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Protective effect of chaperones on polyglutamine diseases

Yasushi Kobayashi and Gen Sobue*

Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

ABSTRACT: Polyglutamine diseases are inherited neurodegenerative diseases caused by the expansion of polyglutamine tract in the disease causing gene products. Studies of polyglutamine disease patients and transgenic mice have revealed that nuclear inclusions formed by the disease protein are a common pathological feature of these diseases. The finding that nuclear inclusions are ubiquitinated raises the possibility that alterations in the major intracellular system for degrading proteins, the ubiquitin-proteasome pathway, may be involved in the pathogenesis of polyglutamine diseases. Perturbations in proteasome function are associated with altered expression levels of stress-response or heat shock proteins. Heat shock proteins function as molecular chaperones, which recognize and renaturate misfolded protein (aggregate). In this article, we review the role of chaperones in the development of polyglutamine diseases. Overexpression of chaperones reduces aggregate formation and suppresses apoptosis in several polyglutamine disease models including spinal and bulbar muscular atrophy. These facts indicate that chaperones may be one of the key factors in the development of polyglutamine disease, and suggest that increasing expression level or enhancing the function of chaperones will provide an avenue for the treatment of polyglutamine disease. © 2001 Elsevier Science Inc.

KEY WORDS: Chaperone, Polyglutamine, Hsp70, Hsp40, SBMA.

INTRODUCTION

Polyglutamine diseases are inherited neurodegenerative diseases caused by the expansion of CAG repeats (polyQ) in the disease causing genes [55]. For example, in spinal and bulbar muscular atrophy (SBMA) patients, a normally polymorphic CAG repeats (10–36 CAGs) expands to 38–66 CAGs in the first exon of *androgen receptor* gene. The number of CAGs is inversely correlated with the age of onset of the disease [15,32]. To date, several polyQ diseases have been identified, including SBMA, Huntington's disease (HD) [50], dentatorubralpallidoluysian atrophy [31, 41], Machado-Joseph disease (MJD) [27], and others. The number of identified polyQ diseases is still increasing. The polyQ diseases have different disease-causing genes, suggesting that these disorders share a common pathogenesis involving the gain of a toxic function associated with the expanded polyglutamine tract.

Processing of the polyglutamine-containing disease protein by proteases (e.g., caspase family) may liberate truncated fragments with the polyglutamine tract [17,30]. Truncated proteins with the expanded polyglutamine tracts cause neurodegeneration in transgenic mice as well as *Drosophila*, and cell death in transfected cells [13,22,38,52]. In addition to cellular toxicity, truncated proteins with the expanded polyglutamine tracts have been shown to form aggregates, likely through hydrogen bonding or transglutaminase activity [25,44,49]. Studies of polyQ disease patients and transgenic mice have revealed that nuclear inclusions (NI) formed by the disease protein are a common pathological feature of these diseases [14,21,33,34,43,47]. In SBMA, NIs containing androgen receptor (AR) protein have been mainly observed in the regions of SBMA central nervous system susceptible to degeneration, including the brain stem motor nuclei and spinal motor neurons [33,34]. The finding that NIs are ubiquitinated raises the possibility that alterations in the major intracellular system for degrading proteins, the ubiquitin-proteasome pathway, may be involved in the pathogenesis of polyQ diseases. The proteasome is a large multicatalytic protease complex that is critical for many cellular processes including cell cycle control, differentiation, antigen presentation, and cell survival [11]. Perturbations in proteasome function are associated with altered expression levels of stress-response or heat shock proteins (Hsps) [2]. These proteins function as molecular chaperones, which recognize and renaturate misfolded proteins under normal and stressed conditions. In addition, chaperones may maintain proteins in an appropriate conformation [20]. The possible role of chaperones in the development of polyQ diseases is raising considerable interest.

HSP70 AND HSP40 AS MOLECULAR CHAPERONES

It has been postulated that most Hsps have a molecular chaperone activity involved in various aspects of protein metabolism [20]. A molecular chaperone is a substance that binds to a substrate protein irrespective of stability, and facilitates its fate in the right way *in vivo*, i.e., folding, oligomeric assembly (switching active/inactive conformations), or transport to a subcellular compartment. The mechanism of this function is to control binding and releasing the substrate proteins [16]. Hsp70 and the Hsp40 chaperone family members act together as molecular chaperones [4]. Hsp40 family regulates the chaperone activity of Hsp70 family through upregulation of their ATPase activity [19]. This chaperone complex system works ubiquitously from bacteria to mammals [4]. Hsp70 in cooperation with Hsp40 has been demonstrated to renaturate heat-induced aggregation *in vitro* and *in vivo* [39,40]. This function to renaturate aggregation is dependent on Hsp40-enhanced adenosine triphosphate hydrolysis [40]. Most Hsps, including Hsp105 [53], Hsp90 [6], Hsp60 [2], Hsp70/40 [39], and Hsp27 [36], work as molecular chaperones, and participate in various

* Address for correspondence: Dr. Gen Sobue, Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Fax: +81-52-744-2384; E-mail: sobueg@med.nagoya-u.ac.jp

aspects of protein fate and, consequently, in the protection of living cells from deleterious environmental stresses.

