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Polyglutamine Diseases-Understanding the Mechanism of Pathogenesis

Sonali Sengupta[1*] **and Saurabh Bhattacharya**[2]

[1]*Division of Biomolecules and Genetics, School of Biosciences and Technology (SBST), VIT University, India*

[2]*Cytogenetics Department, Dr. Lal Path Labs Pvt. Ltd, National Research Laboratory, New Delhi, India*

[*]**Corresponding author:** Sonali Sengupta, Division of Biomolecules and Genetics, School of Biosciences and Technology (SBST), VIT University, Tamil Nadu, India, Tel: +919566812955; E-mail: sonalisengupta@vit.ac.in

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Abstract

Protein misfolding has been implicated in a large number of diseases, which are now grouped under the name of Protein conformational disorders (PCDs). Few examples of diseases that fall in this group are Alzheimer's disease, Parkinson's disease and Huntington's disease. All these disorders are characterized by sets of protein that misfold and aggregate in specific tissues. In order to identify and develop possible routes of therapeutic strategies, scientists have discovered several modifiers for these fatal diseases. These modifiers, primarily identified using models systems, include heat shock proteins, components of UPS pathway and autophagy, transcription factors, detoxifying enzymes, several RNA binding proteins, and RNA species, among other examples. These reviews will focus primarily on cellular processes that are affected in Polyglutamine disorders.

Keywords: Polyglutamine disease; Neurodegeneration; Aggregates/ Inclusion; Huntington`s disease; Spinocerebellar ataxia

Introduction

Expansion of CAG trinucleotide repeat sequences has been associated with a number of inherited human disorders [1-3]. These neurodegenerative diseases include Huntington's disease (HD), Spinocerebellar ataxias, type 1 (SCA-1), type 2 (SCA-2), type 3 (SCA-3, also known as Machado-Joseph disease), type 6 (SCA-6), type 7 (SCA-7), Dentatorubropallidoluysian atrophy (DRPLA) and Spinobulbar muscular atrophy (SBMA) [4-9]. With the exception of SBMA, all these neurodegenerative diseases are inherited in an autosomal dominant manner. The CAG repeat expansion occurs in the translated region of the gene that encodes a stretch of polyglutamines. In SCA-2, SCA-3, HD, and SBMA, repeats are found in the first exon, while those in the SCA-1, SCA-7 and DRPLA are located in the eighth, third and fifth exons, respectively [2,4-6,8-10]. The expansion of CAG repeats in all these cases has been classified as "dynamic mutation" in which the repeat number changes during intergenerational transmission. Polyglutamine expansion disorders are characterized by "*genetic anticipation*", i.e. there is a progressively earlier onset and increased severity in successive generations in a family [11-13]. Anticipation has a sex bias and is most pronounced on paternal transmission [14]. A common feature of these diseases is that they become clinically evident only late in life. These diseases are also characterized by selective vulnerability of neurons despite widespread expression of the diseased protein in brain and other tissues [15]. It is important to note that the genes causing these diseases show no homology to each other, except in the highly polymorphic CAG tract but pathogenicity results when the CAG tract in the disease causing allele expands beyond a threshold of 35-40 CAG repeats [16-20] (Figure 1).

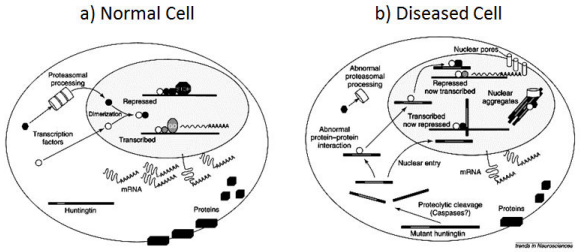

Figure 1: Mechanism of cellular pathogenesis in Poly-Q expansion diseases. a) Normal cell showing normal transcription and protein turnover. b) Diseased cell expressing expanded hunting tin protein that undergoes proteolytic cleavage and interacts with transcriptional and Proteasomal machinery inside the cell. This aberrant interaction leads to transcriptional dysregulation and proteasomal blockage. (Image adapted from Jhang Ho Cha, Trends in Neurosciences, 2000).

