Heat Shock Proteins: Still Hot in Neurodegenerative Disease?

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Abstract

A common pathological hallmark of a number of neurodegenerative diseases, including Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntingdon's disease (HD) and prion disease, is the accumulation of proteins in the parenchyma of brain. While these proteins have a normal function in healthy individuals, they accumulate by unknown mechanisms contributing to disease onset. Heat shock proteins are an important group of proteins that regulate cellular protein homeostasis (proteostasis) and understanding their role in aberrant protein homeostasis may lead to the identification of new drug targets.

Keywords: Heat Shock Proteins; Parkinson'sDisease; Alzheimer's Disease; SH-SY5Y neuronal cells

Introduction

Neuronal proteostasis, or proteome homeostasis is the balance of all cellular proteins in the concentration, location and conformation necessary to maintain metabolic function [1]. Not surprisingly, the balancing of this process is highly regulated, unique to each cell type and requires a number of specific, cellular regulatory proteins. These proteosome regulators are involved in a number of pathways, ranging from protein biogenesis and folding to eventual protein destruction [2]. Furthermore, salvage pathways are required to remove damaged, misfolded or mutated proteins produced in error or in response to stress, such as during aging and periods of energy depletion. Chaperone proteins are important regulators of these processes, enabling folding and removing errors by guiding aberrant, and potentially toxic, proteins into an isolated cellular compartment, and subsequently degrading them by autophagy or by targeting them for removal by the ubiquitin-proteasome system (UPS) [2,3].

Protein aggregation is thought to occur when proteostasis is unbalanced, suggesting that the underlying regulators of this complex process have failed or are overwhelmed [3]. Postmortem studies of age-related neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD), demonstrate the presence of protein deposits in brain tissue, implicating cellular aging as significant in the regulation of proteostasis. The observed increase in protein aggregation during aging may reflect a decline in proteosome regulators or a decrease in functionality. Alternatively, as aging progresses, neurons may contain an increased number of proteins that require attention due to senescence-related events including accumulated oxidative stress or somatic DNA mutations that effect cellular proteins with increasing frequency as the neuron naturally ages. With the decline in proteosome capability, aggregation starts, and continues, through the course of the disease. It is important, therefore, to understand the mechanisms involved in pathological accumulation in order to halt neuronal damage at a critical disease preventing point.
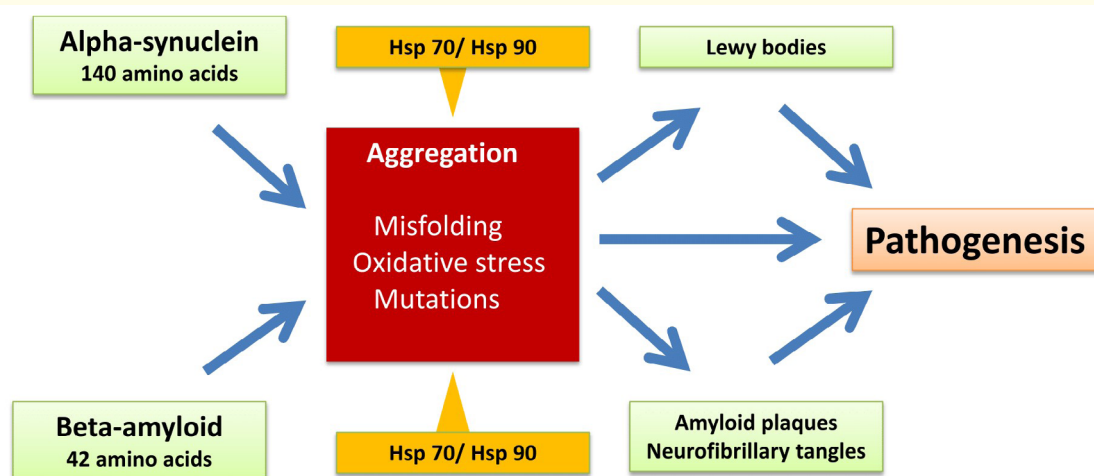
Aggregates and disease

