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REVIEW

Polyglutamine-Mediated Neurodegeneration: Use of Chaperones as Prevention Strategy

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Abstract—Polyglutamine diseases are a class of inherited neurodegenerative disorders caused by the expansion of a polyglutamine tract within the respective proteins. Clinical studies have revealed that the forming of neuronal intranuclear inclusions by the disease protein is a common pathological feature of polyglutamine diseases. Although there has been considerable progress in understanding polyglutamine diseases, many questions regarding their mechanism are still unanswered. The finding that molecular chaperones are associated with ubiquitinated intranuclear inclusions clearly indicates a crucial role of molecular chaperones in the generation of these fatal diseases. Molecular and chemical chaperones have been found to be a good agent for suppressing many polyglutamine diseases in several animal models. In this review, I discuss the roles of the ubiquitin–proteasome pathway and molecular chaperones in the development of polyglutamine diseases and probable approach for the prevention of many of these fatal disorders using molecular chaperones as a therapeutic agent. Newly found chemical chaperones have been demonstrated to be potentially useful and could be used as a therapeutic strategy in preventing many versions of polyglutamine diseases.

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Key words: polyglutamine disease, heat shock protein, ubiquitin–proteasome pathway, chemical chaperones, Huntington's disease, spinobulbar muscular atrophy

Polyglutamine diseases are a class of autosomal neurodegenerative disorders caused by unusual CAG repeats in the polypeptide. This is a family of neurodegenerative diseases caused by mutations in DNA, in which an expanded CAG repeat tract results in long stretches of polyglutamine (polyQ) in the encoded protein [1]. So far, nine such disorders have been recognized, including Huntington's disease (HD), spinobulbar muscular atrophy (SBMA), and several spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17).

The common feature of all these disorders is the expansion of CAG repeats that encodes an abnormally long glutamine repeat. Perhaps this is the basis of toxicity of polyQ diseases, and this toxicity increases with longer glutamine repeats (shown in Table 1). In all cases, the neuropathology of these diseases is characterized by the presence of nuclear, and in some cases extranuclear, aggregates that are insoluble and immunoreactive for the mutant protein and for ubiquitin [2]. Although it is likely that the misfolding and accumulation of mutant polyQ is central to the pathogenesis of these diseases, the structure of the intermediates involved in aggregate formation, the stage at which this process is first detrimental to cells, and the relationship of the aggregation process to neuronal dysfunction and neuronal cell death are not clearly understood [3-8].

Studies of polyQ disease patients and transgenic mice have revealed that in seven of nine diseases, nuclear inclusions (NI) formed by the disease protein are a common pathological feature of these kinds of disorders [9-13]. The finding that NIs are ubiquitinated has clearly raised the possibility that alterations in the major intracellular system for degrading proteins, the ubiquitin–proteasome pathway, may be involved in the pathogenesis of polyQ disease. This pathway is responsible for the normal

Abbreviations: 17-AAG) 17-allylamino-17-demethoxygeldanamycin; AR) androgen receptor; DMSO) dimethylsulfoxide; GGA) geranylgeranylacetone; HD) Huntington's disease; Hsp) heat shock protein; Htt) huntingtin; NI) nuclear inclusions; SBMA) spinobulbar muscular atrophy; SCA) spinocerebellar ataxia; TMAO) trimethylamine N-oxide.

Table 1. Summary of polyglutamine diseases [4, 72, 73]

Disease	Protein	Mw, kD	CAG repeat length		Type of aggregate
			wild type	mutant	
HD	huntingtin	348	6-35	38-180	NI, neuropil aggregates
SBMA	androgen receptor	99	9-36	38-65	NI
DRPLA	atrophin-1	124	3-36	49-88	NI
SCA1	ataxin-1	87	6-44	39-83	NI
SCA2	ataxin-2	90	14-32	34-59	cytoplasmic accumulation
SCA3	ataxin-3	42	12-40	55-84	NI
SCA6	$\alpha 1_A$-voltage-dependent calcium channel subunit	160-250	4-18	21-30	cytoplasmic accumulation
SCA7	ataxin-7	95	7-17	31→200	NI
SCA17	TATA-binding protein	41	29-42	47-55	NI

regulation of protein metabolism as well as the destruction of misfolded or damaged proteins. The proteasome is a large multicatalytic protease complex that is critical for many cellular process including cell cycle control, differentiation, antigen presentation, and cell survival [14].