Aggregates and PolyQ Toxicity

A morphological feature that characterizes these polyQ expansion disorders is the presence of intracellular aggregates in neurons of affected patients, *in vitro* cell culture studies and also in mouse and fly models [21-25]. These aggregates are truncated polyglutamine fragments. Aggregates are found both in cytoplasm and nucleus and are often referred to as cytoplasmic and nuclear inclusion bodies, respectively. Whether inclusion bodies are pathogenic or are protective to a cell is still debated [14,26]. But their presence in neuronal cells that later undergo degeneration and death intrinsically links them to pathogenecity [21-23]. Furthermore studies have shown that mice expressing full length huntingtin [27] and mice expressing ataxin-1 lacking the self association domain of the protein develop specific neuronal loss characteristic of the disease in the absence of aggregates

[17]. Thus aggregates may not be required for neuronal loss but may be representative markers of neurotoxicity.

Cellular Processes Affected in Polyglutamine Diseases

All the CAG-repeat diseases share common features like adult onset, progressive neurodegeneration, generational anticipation and a remarkable threshold-expansion length which suggests that these diseases may share a common pathogenetic mechanism [14]. Genetic data suggests new gain of function acquired by these expanded proteins to be important in manifestation of the disease phenotype [19].

As discussed below, studies in cell culture and in model organisms have implicated several cellular processes to be affected in these diseases [28].

Transcription

The possibility that the mutant polyQ proteins may affect nuclear functions arose when it was noted that nuclear localization of the protein increases toxicity. Transcriptional dysregultion may be a primary pathogenic process affected in polyglutamine diseases [17,29-31]. Many transcription factors contain glutamine rich regions. Other proteins that contain significant polyglutamine stretches include transcriptionally active molecules like N-Oct-3 (a nervous system specific POU domain transcription factor) [32,33], TATA binding protein (TBP), a transcriptional co-activator with intrinsic histone acetyltransferase activity, [34-36], CREB-binding protein (CBP), a transcriptional co-activator with histone acetylase function [37,38] etc. Interaction of polyQ aggregates with transcriptional factors can be mediated via the polyglutamine-tract present in the other proteins. Several studies have shown that nuclear inclusions of mutant Htt contain transcriptional co-activators such as CBP, an acetyl transferase (AT) etc. [38]. CBP and other histone acetyl transferases act as co-activators of transcription by modifying histones and other proteins to increase transcription. Whether sequestration of transcriptional factors is relevant to neuropathology *in vivo* was examined in *Drosophila* models. Using *Drosophila* model of Huntington's disease, a direct relevance of reduced acetylation activity and/or enhanced deacetylation was established. Histone deacetylases (HDACs) work in concert with histone acetyl transferases (HATs) to modify chromatin and regulate transcription [39]. In *Drosophila* model of Huntington's disease, genetic reduction of Sin 3A co-repressor activity or introducing inhibitors of histone deacetylases like SAHA have been shown to rescue the neurodegenerative phenotype associated with Huntington's disease [38]. Following the promising finding in *Drosophila*, the therapeutic potential of SAHA (Suberoylanilide hydroxamic acid) and another HDAC inhibitor, phenylbutyrate was tested in HD transgenic mouse models. Two mice models, R6/2 and HD-N171-82Q, expressing truncated portions of Htt was used in the study [40,41]. SAHA dramatically improved the motor impairment in R6/2 mice and phenylbutyrate revealed an overall improvement in the condition of HD-N171-82Q transgenic mice which showed decreased striatal atrophy [40,41] .These study validated this class of compounds as HD therapeutics [41].

