cortex
SBMA	<i>AR</i>	androgen receptor	Cytoplasm and nucleus	Nuclear receptor	Spinal cord, brainstem
DRPLA	<i>ATN1</i>	atrophin-1	Nucleus	Nuclear receptor corepressor	Cerebellum, cerebral cortex, basal ganglia, subthalamic nuclei

mation of insoluble aggregates, which subsequently accumulate within neurons as inclusion bodies. Eventually neurodegeneration is induced.

2.1 Conformational transition The expanded polyQ protein can aggregate spontaneously *in vitro* and in specific cellular contexts. The expanded polyQ tract within a target protein facilitates the toxic aggregate-prone conformational transition. Aggregation of proteins containing polyQ tracts can be detected when the tract expansion exceeds a critical length. The conformational structure of the protein is essential for understanding the causative role of expanded polyQ protein in disease progression. Previous studies have demonstrated that the expanded polyQ tract can easily form the stable β -sheet structure^[2-4]. Annular structures that are made up of β -sheets have been shown by molecular simulation systems^[5] and proved by experimental observation^[6]. Besides, these structures are nearly identical to the water-filled β -helix nanotube model proposed by Perutz *et al.*^[7]. The β -helix structure is formed by parallel β -sheet strands of polyQ tracts in a

helical pattern and residues in neighboring turns are linked by hydrogen bonds involving not only main-chain but also side-chain amides. It has been shown that if polyQ has a length of at least 37 residues, it would spontaneously fold into a β -helix structure^[8]. Molecular dynamics simulation^[9-11] reveals that when containing more than 40 residues, the β -helix structure formed by the polyQ tracts is stable and regular. Besides, the ellipsoid-shaped helical structures can be easily formed in this conformation, and are similar to the nanotube. The nanotube model can explain that polyQ aggregation is dependent on the tract length. As illustrated by the nanotube mode, polyQ tracts of 40 residues or longer could form stable double-turn structures through hydrogen bonds between the turns, serving as a nucleus for growth of a nanotube, while tracts shorter than 40 residues would not be able to form the whole two-turn structure because of the reduced stability due to fewer hydrogen bonds^[7]. This prediction is in qualitative agreement with the molecular dynamics simulation results^[11]. It has been proposed that the Perutz's nanotube

structure can spontaneously integrate into the bilayers of membranes to form aqueous pores and produce permeable channels in appropriate neuronal membranes, which may be responsible for the toxicity of proteins with expanded polyQ^[12]. However, this proposition is controversial, according to the results of X-ray diffraction patterns that suggest that the peptides are in a stacked β -sheet conformation similar to the classical fibril structure^[13], thus needing to be reinterpreted. In addition, recent data indicate that the polyQ β -sheets can stack to form slab-like fibrils^[14]. More recently, the atomic architecture of the spine from the fibril-forming segment was examined by X-ray microcrystallography, and the results indicate that the fundamental unit of amyloid-like fibrils is a steric zipper, formed by 2 tightly interdigitated β -sheets^[15].

Although the detailed conformational changes remain unclear, current evidence suggests that proteins with expanded polyQ tracts can form compact structures that can not be degraded by the protein quantity control systems, such as the proteasome and the lysosome, leading to abnormalities in various intracellular pathways and neuronal dysfunction.

2.2 Roles of the flanking sequences Although toxicity is derived from expanded polyQ, the protein context determines the sites of affection in brain or the affected sub-population of neurons. Thus, polyQ cellular toxicity can be modified by the flanking sequences.