In general, the misfolding and aggregation of proteins are prevented by molecular chaperones in cells [15-17]. Heat shock proteins (Hsps) are a class of molecular chaperones that bind with non-native proteins and assist them to acquire native structure and thus prevent misfolding and the aggregation process. Some Hsps such as Hsp70 and Hsp40 have recently been found to be associated with NIs formed by polyglutamine disease proteins (SCA1 and MJD/SCA3) in brain tissues from patients and transgenic mice [18, 19]. The presence of Hsp in nuclear aggregates is an important indicator of a crucial role of chaperones in the development of polyglutamine diseases. Hence, there is a possibility for controlling the progression of these diseases by modulating the function or expression level of these Hsps. In fact, overexpression of both Hsp70 and Hsp40 chaperones has been found to reduce aggregate formation by expanded polyglutamine tract and cytotoxicity induced by aggregate formation with disease gene product in many polyQ diseases [16, 20-23].

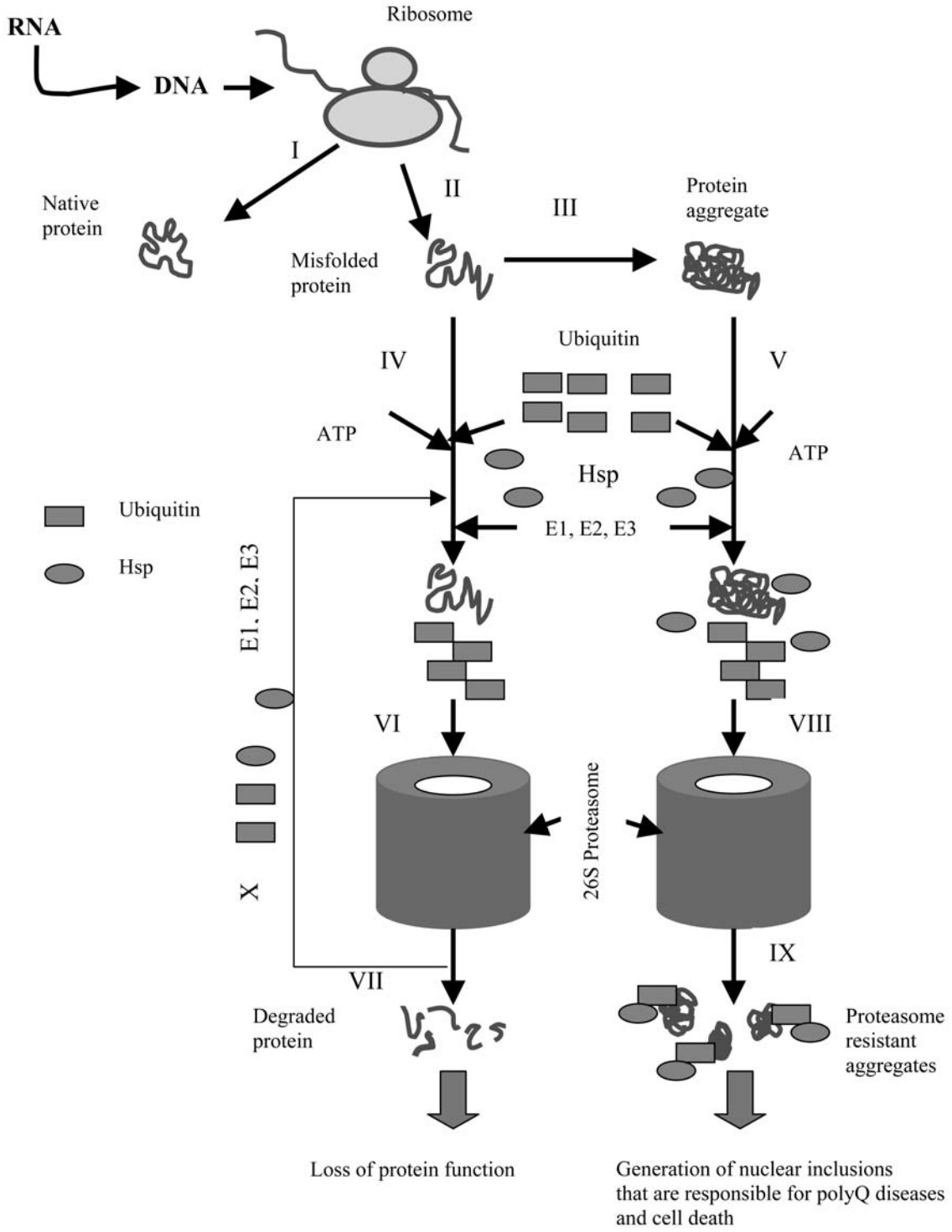
In parallel, several chemical chaperones have also been tried to suppress polyQ neurodegeneration [24-30]. Glycerol, dimethylsulfoxide (DMSO), and trimethylamine N-oxide (TMAO) are well known chemical chaperones that have been demonstrated to reduce polyQ-mediated neurodegeneration in many disease models [25, 26]. All these evidences suggested that increasing the expression level or enhancing the function of molecular chaperones and optimized use of chemical chaperones could be a good therapeutic agent and may provide a way for the treatment of polyQ diseases. However, further studies of cellular and animal models are needed to determine the precise mechanism of neurodegeneration in polyQ disease mediated by expanded polyglutamine tract.