Pathological protein aggregates have been observed in a number of progressive neurodegenerative disorders including Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntingdon's disease (HD) and prion disease. While some of these protein aggregations result from a genetic mutation, as in the case of HD, in the majority of neurodegenerative disease, most accumulations occur as the result of an unknown, sporadic event. Further, it is unknown as to whether the protein aggregates themselves are toxic, leading to disease pathology or whether they are the manifestations of an underlying aberrant cellular process. Confounding the issue further are the location and forms of the aggregates, which may be found within the cell, as an inclusion, or outside the cell as an extracellular deposit. Understanding the mechanism of aggregation in AD and PD is critical as the number of afflicted individuals rises annually with increased population longevity.

The clinical characteristics of PD include bradykinesia, tremor, postural instability and rigidity [4]. It affects approximately 1% of the population over the age of 60 and is accompanied by pathological brain changes, that include a loss of dopamine neurons and the deposition of significant amounts of α-synuclein in the form of protein aggregates or Lewy bodies [5]. α-synuclein is a membrane protein that functions in synaptic plasticity and neurotransmission [6] and mutations of the α-synuclein gene lead to the onset of familial PD, a genetic form of PD that afflicts less than 0.1% of all patients with PD [7].

The pathological hallmarks of AD include the deposition of amyloid protein (β-amyloid) as plaques and the hyperphosphorylation of tau leading to the formation of neurofibrillary tangles [8]. Familial AD results from a mutation in a number of key genes, including in the amyloid precursor protein (APP) gene, which afflicts less than 1% of the affected, leading to earlier deposition of β-amyloid [9]. Whether early or late in onset, the cognitive changes in AD become progressively disabling as the disease progresses, leaving the afflicted unable to care for themselves.

With both PD and AD, one protein aggregate is found in greater quantities than others, and is more disease specific, however, it should be noted that the aggregated protein is not homogenous. Indeed, α-synuclein, β-amyloid, and many other proteins including superoxide dismutase may occur together in both PD and AD brains [10,11]. Furthermore, AD symptoms are believed to be more aggressive when Lewy bodies are present [12] giving rise to the suggestion that α-synuclein, β-amyloid and tau can promote each other's aggregation [10,13]. These observations suggest a common disease pathway (Figure 1).

Figure 1: *Accumulation of pathogenic proteins in Parkinson's and Alzheimer's diseases.*

Alpha-synuclein aggregation leads to the formation of Lewy bodies in Parkinson's disease whereas beta amyloid plaques and neurofibrillary tangles are the majority aggregates of Alzheimer's disease. Pathogenesis may result through chaperone protein impairment or increased cell stress, which ordinarily guide proteins to the correct compartment.

Heat shock proteins (Hsp)

Aggregations of misfolded proteins are characterized by activation of the Unfolded Protein Response (UPR), which is an evolutionary conserved response triggered by the endoplasmic reticulum (ER) in response to stress [3,14]. Multiple stimuli, including hypoxia, energy depletion and calcium dysregulation trigger UPR which is believed to protect the ER from stress. UPR prevents the accumulation of misfolded proteins in the ER and adapts the cell function to stress by inhibiting protein synthesis as well as producing chaperone proteins [15]. To-date, multiple heat shock proteins have been linked to a variety of age-related diseases, including cancer development, cardiomyopathy and neurodegeneration [1,14,15]. In particular, the heat shock protein (Hsp) family, Hsp 70/Hsp 90, which contains multiple members classified based on molecular weight, are significant in neurodegenerative processes [16].