Although much attention has been paid to the mechanism underlying the role of polyQ tract in the pathogenesis of polyQ diseases, several studies show that the flanking sequences of polyQ tract are also of great importance and worth further investigation^[16-19]. In the exon-1 segment of huntingtin, the polyQ tract is directly followed by a proline-rich region. Recent research has shown that the proline-rich region modulates the polyQ domain structure, diminishes the tendency to aggregate and decreases the polyQ toxicity^[17,20,21]. Using a yeast model, Duennwald *et al.* have found that sequences flanking the polyQ region of human huntingtin exon-1 can convert a benign protein to a toxic species and vice versa^[19]. An *in vitro* study has indicated that the AXH domain-containing ataxin-1 has an intrinsic tendency to aggregate, while ataxin-1 aggregation is notice-

ably reduced when AXH is deleted or replaced by a sequence with no tendency^[18]. Besides, although ataxin-3 lacking a polyQ tract can also form aggregates, the presence of an expanded polyQ tract can accelerate the formation of large and stable aggregates^[22]. In addition to their effect on protein character, the flanking sequences also play a role in modulating the protective effect of the co-chaperone C-terminus of hsc70-interacting protein (CHIP), an E3 ligase that mediates the ubiquitylation of polyQ proteins and suppresses the aggregation of these proteins. Although CHIP is not effective in suppressing the toxicity caused by a bare 127Q tract, it is very efficient in suppressing the toxicity caused by a 128Q tract in the context of an N-terminal huntingtin backbone^[23].

In summary, these data demonstrate the importance of flanking amino acid sequences of polyQ tract in modulating polyQ toxicity.

3 Loss-of-function vs gain-of-function mutation

PolyQ diseases are dominantly inherited disorders caused by expanded polyQ tract, through 2 different but not mutually exclusive ways: loss of normal protein function and gain of toxic function. Knowing the normal functions of these essential proteins can contribute to a better understanding of the pathogenesis of polyQ diseases. Transcriptional dysregulation and organelle dysfunction may induce polyQ toxicity mutation.

3.1 Perturbation of normal protein function Overexpression of wild-type huntingtin in transgenic mice can alleviate the mutant huntingtin toxicity^[24,25], indicating that the wild-type huntingtin may have an essential cellular function. Previous reports have shown that wild-type huntingtin plays a role in promoting transcription^[26] and intracellular trafficking of brain-derived neurotrophic factor (BDNF), which is required for normal synaptic activity between the striatum and the surrounding cortex axons^[27]. Also, huntingtin has been shown to play a role in the intracellular trafficking of proteins required for the construction of extracellular matrix, including components involved in cellular adhesion, receptor binding and hormone activity^[28]. Besides, during endoplasmic reticulum (ER) stress, autophagy can be induced^[29], and

huntingtin is released from ER and vesicle membranes, shuttling between the nucleus and cytoplasm. A recent review proposes that wild-type huntingtin may be a potential regulator of autophagy in response to ER stress^[30], based on the findings that huntingtin is an ER-associated protein and is co-localized with Rab7- and LC3-positive late endosomes and lysosomes^[31]. On the other hand, knockdown of huntingtin results in a variety of developmental defects. In a zebra fish model, huntingtin knockdown leads to pronounced signs of cellular iron deficiency in many of the regions with neurodegeneration, suggesting a role of huntingtin in facilitating cellular utilization of endocytosed iron^[32].

In the case of SCA2, ataxin-2 interacts with a component of P-bodies and stress granules, which are main compartments for regulating and controlling mRNA degradation, stability and translation, and is important for their assembly^[33]. SCA6 is caused by polyQ expansion in P/Q-type calcium channel and is characterized by predominant degeneration of cerebellar Purkinje cells. PolyQ expansion in CACNA1A alters the channel function and causes a proportional negative shift of voltage-dependent inactivation, resulting in a reduction of calcium influx, which may contribute to Purkinje cell death^[34]. Interestingly, polyQ repeat variation in the CACNA1A calcium channel subunit could modify the onset age of SCA2^[35]. Ataxin-3, a polyQ disease protein in SCA3, is a deubiquitinating enzyme^[36,37] and its catalytic activity mainly depends on the Josephin domain, while the ubiquitin interaction motifs (UIM) region binds to the substrate polyubiquitin during catalysis^[36,38]. By using the *Drosophila* model, researchers have reported that the normal human ataxin-3 can strikingly suppress neurodegeneration caused by expanded polyQ ataxin-3, and this effect depends on its ubiquitin-binding activity and proteasome activity^[39]. Furthermore, the full-length wild-type ataxin-3 is recruited to aggregates formed by polyQ-expanded ataxin-3^[40]. These findings imply that the pathology may partly result from the loss of function of ataxin-3, and that the expanded polyQ tract within ataxin-3 may diminish the deubiquitinating effect by modulating the Josephin domain. In addition, the expanded polyQ domain of ataxin-3 stimulates Ku70 acetylation, which in turn promotes Bax activation and leads to cell apoptosis^[41].