In this review, I discuss the role of chaperones in the development of polyQ disease, and I describe from many reported experimental findings how enhanced expression of molecular chaperones suppresses the phenotypes of many polyQ neurodegenerations. I also discuss the finding of few chemical chaperones and their use in preventing the cytotoxicity and aggregate formation in different versions of polyQ-mediated disorders.

ROLE OF THE UBIQUITIN–PROTEASOME SYSTEM AND MOLECULAR CHAPERONES IN THE DEVELOPMENT OF polyQ DISEASES

The ubiquitin–proteasome system seems to have a role in the response to an expanded polyglutamine tract. The ubiquitin–proteasome pathway is one major intracellular pathway for the recognition and handling of abnormal/misfolded proteins in cells. The proteasome complex is responsible for the ubiquitin-dependent degradation of most cytosolic proteins. Proteasomes are abundant in neurons, where precisely controlled protein degradation is required to maintain neuronal function.

Ubiquitins and proteasome components are associated with polyglutamine inclusions in both disease models and patient tissue in SCA1 and SCA3 [4, 18, 31, 32]. Hence, the presence of ubiquitin and proteasome in polyQ aggregates indicates that cells have attempted to destroy polyglutamine-containing proteins and that these substrates may be resistant to proteasomal degradation. The figure shows how the proteasome complex tries to remove misfolded polyQ proteins with the help of ubiqui-



Fate of polyglutamine diseased polypeptide after glutamine repetition. I. Nascent polypeptide chain is converted into native protein. II. Polypeptide chain forms misfolded structure because of mutation in DNA that leads to CAG codon repetition and subsequently produces a long glutamine tract. III. Misfolded structures interact with each other and form protein aggregates. IV, V. Misfolded and aggregated proteins are ubiquitinated with the help of ubiquitin proteins, Hsps, E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzyme), E3 (ubiquitin ligase), and ATP, and enter into the 26S proteasome system. VI. Misfolded polypeptides are digested by many proteases secreted by the proteasome system and converted into small peptides. VII. Misfolded proteins are removed or metabolised after being degraded into small peptides by the proteasome system. VIII, IX. Aggregates or fibrillar amyloids try to be digested with the help of ubiquitins, Hsps, and many other proteases. As these aggregates become resistant to proteasomal attack, the 26S proteasome system cannot digest them and therefore they interact with each other to form nuclear inclusions, which are toxic in nature, and accumulate in different cellular spaces and consequently cause polyglutamine disease. X. Ubiquitins and Hsps are regenerated

tins and many other heat shock proteins, and the fate of the polyglutamine disease proteins.