The Hsp70 family of proteins includes the glucose- regulated protein 78 (HSPA5/GRP-78) which binds to hydrophobic residues in unfolded regions of proteins, and maintains them in a state for later folding [17]. ATP binding and hydrolysis is necessary for allosteric changes in the Hsp 70/Hsp 90 family of proteins enabling them to interact with their associate targets, for example client proteins or the activation of other stress sensor proteins [18]. Depending on the toxic driver, the UPR may activate multiple, overlapping or restricted second messenger pathways in two phases: an early pro-survival phase and a later pro-apoptotic phase. If the original stress is not corrected, apoptotic death will occur, which has been reported in both AD and PD. In addition, genetic studies have implicated mutation of UPR proteins in familial PD. Supportive evidence has come, not only, from studies in cell culture and animal models, but also from studies of stress inducing agents and aging models. For example, HSPA5/GRP-78 has been shown to decline during aging, as well as when modeled by RNA knockdown, in rat neurons, with the result of increased α-synuclein toxicity [19]. Because the Hsp 70/ Hsp 90 family of proteins interact with exposed hydrophic domains of proteins, in the absence of chaperones self-association of proteins such as α-synuclein is augmented, leading to pathogenesis. Furthermore, *in vitro* models have shown that energy depletion, modeled by serum withdrawal, increases the expression of HSPA5/GRP-78 in dopaminergic neurons over three days in culture when compared to cells cultured with serum (Figure 2). However there is no additional increase in expression when cells are stressed for 5 days (Figure 3) and dompanergic neurons are lost. By 5 days, increases in alpha-synuclein are also apparent in the dying neurons (Figure 3).

Figure 2: Figure 2: Energy depletion, modeled via serum withdrawal promotes upregulation of GRP-78 in SH-SY5Y dopaminergic cells in culture.
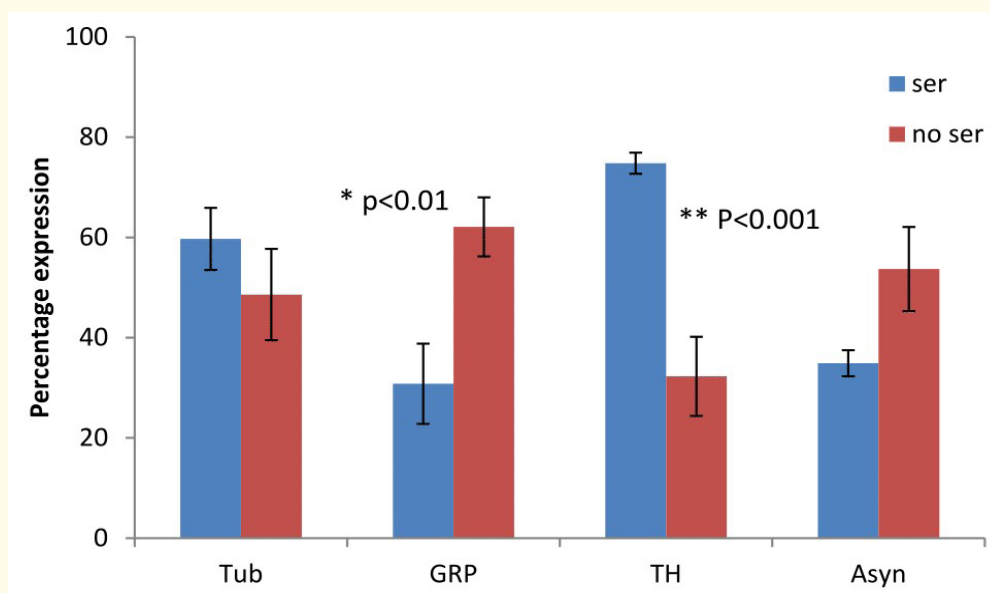
After culturing SHSY5Y cells without serum for 3 days, GRP expression increased significantly from 30.8 % to 62.1% (p > 0.01, shown in columns 3 and 4) . The percentage of cells expressing tyrosine hydroxylase, a marker for dopaminergic neurons, declined from 74.8 % to 32.2%, (p < 0.001). Tubulin, a marker used as control, did not change significantly when cells were cultured with or without serum and no significant change in alpha-synuclein expression was observed in cells cultured in these conditions for 3 days. Immunocytochemistry with specific antibodies was used to determine biomarker expression, as described previously [22].