Recent evidence shows that levels of Bcl-2 protein and mRNA are both decreased in the presence of expanded ataxin-3^[42], which also promotes apoptosis and triggers the degeneration of neurons. Ataxin-1 directly binds to the transcription repressor Capicua and modulates its activity *in vivo*, and the polyQ tract within ataxin-1 is proposed to alter its conformational state, which in turn alters the conformational or functional state of the Ataxin-1-Capicua complex and decreases its activity^[43]. The transcriptional role of ataxin-1 is further investigated. By performing cerebellar gene expression profiling, ataxin-1 and specific protein 1 (Sp1) are identified to co-regulate dopamine receptor D2 expression. This interaction and the transcriptional effects are mediated by AXH domain and diminished by expanded polyQ within ataxin-1^[44].

Although the removal of the causative protein, genetically or by other means, fails to induce disease symptoms in either human or animal models^[45], all the above data indicate that the pathogenesis of polyQ disease may partly result from polyQ expansion-induced perturbation of the normal function of the protein.

3.2 Transcriptional dysregulation Transcriptional dysregulation has been indicated as a common feature of polyQ diseases. Nevertheless, the underlying mechanisms remain largely obscure. The transcriptional regulation of some polyQ proteins, such as ataxin-1 and huntingtin, has been discussed above. Accumulating lines of evidence suggest that polyQ proteins are involved in transcriptional functions. Ataxin-1 has been proved to repress transcription through interacting with the co-repressor silencing mediator of retinoid and thyroid hormone receptors (SMRT)^[46,47]. Ataxin-7 is a subunit of a histone acetyltransferase co-activator complex called STAGA/TFTC^[48]. Moreover, the aberrant interactions between expanded polyQ proteins and transcriptional factors/co-factors, such as CBP, p/CAF, p53, Sp1, TAF4 and PQBP-1, have been reported^[49]. These interactions would interfere with the normal functions of the transcriptional factors, thereby contributing to the abnormalities. Evidence shows that mutant huntingtin can induce dissociation of transcription factor Sp1 from target promoters, thus inhibiting the transcription of certain genes^[50]. Other reports also indicate that expression of expanded polyQ proteins down-regu-

lates NF- κ B-dependent transcriptional activity^[51], the latter of which is crucial for certain cell survival pathways. In SCA17-transgenic mice, the expanded TATA box binding protein (TBP) perturbs the interaction between the general transcription factor IIB (TFIIB) and the promoter region of *Hspb1*, thus down-regulating the expression of small heat shock protein HSPB1^[52].