Perturbations in normal proteasome function are associated with enhanced expression of several highly specific Hsps. They function as molecular chaperones by recognizing misfolded proteins, helping them in reaching the native structure or directing them to the ubiquitin–proteasome pathway for proteolysis [33-36]. Hsp70 and the Hsp40 chaperone family members normally act together as molecular chaperone inside cells [37]. The Hsp40 family regulates the chaperone activity of the Hsp70 family through upregulation of their ATPase activity [38].

Both Hsp70 and Hsp40 co-localize with the polyglutamine aggregates in both cell-based polyglutamine expression systems and patients' tissues. Human DnaJ (Hsp40) associates with ataxin-1 aggregates in cell culture and in patients' tissues [18]. Hsp40 and Hsp70 proteins co-localize with intranuclear aggregates of expanded ataxin-3 and androgen receptor in the cell culture models [19, 39, 40]. Hsp105α has been found to be localized in nuclear inclusions formed by truncated ARs (androgen receptors) containing an expanded polyglutamine tract in tissues of patients and transgenic mice with SBMA [41]. It has recently been shown that a progressive decrease in Hdj1, Hdj2, Hsp70, αSGT, and βSGT levels likely contributes to disease pathogenesis in the R6/2 mouse model of HD and as evidence Hdj1, Hdj2, Hsc70, αSGT, and βSGT were found to co-localize with nuclear aggregates [2]. In addition, Hsp84 has been reported to co-localize with nuclear aggregates in HD transgenic mouse brains [42]. Therefore, all these evidences clearly indicate that molecular chaperones have a crucial role in the development of polyglutamine diseases.

MOLECULAR CHAPERONES CAN SUPPRESS NEURODEGENERATION IN polyQ DISEASES

Overexpression of human Hsp70 completely suppressed the external eye defects mediated by the expression of expanded ataxin-3 protein in a *Drosophila melanogaster* model, and partially restored retinal structure [23]. Expression of the expanded polyQ protein in a fly line bearing a dominant-negative drosophila Hsp70 augmented the severity and kinetics of neurodegeneration, suggesting endogenous fly Hsp70 may partially mitigate the toxic effects of the expanded polyQ proteins [23]. In another remarkable finding, increasing levels of Hsp70 in a mouse model of SCA1 mitigates neurodegeneration [21].

Overexpression of Hsp70, together with Hsp40, inhibits toxic accumulation of abnormal polyglutamine protein and suppresses cell death in a variety of cellular models of polyglutamine diseases including SBMA [43-45]. Hsp70 has also been shown to facilitate proteasomal degradation of abnormal AR protein in a cell culture model of SBMA [46]. In one report by Gong and Golic, it was demonstrated that in *D. melanogaster* lack of Hsp70 confers enhanced sensitivity to a temperature-sensitive lethal mutation and to the neurodegenerative effects produced by expression of a human polyglutamine disease protein [47]. Overexpression of molecular chaperone Hsp70, which renatures misfolded mutant AR, ameliorates neuromuscular phenotypes of the transgenic mouse by reducing nuclear-localized mutant AR [48].

Exogenously administered biotinylated Hsp70/Hsc70 into a model of HD culture medium followed by 3-6 h of incubation was correlated with a reduction in apoptotic cells by 40-50%, showing that preparations based on Hsp70 may have some potential as therapies for a variety of neurodegenerative pathologies [49].

In a cellular model of HD, it has been shown that Hsp27 suppressed polyQ-mediated cell death, protecting from the increase in reactive oxygen species caused by huntingtin (Htt) [50]. However, in two of the fly model studies [16, 23] and in the mouse model [21], suppression of neurodegeneration was not accompanied by suppression of aggregation. The mechanism of suppression of neurodegeneration is not clearly understood.

In another case when Hsp105α was overexpressed with tAR97 (truncated androgen receptor) in the cells, Hsp105α was co-localized to aggregates of tAR97, and the aggregation and cell toxicity caused by expansion of the polyglutamine tract were markedly reduced [41].