Further work with a poly-Q *Drosophila* model showed complete rescue of neurodegenerative phenotype by over expression of a endogeneous *Drosophila* CBP (dCBP) [42]. Rescue of phenotype was also associated with eradication of polyglutamine aggregates, recovery of histone acetylation level and normalization of transcription profile [42]. It is important to note that this was the first report of rescue of neurodegenerative phenotype which was associated with lessening of burden of mutant polyQ aggregates in cells. This finding is in contrast to other findings where previously known suppressors of polyglutamine toxicity such as *Drosophila* heat shock protein 70 (dHSP70), *Drosophila* heat shock protein Hdj1 (dHDJ1), *Drosophila* tetratrico repeat protein (dTPR2) and *Drosophila* myeloid leukemia factor (dMLF) rescued phenotype but did not have any effect on formation of aggregates in diseased cells [24,43,44]. Thus the transcriptional dysregulation seems to be an important component of pathogenesis in polyglutmine induced neurodegeneration.

Protein folding and turnover

The mutational mechanism associated with the neurodegenerative diseases is a dominant toxic gain of function attained by the expanded proteins rather than loss of function [14]. The different CAG repeat disease proteins do not share any homology except in the polyglutamine tract. PolyQ expansion confers a dominant toxic property on the protein that leads to neuronal dysfunction and degeneration [16,18-20]. Evidence suggests that polyglutamine expansion increases the probability that the protein will attain an abnormal conformation. *In vitro* studies have shown that PolyQ self associates to form amyloid like fibrils and several studies in diseased tissue, transfected cells and in animal models have demonstrated expanded polyQ protein to form intracellular inclusions [45-47]. These inclusion bodies have been shown to be associated with various molecular chaperones and proteasome components [14]. The association of inclusion bodies with proteasome components impairs the function of Ubiquitin-Proteasome system (UPS) [48,49]. Since the UPS normally controls the quality of proteins by degradation, a blockage of UPS might result in accumulation of misfolded proteins that are produced during normal protein turnover. Thus it appears that cells recognize aggregated disease protein as abnormal protein and recruitment of chaperone and proteasome to the inclusion bodies is for refolding and or degradation of the mutant protein [14]. Recently, it has been reported ataxin 3 physically interacts with VCP and regulates proteasomal degradation of substartes derived from ER [50]. Ataxin-3, is a 42 Kda cytoplasmic protein and expansion of poly-Q repeat in ataxin -3 causes the most common form of autosomal dominant SCA, also known as SCA-3 or Machado-Joseph disease [51]. Using a *Drosophila* melanogaster model of SCA 3, it was shown that over-expression of molecular chaperones results in suppression of the neurotoxicity associated with these diseases [52,53].

Concluding Remarks

Understanding the molecular basis of neurological diseases will help us to find drugs that can prevent or to some extent delay the onset of these fatal disorders. With the advent of animal models it has been possible to establish the sequence of pathological changes that characterizes these diseases. Transgenic mice expressing diseased genes have allowed studies of the early phenotypic changes as patient material for such a kind of analysis is seldom available. These animal models also provide an opportunity to test several potential therapies, which can be aimed to block or slow down the progressive neuro-pathological phenotype [14,52].

Therapeutic Strategies/Future Directions

As described in the sections above, transcriptional dysregulation is the primary cause of pathogenesis in *Drosophila* and mouse models of Huntington`s disease. Mutant huntingtin has been shown to disrupt the activity of transcriptional factors with acetyltransferase activity. HDAC inhibitos like suberoylanilide hydroxamic acid, sodium butyrate, and phenylbutyrate have been shown to rescue neurodegenerative phenotype in various animal models of HD diseases [40,41,53,54]. Activation of cellular protein clearance pathway helps to target the misfolded/aggregated disease protein. Geldenamycin and Geranylgeranyla that stimulates the production of molecular chaperone, Hsp70, has been a focus of therapeutic strategy for several years [55]. Interestingly, mTOR inhibitior rapamycin that stimulates autophagy and helps in clearance of aggregated proteins has been proved to beneficial in cell, *Drosophila* and mouse model of poly Q expanded diseases [56].

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