3.3 Organelle dysfunction ER stress is caused by structural and functional disturbances of ER, with accumulations of misfolded proteins and alterations in calcium homeostasis. ER stress is an early event in polyQ toxicity^[53] and is indicated by the co-localization of mutant polyQ proteins with various molecular chaperones^[54]. During ER stress, a series of responses will be triggered, such as the unfolded protein response (UPR), which includes attenuation of general protein synthesis and transcriptional activation of the genes encoding chaperones and molecules involved in the ER-associated degradation (ERAD)^[55]. ER stress can also induce apoptosis. The activation of the apoptosis signal regulating kinase 1 (ASK1) is essential for polyQ-induced ER-mediated cell death. ASK1 can form a complex with iron responsive element (IRE) and TNF receptor-associated factor 2 (TRAF2) proteins in ER, and subsequently activates the downstream signals, such as the c-Jun NH2-terminal kinase (JNK)^[56,57]. When phosphorylated, JNK can cause apoptosis, either by activating the death effector p53 or by stimulating the release of pro-apoptotic factor cytochrome *c* from mitochondria^[58]. It has been increasingly clear that mitochondrial dysfunction and oxidative damage are major inducers of neuronal loss. Free radicals, typically those generated by mitochondrial respiration, cause oxidative damage to nucleic acids, lipids, carbohydrates and proteins^[59-61]. Increased mitochondrial depolarization and early calcium defects have been detected in HD patients, and in a transgenic mouse model^[45]. Mutant huntingtin has been proposed to influence the mitochondria, via direct interaction^[62,63], causing impairment of mitochondrial trafficking^[64,65] and decreasing the expression of peroxisome proliferator-activated receptor [PPAR]- γ coactivator 1 α (PGC-1 α), a transcriptional co-activator that regulates mitochondrial biogenesis and respiration^[66,67]. Similarly, mutant ataxin-3 and ataxin-7 are both re-

ported to activate the mitochondrial apoptotic cascade^[68,69]. Mitochondrial abnormalities also exist in SBMA^[70]. The downstream death-triggering mechanisms, including excitotoxicity, apoptosis and oxidative damage, are implicated in the neuronal degeneration diseases.

4 Late-onset and proteolytic stress

The symptoms of these diseases usually appear in late 40s, and progressively deteriorate before death. They also generally demonstrate an earlier onset and increased severity in the subsequent generations with longer CAG repeats. This suggests that the cells can sufficiently deal with these misfolded proteins for many years until the proteolysis capacity is compromised.

For these diseases, one contributing factor is the decreased proteolytic capacity during aging. On one hand, the degradation of these misfolded proteins is a challenge for aged cells. On the other hand, the mutant proteins can impair the ubiquitin-proteasome system (UPS)^[71-74], the major intracellular proteolytic pathway, leading to the failure in degradation of these misfolded proteins. Evidence comes from the findings of mutant protein aggregation and inclusion bodies containing components of the UPS components^[75]. An elevation in the ubiquitin level, which enhances the activity of proteasomes, can suppress polyQ toxicity in cells and animal models of HD^[76,77].

Autophagy is another critical mechanism involved in the turnover of intracellular organelles and long-lived proteins. Studies have indicated that autophagy is induced in polyQ diseases to alleviate the toxicity. Expression levels of autophagy-related genes are up-regulated^[78] and the mammalian target of rapamycin (mTOR) is sequestered into aggregates, resulting in activation of autophagy and enhanced clearances of mutant polyQ proteins^[79]. However, the polyQ proteins such as mutant huntingtin, can also recruit Beclin 1 and reduce the activation of autophagy^[80].

Moreover, family studies reveal that the CAG repeats have a capability of further expansion in successive generations. With the increase in length, polyQ sequence can form more compact structures, being more difficult to be degraded, which may account for the anticipation.

5 Selective neuronal loss

Although the mutant polyQ proteins are ubiquitously expressed in the neuronal system, only some specific neurons in distinct brain regions are affected by polyQ toxicity.

In HD, neuronal loss occurs mostly in the medium spiny neurons (MSNs) of the dorsal striatum. Here the *N*-methyl *D*-aspartate receptor (NMDAR)-mediated excitotoxicity is used as an example to explain the specific pathology of HD^[81]. NMDARs are ligand-gated ion channels composed of a basic subunit NR1 and regulatory NR2 and NR3 subunits. There are 4 types of NR2 subunits, namely NR2A, NR2B, NR2C and NR2D, according to the pharmacological specificity^[82]. Glutamate-induced excitotoxicity is mainly caused by activation of the extra synaptic NR2B-containing NMDARs^[83]. It is worth noting that the NR2B subtype of NMDAR is expressed at a very low level in many mature neurons while at a high level in MSNs^[84]. The mutant huntingtin is proposed to selectively interact with NR2B-containing NMDARs^[81] and inhibit glutamate uptake by synaptic vesicles in striatal neurons^[85], resulting in the promotion of excitotoxicity. These may explain the selective striatal neuronal loss. However, other mechanisms also contribute to the specific neurodegeneration.