Small Hsps like Hsp26 and Hsp42 facilitate the Hsp104-mediated solubilization of polyglutamine aggregates in yeast [51]. Small Hsps partially suppress toxicity, even in the absence of Hsp104, potentially by sequestering polyglutamine from toxic interactions with other proteins.

It was also reported that overexpression of yeast Hsp104 reduced aggregate formation and prolonged the lifespan of a transgenic mouse model of HD by 20% [52]. However it is not clear whether this protection was mediated at the level of changing the conformation of a putative toxic monomer, reversing the oligomerization or aggregation, reducing the levels of oligomeric species or aggregates, or combinations of these non-mutually exclusive possibilities.

Another interesting observation was that the hetero-oligomeric chaperonin TRiC (also known as CCT) physically interacts with polyglutamine-expanded variants of Htt and effectively inhibits their aggregation [53]. Reducing of TRiC level enhances polyglutamine aggregation in yeast and mammalian cells [53]. In a separate case study, overexpression of a single TRiC subunit, CCT1, was found to be sufficient to remodel Htt-aggregate morphology *in vivo* and *in vitro*, and reduce Htt-induced toxicity in neuronal cells [53, 54]. Overexpression of all eight subunits of CCT suppressed Htt aggregation and neuronal cell death [54]. These results indicate that CCT has

an essential role in protecting against the cytotoxicity of polyQ proteins by affecting the course of aggregation.

Ubiquilin, a brain protein involved in the stabilization of other brain proteins, has emerged as another possible therapeutic agent for Huntington's disease. Overexpression of ubiquilin in HeLa cells and primary neurons reduced aggregation of polyglutamine-containing proteins and cell death induced by overexpression of a GFP–Htt fusion protein containing 74 polyglutamine repeats, in a dose-dependent manner [55]. These results suggest that ubiquilin might be a novel therapeutic agent for treating polyQ diseases.

The molecular mechanism for the reduction of cytotoxicity of polyQ disease by overexpression of chaperones is not yet clearly understood. In one recent study, the Hsp70/Hsp40 chaperone system was shown not only to enhance the solubility of expanded polyQ proteins, but also to increase the degradation of these proteins through the 26S proteasome [56]. The increase in polyQ solubility was accompanied by a 40% decrease in the half-life of the expanded polyQ proteins. This important result indicates that excess amounts of chaperones can shift the equilibrium between amorphous (detergent-soluble) and fibrillar (detergent-insoluble) aggregates such that the cell's proteolytic machinery can more efficiently turn over the toxic polyQ proteins. Recent study hypothesized that a pre-fibrillar intermediate, which is called protofibrils, and not mature fibrils, may be the cause of toxicity of the polyQ disease [57]. So it might be assumed that Hsps bind to protofibrils and induce a conformational change that leads to the generation of amorphous aggregates.

Another possibility is that overexpression of chaperones enhances the function of the ubiquitin–proteasome pathway for mutant protein degradation, because the function of the ubiquitin–proteasome pathway is associated with the expression level of chaperones [36]. The report that the inhibition of proteasome function accelerates aggregate formation by polyQ tract also implies that the ubiquitin–proteasome pathway plays a direct role in modulating aggregation in polyQ disease [19]. Expanded polyglutamine tract would interfere with ubiquitin-dependent protein degradation pathway and lead to neuronal damage in polyQ disease. Therefore, the overexpression of chaperone perhaps positively influences the function of proteasome that leads the protection of cells from toxicity of expanded polyglutamine tract.

CHEMICAL AND PHARMACOLOGICAL CHAPERONES CAN REDUCE AGGREGATE FORMATION AND TOXICITY IN polyQ DISEASES

Chemical chaperones are low molecular weight compounds that stabilize proteins in their native conformation by influencing protein folding against thermally and chemically induced denaturation [58]. These compounds include the organic solvent DMSO, cellular osmolytes glycerol, TMAO, and many others mentioned in Table 2.