Figure 3: *Energy depletion modeled with serum withdrawal alters biomarker expression in SH-SY5Y dopaminergic cells.*
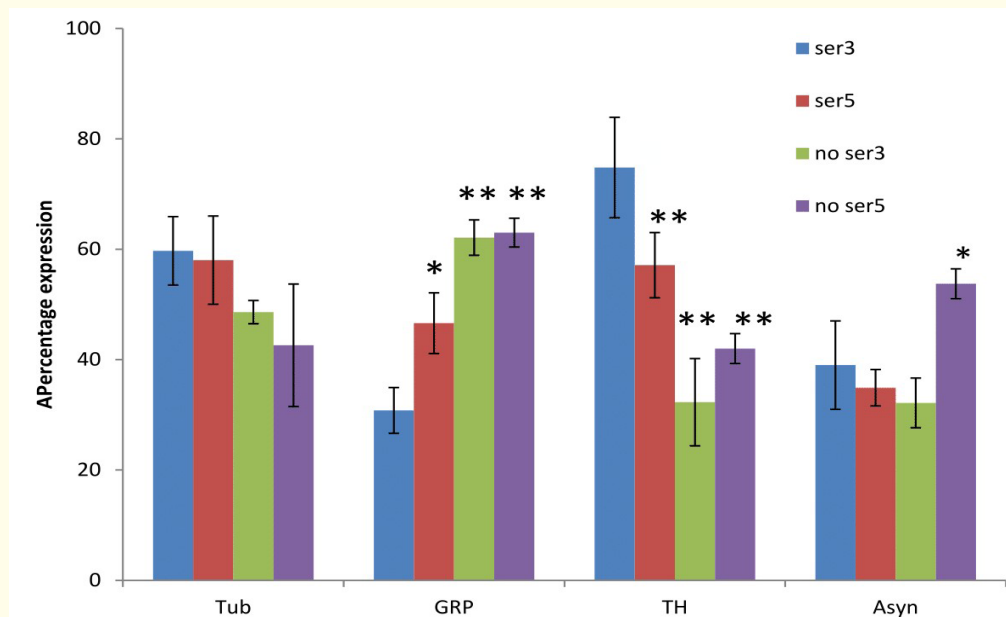
After culturing SH-SY5Y cells with (blue and red bars) and without serum (green and purple bars) for 3 or 5 days GRP expression increased significantly (*p < 0.05 and **p < 0.001 versus ser 3). The percentage of cells expressing tyrosine hydroxylase, a marker for dopaminergic neurons declined overtime and significantly (**p < 0.001 versus ser 3). Tubulin, a marker used as control, did not change significantly when cells were cultured with or without serum and no significant change in alpha-synuclein expression was observed in cells cultured for 3 days, but was significant (P < 0.03) after 5 days when neurons were cultured without serum.

While there are commonalities in age-related neurodegenerative pathways, certain brain regions are more vulnerable to degeneration than others. For instance dopaminergic neurons of the substantia nigra are the first to show signs of PD, while in AD it is the entorhinal cortex. From these areas, the pathology spreads, suggesting it is a molecular event in region specific areas that trigger the process. Freer., *et al.* (2016) correlated gene expression and proteins known to co- aggregate with protein inclusions [20] using data from the Allen Brain Atlas which contains information from 500 regions in six brains from healthy people with Braak disease staging for AD to define where, when and if neurofibrillary tangles develop. They found that genes encoding proteins known to promote aggregation, such as Hsp 70/ Hsp 90, were also active in early Braak regions, compared to others. This exciting observation pinpoints chaperone proteins as the earliest sensors and targets for the prevention of neurodegenerative disease.

Concluding Remarks

Protein aggregation contributes to multiple diseases including complex age – associated central nervous system disorders. Reported aberrations in chaperone proteins of the proteosome are promising targets for therapeutic intervention as are the co-chaperone proteins

they may interact with [21]. Furthermore, laboratory tests with probes to detect chaperones and/or oligomers of aggregates [23] may be useful for early disease detection. Advancing knowledge of heat shock proteins and chaperones offers a growing number of drug targets with potential for complex neurodegenerative diseases with unmet medical need.

Acknowledgements

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