An alternative mechanism is the increase in the copy number of CAG repeats in the suffered neurons. A number of studies have demonstrated that the CAG repeat mutation is unstable in somatic tissues, most remarkable in the striatum, derived from the knock-in HD mice and HD patients^[86-88]. The somatic length of CAG repeats, which is mainly determined by inheritance^[89], is found to be a significant predictor of the disease onset. Longer CAG repeats are associated with earlier disease onset, and also with the specific neuronal death^[90]. The CAG mutation instability has also been occasionally found in DRPLA mice. The Q129 mouse line obtained unexpectedly based on the somatic instability of 76 CAG repeats *in vivo*, exhibit devastating progressive neurological phenotypes similar to that of DRPLA patients^[91]. The fact that CAG repeat length instability occurs in non-replicating neuronal cells suggests that processes such as inappropriate mismatch repair, are responsible for the instability

of CAG repeat expansion in somatic tissues.

Although evidence for this proposition remains largely obscure, the role of protein quantity control systems in the selective neuronal death has been considered. The heat shock protein 70, a well-known chaperone molecule protecting neurons in the polyQ pathology, is dramatically up-regulated only by mutant HTT, selectively in the granule cells of the cerebellum^[92]. In the glia, the activities of UPS is selectively higher than that in neurons, which may account for the lower vulnerability of glia^[93].

Mechanisms underlying the selective neuronal loss in other polyQ diseases are rarely reported, except the transcriptional repression in the specific pathology of SCA7^[45]. Here we propose that the distinct roles of various mutant polyQ proteins in diverse neurons may account for the selective death. Accumulation of mutant proteins in certain neurons can interrupt the cellular processes, such as signal transduction and intracellular trafficking. Li XJ shows that the mutant HTT aggregates in the axon, which can block the axonal transport, leading to neuronal dysfunction at the early stage^[94]. This is supported by the fact that the neuronal cell death is often undetectable in mildly affected HD patients despite the presence of definite clinical symptoms^[95]. The neurons containing mutant proteins would be eventually lost if the abnormal aggregates are not cleared by intracellular defense mechanism.

6 Conclusion

Currently, although the mechanisms of polyQ disease pathogenesis are not fully clear, the triggering role of CAG repeat mutation has been widely recognized. For clinical diagnosis, PCR method has been used to detect the CAG repeat size in the corresponding gene. Current treatment for polyQ diseases is non-specific, and it merely relieves the symptoms, but not restoring neuronal function or stopping the progressive loss of specific neurons. New insights into the molecular mechanisms underlying the polyQ diseases may provide important clues for therapeutic intervention. Potential therapies include inhibiting protein misfolding, clearing the mutant proteins, and protecting the intracellular organelles and neurons. Currently, many drugs and therapies

have been developed^[45,96,97]. However, more efforts are needed to fully understand the pathogenesis of these diseases and improve the disease management.

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多聚谷氨酰胺疾病分子发病机制的研究进展

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摘要: 多聚谷氨酰胺疾病是一类中枢神经系统退行性疾病, 目前已知的包括亨廷顿氏舞蹈病、脊延髓肌萎缩症、齿状核红核苍白球丘脑下部核萎缩以及其它几种脊髓小脑共济失调亚型。多聚谷氨酰胺疾病是由疾病相关基因的外显子内CAG三核苷酸重复序列异常扩展引起的, 后者导致其编码的多聚谷氨酰胺链的异常延长, 引起相关蛋白质的错误折叠。本文主要探讨多聚谷氨酰胺疾病分子发病机制的一些共同特征, 包括蛋白质构象变化及其对蛋白功能的影响、蛋白水解能力下降与疾病迟发性的相互关系以及致病蛋白广泛表达与神经元选择性死亡的关系。

关键词: 多聚谷氨酰胺; 中枢神经系统; 神经退行性疾病; 迟发型障碍; 泛素; 自噬