In a set of experiments, a truncated HD gene was expressed which causes polyQ degeneration in bacterial cells. Surprisingly, it was found that the addition of DMSO or glycerol to the medium partially reduced bacterial death with a consequent decrease in aggregated material [25]. The same team carried out a similar kind of experiment where the polyQ degeneration caused by a truncated form of mutated ataxin-3 in Machado–Joseph disease SCA-3 *in vivo* and *in vitro*, was reduced significantly. Addition of DMSO, glycerol, and TMAO individually was shown to reduce the aggregate formation and cytotoxicity of the diseased cells [26].

In a cell model of SBMA, geranylgeranylacetone (GGA), a nontoxic anti-ulcer drug, has been shown to potently induce Hsp expression (e.g. Hsp70, Hsp90, and

Table 2. Chaperones used to suppress the aggregation and toxicity generated by expanded polyQ tract of various disease proteins

Disease	Protein	Chaperone type	Chaperone used	References
HD	huntingtin	molecular chemical	Hsp70, Hsp40, Hsp27 glycerol, DMSO, Congo Red, ubiquilin, disaccharide trehalose	[43-45, 50] [25] [64] [55] [29]
SCA1	ataxin-1	molecular	Hsp40/Hsp70	[21]
SCA3	ataxin-3	molecular chemical	Hsp70, Hsp40 glycerol, DMSO, TMAO, ectoine	[19] [26] [26, 30]
SBMA	androgen receptor	molecular chemical	Hsp70, Hsp40, Hsp105α GGA, 17-AAG	[43-45, 59] [59, 60]

Hsp105) and inhibit cell death and the accumulation of pathogenic AR. Oral administration of GGA also up-regulated the expression of Hsps in the central nervous system of SBMA-transgenic mice and suppressed nuclear accumulation of the pathogenic AR protein, resulting in amelioration of polyglutamine-dependent neuromuscular phenotypes [59].

17-Allylamino-17-demethoxygeldanamycin (17-AAG), a novel Hsp90 inhibitor and new derivative of geldanamycin, was demonstrated for its efficacy and safety in a mouse model of SBMA. In one study, it was demonstrated that the administration of 17-AAG significantly ameliorated polyQ-mediated motor neuron degeneration by reducing the total amount of mutant AR in SBMA [60]. In another report, it has been demonstrated that rapamycin, an autophagy inducer, enhances mutant Htt fragment clearance and attenuated toxicity in cell, fly, and mouse models [61]. Rapamycin enhances the autophagic clearance of different proteins with long polyglutamine tracts, and reduces their toxicity.

In one finding, polyglutamine-binding peptide 1 (QBP1) has been identified, and it was shown that a tandem repeat of the inhibitor peptide QBP1, $(QBP1)_2$, significantly suppresses polyQ aggregation and polyQ-induced neurodegeneration in the compound eye of *Drosophila* polyQ disease models, which express the expanded polyQ protein under the eye-specific promoter [62]. The efforts also identified a potent compound (IC_{50}) with long-term inhibitory effects on polyQ aggregation in HD neurons [63]. Testing of this compound has been reported to suppress neurodegeneration *in vivo* in a *Drosophila* HD model.

All the above results indicated the potentially useful therapeutic strategy of the chemical chaperones in preventing cell death in polyglutamine disease. Perhaps the most tantalizing results were obtained using the azo-dye Congo Red. This chemical not only blocks oligomerization and aggregation of expanded polyglutamine, but can also disintegrate existing aggregates. Intriguingly, Congo Red prevented much of the toxicity of expanded polyglutamine both in transfected cells and in one transgenic mouse model of Huntington's disease [64]. In the mouse model, administration of Congo Red well after the formation of nuclear inclusions and appearance of neurological symptoms resulted in significant improvement of motor performance and extended lifespan. In another study, it has also been reported that the chemical compound Congo Red inhibits HD exon 1 protein aggregation in a dose-dependent manner [15]. In the same study, it was observed that the Congo Red dye at micromolar concentrations reduced the extent of HD exon 1 aggregation in transiently transfected COS cells. Thus, the use of Congo Red (or its analogs) represents a promising avenue in treatment of polyglutamine diseases.

It was also demonstrated that many other small molecules like mAb 1C2, thioflavine S, chrysamine G, and DFY (Direct Fast Yellow) inhibit HD exon 1 protein aggregation [15]. In cell and tissue culture models of HD, intracellularly expressed single-chain Fv (sFv) antibodies (intrabodies) reduce aggregation and cellular toxicity [65].