CHAPERONE AND POLYQ DISEASES

Recently, overexpression of Hsps has been reported to decrease aggregate formation by expanded polyglutamine tract [12]. We hypothesized that the ability of Hsp70 and Hsp40 chaperones to facilitate refolding or proteolysis of mutant protein may be a key factor for neuronal cells to defend themselves against the toxic properties of expanded polyglutamine tract. Immunohistochemical studies revealed that polyQ-formed nuclear aggregates colocalized with several Hsps and proteasome [7,12,48,54], however, naïve cellular Hsps and proteasomes could suppress neither aggregate formation nor cellular toxicity induced by polyQ. To determine whether a sufficient amount of chaperones could be effective to protect cells against toxicity of expanded polyglutamine tract, we overexpressed chaperones in the cell model of SBMA [29]. Our results reveal that a combination of Hsp70 and Hsp40, or Hsp70 alone, has a favorable effect on cellular protection as well as suppression of aggregate formation, and that combination of Hsp70 and Hsp40 has the strongest effect among them. Our results were in accordance with the reports that the chaperone function of Hsp70 is critically dependent on the cooperation with Hsp40 [4]. Hsps have been confirmed to suppress aggregate formation and cellular toxicity in other polyQ disease models [5,9,12,24,29]. In other conformational diseases, the study of mutant Cu/Zn-superoxide dismutase associated with familial form of amyotrophic lateral sclerosis also displayed that increasing the level of Hsp70 reduced formation of mutant SOD-containing aggregates in cultured primary motor neurons expressing mutant SOD-1 and prolonged their survival [1]. It is therefore reasonable to consider that disease gene product-formed aggregates are directly associated with the induction of neurodegeneration in polyQ disease and other conformation diseases. We thus reasoned that overexpression of both Hsp70 and Hsp40 chaperones reduces cytotoxicity induced by aggregate formation with disease gene product in polyQ disease and other conformation diseases.

The molecular mechanism for the reduction of cytotoxicity through inhibition of expanded polyglutamine tract formed-aggregate by overexpression of chaperones needs to be examined. Although it has been proposed that expanded polyglutamine tract formed-aggregates participate in inappropriate protein-protein interactions that lead to cell death, the nature of such interactions and the mechanism by which cell death is induced remain unclear. Molecular chaperones could be involved in the actual formation of expanded polyglutamine tract formed aggregates by stabilizing the unfolded protein in an intermediate conformation which has the propensity to interact with self or other proteins. To date, several proteins interacting with polyglutamine tract-containing disease gene product have been cloned, including huntingtin-associated protein [35], huntingtin-interacting protein [26], glyceraldehyde 3-phosphate dehydrogenase [3], leucine-rich acidic nuclear protein [37], PQBP-1 [51], and TAF130 [46]. These interacting proteins are candidate players in the pathogenesis of polyQ disease. Chaperones might reduce cytotoxicity of expanded polyglutamine tract through inhibition of the interaction of expanded polyglutamine-formed aggregate with these proteins.

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 related with the expression level of chaperones [2]. Nuclear aggregates are ubiquitinated and are colocalized with chaperones and proteasome, implicating the ubiquitin-proteasome degradation pathway in the pathogenesis of polyQ disease [12,33,34]. The report that the inhibition of proteasome function accelerates aggregate formation by polyglutamine tract also implies that the ubiquitin-proteasome degradation pathway plays a direct role in modulating aggregation in polyQ disease [8]. In Alzheimer‘s disease, one of the conformational disease, amyloid protein, which is a major component of senile plaques, could interfere with ubiquitin-dependent protein degradation pathway by inhibiting the 26S proteasome. Consequently, this mechanism could lead to neuronal damage observed in Alzheimer‘s disease [18]. Similarly, expanded polyglutamine tract would interfere with ubiquitin-dependent protein degradation pathway and lead to neuronal damage in polyQ disease. Thus, overexpression of chaperones would enhance the function of proteasome, leading to the protection of cells expressing truncated and expanded AR against a cellular toxicity of expanded polyglutamine tract.

It has been recently reported that overexpression of Hsdj/Hdj2 in Hela cells decreases the frequency of mutant ataxin-1 and mutant AR aggregation [12,48]. However, we found that overexpression of Hsdj/Hdj2 has little effect on the reduction of aggregate formation and the protection from cytotoxicity in our report [29]. Such differences in the results may derive from the difference in cell lineage, the different origin of Hsdj/Hdj2 or expression level of Hsdj/Hdj2 in transfected cells. Previous studies used a non-neural cell line (Hela cell) [12,48], whereas we used a neural cell line (Neuro2a) [29]. The difference in cell lineages could influence the relations of chaperones, aggregation and cell death. Although Hsdj we employed in this study [29] is 99% identical to Hsdj2 employed in other reports [12,48] at the level of amino acid sequence [10,42], the relationship between Hsdj and Hdj2 remains to be studied.

In contrast to our results, evidence against a critical role of intranuclear aggregates in neuronal cell death has been provided [28,45]. Such discrepancies could arise from the difference of cell type/animal (primary neuron/animal vs. cell line) or interventions (neurotrophic factors/suppression of ubiquitin-conjugating enzyme/inhibition of ataxin-1 self-association vs. overexpression of chaperones). However, we cannot rule out the possibility that our finding of a parallel correlation between the reduction of aggregate formation and suppression of apoptosis in our system [29], independently might occur. Thus, Hsps could reduce aggregate formation as a molecular chaperone, and independently suppress apoptosis in our cell system through a different molecular mechanism. Although Hsp70 was reported to have an anti-apoptotic effect through veiled mechanism [23], the mechanism of such effect needs to be examined in further studies.

Finally, we highlighted that chaperones play a 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. Further studies of cellular and animal models are required to determine the precise mechanism of neurodegeneration of polyQ disease mediated by expanded polyglutamine tract as well as therapeutic approach.

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