The disaccharide trehalose has been recently found to be a good therapeutic agent against polyglutamine-induced protein aggregation and cell death in an *in vitro* cellular model and an *in vivo* mouse model of HD [29]. In a recent report it was demonstrated that ectoine, or 2-methyl,4-carboxy-3,4,5,6-tetrahydropyrimidine, a widely distributed natural osmoprotector in bacteria cells, reduces polyglutamine-induced toxicity [30]. In this study, the ectoine effect was studied on mutant ataxin-3 fragment-induced aggregate formation and apoptotic cell death. Addition of ectoine reduced total amount of aggregates and also decreased cell death.

Apoptosis is a highly regulated cellular death pathway that involves the activation of a cascade of proteases known as caspases. Evidence for caspase activation has been observed in polyglutamine diseases. For example, in HD brain [27, 66] some polyQ containing proteins have been reported to be the substrates of caspase protease. That truncation of huntingtin increases its cellular toxicity is evidence that proteolytic cleavage of huntingtin assists in the progress of this disease [67]. Hence, inhibition of caspase activity could reduce the disease progression. In fact, two different caspase inhibitors, zVAD-fmk [27] and minocycline [28], have shown to slow disease progression in the R6/2 mouse model.

CONCLUSION

A considerable effort has been made to find molecules that suppress polyglutamine aggregation and cell death/toxicity for therapeutic purposes. I reviewed that chaperones play a crucial role in the development of polyQ disease. These evidences suggested that increasing expression level or enhancing the function of chaperones provide an avenue for the treatment of polyQ disease. More cellular and animal models are required for various kinds of study to determine the precise mechanism of neurodegeneration of polyQ disease mediated by expanded polyglutamine tract, which in future might be a great help to find a suitable therapeutic approach against the lethal character of various forms of polyglutamine diseases. The mechanism that chaperones use to facilitate clearance of misfolded proteins by the action of proteasomes remains to be elucidated, and might indicate a useful agent for pharmacological intervention.

Since Hsps function in a cooperative manner *in vivo* and many of them work in tandem in the cell, it seems that increasing the expression level of multiple chaperones might be required to obtain a beneficial impact on disease progression and severity in patients with neurodegenerative disorders. Identification of small molecules

which up-regulates chaperone activity could be a potential agent against disease caused by long glutamine tracts. For example, a small molecule antagonist of the chaperone Hsp90 is currently in clinical trials for the treatment of breast cancer [68].

Chemical chaperones, like DMSO, glycerol, and TMAO, also have been shown to be highly effective in preventing protein misfolding and aggregation. Although GGA- and 17-AAG-like molecules have been found as effective chemical chaperones [59, 69], there are not ample reports available regarding their use against neurodegenerative disease progression. One interesting finding could be a combined application of molecular chaperones like Hsp70, Hsp40, Hsp105α and chemical chaperones in the prevention of polyglutamine disease and its toxicity. In a separate finding it was reported that geldanamycin, an antibiotic, not only prevented protein aggregation in a fruit fly model of neurodegeneration, but also stopped the degeneration. Geldanamycin might be a potential agent and probable treatment for HD [70]. It has also been demonstrated that geldanamycin blocks the development of aggregates of the expanded glutamine androgen receptor (AR112Q) of Kennedy disease (SBMA) in Hsf1(−/−) mouse embryonic fibroblasts [71].

It is quite clear from many experimental findings that chaperones can have a profound influence on solubility, aggregation, fibril formation, and toxicity of proteins that cause polyglutamine neurodegeneration, but many issues remain not yet understood. Details underlying pathogenesis are largely unknown. Further study of these diseases should provide important information for unraveling the molecular pathogenesis of neuronal cell degeneration as well as for the development of future therapeutic interventions. Molecular chaperones appear to be effective suppressors of polyQ toxicity in various experimental models. Understanding the protective role of chaperones might not only help us to understand the biology of polyglutamine toxicity, but also, and perhaps more importantly, inspire the design of novel therapeutic strategies.

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