Heat shock proteins (HSPs), known as molecular chaperone to assist protein folding, have recently become a research focus in Parkinson’s disease (PD) because the pathogenesis of this disease is highlighted by the intracellular protein misfolding and inclusion body formation. The present review will focus on the functions of different HSPs and their protective roles in PD. It is postulated that HSPs may serve as protein folding machinery and work together with ubiquitin-proteasome system (UPS) to assist in decomposing aberrant proteins. Failure of UPS is thought to play a key role in the pathogenesis of PD. In addition, HSPs may possess anti-apoptotic effects and keep the homeostasis of dopaminergic neurons against stress conditions. The critical role of HSPs and recent discovery of some novel HSPs inducers suggest that HSPs may be potential therapeutic targets for PD and other neurodegenerative disorders.

Key words: Parkinson’s disease (PD), heat shock proteins (HSPs), ubiquitin-proteasome system (UPS), apoptosis

1. Introduction of HSPs

In the eukaryotic cell, heat shock proteins (HSPs) provide an intrinsic mechanism to defend the cell against external diverse physiological stress that may initiate a cascade of events affecting cell structure and function. The high conservation of HSPs throughout the evolution suggests that these proteins may have a vital role in protecting cells from injury. HSPs are composed of different classes of proteins according to their molecular weight, which include high-molecular-mass HSPs (≥100kD), HSP90 (81 to 99kD), HSP70 (65 to 80kD), HSP60 (55 to 64kD), HSP40 (35 to 54kD) and small HSPs (≤34kD) [1]. Different classes of HSPs play a diversity role in governing proper protein assembly, folding, and translocation [1, 2]. Regulation of these HSPs synthesis creates a unique defense system to maintain cellular protein homeostasis and to ensure cell survival [2].

The current understanding of HSPs’ function is based on two main lines of evidence: (1) the clearance of waste proteins requires protein folding machinery called chaperones [1], and (2) HSPs chaperones bind to denatured proteins to promote their degradation [2]. New evidence suggests that HSPs may actively participate in an array of cellular processes, including cytoprotection [3], and HSPs dysfunction may contribute to the pathogenesis of Parkinson’s disease (PD), a disease characterized by conformational changes in proteins that result in misfolding, aggregation and intracellular Lewy Body formation [4].

This review provides an update view of the cytoprotective role of HSPs in PD and the potential therapeutic target of HSPs for the treatment of PD.

2. HSPs and PD Pathophysiology

Many neurodegenerative disorders, including PD, Alzheimer’s disease (AD), amyotrophic lateral sclerosis (ALS), Huntington disease (HD) and other polyglutamine expansion disorders, are associated with degeneration and death of specific neuronal populations due to accumulation of certain abnormal polypeptides or proteins [4]. PD is a neurological disorder characterized by movement disturbance that mostly results from progressive degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc). Numerous studies implicate that at least two components of cellular proteins are associated with PD: the ubiquitin proteasomal system (UPS) and the HSPs [5, 6]. Transcriptional analysis of multiple brain regions in PD indicates the impairment of multiple electron transport chain complexes and the dysfunction of UPS in PD, along with a robust induction of several forms of HSPs [7]. Inclusion bodies called Lewy bodies with aberrant misfolding and aggregative proteins are common pathological hallmark in PD, indicating that abnormality of protein homeostasis may contribute to the pathogenesis of the disease [5]. Hsp70 and Torsin A, a homology to yeast Hsp104 and mutations of the gene causing dystonia, are colocalized with α-synuclein (αSN) containing Lewy bodies [8]. Further, Dedmon et al. [9] found that Hsp70 could inhibit αSN fibril formation through preferential binding to prefibrillar species to change the characteristics of toxic αSN aggregates. This work therefore elucidates a specific role of Hsp70 in the pathogenesis of PD and supports a general concept that chaperone action is a crucial aspect in protecting against the otherwise damaging consequences of protein misfolding [9]. With ageing, the level of HSPs is decreased insufficiently to keep the cellular proteins homeostasis, which may give rise to certain diseases
EGCG, a major monomer of green tea polyphenols, is

DJ-1

It has been reported that Hsp70 is associated with αSN, dopamine transporter (DAT), parkin, proteasome subunits, ubiquitin and UCH-L1 [18]. Hsp70 is believed not only to protect cells from rotenone-mediated cytotoxicity but also to decrease soluble αSN aggregation [12]. Furthermore, Hsp70 can work as a putative anti-apoptotic factor to protect against neuronal cell death in PD [3, 4]. These results highlight the possibility of using Hsp70 as a potential therapy for PD. Recent studies of function and inducer of Hsp90 also indicate its potential therapy for PD [27, 28].

Hsp90

Hsp90 is the main component of the cytosolic molecular chaperone complex that has been implicated in the negative regulation of the heat shock factor 1 (HSF1). HSF1 is responsible for the transcriptional activation of the heat shock genes including Hsp40, Hsp70, and Hsp90 [29], suggesting a regulatory role in Hsp90 synthesis at the transcriptional level. Hsp90 forms a multichaperone complex with Hsp70 and Hsp40 to regulate several regulatory proteins, such as steroid hormone receptors [30] and transcription factors [31], and to modulate the protein translocation from peroxisomal to organelle [32]. The interplay between these chaperones is of crucial importance for cell function and survival. Recently, Uryu et al. demonstrated that Hsp90 was predominantly increased in PD brains, which was in correlation with the elevated level of insoluble αSN. These alterations of Hsp90 in PD brain were recapitulated by neuropathological findings in αSN mutant transgenic mouse model of PD [27]. Furthermore, exposure of cells to proteasome inhibitors resulted in increased levels of Hsp90 [27].

Microglia, which plays a principal role of inflammation in brain [33], express high levels of Hsp90 following excitotoxic lesion in the mouse hippocampus [34]. The protective function of Hsp90 can be very important since inflammation evoked by microglia may increase the risk of PD. Recently, we have demonstrated that (-)-Epigallocatechin gallate EGCG, a major monomer of green tea polyphenols, is
a potent inhibitor of microglial activation [35]. ECGG could directly bind to Hsp90 and stabilize the complex of Hsp90 [36]. Thus ECGG could be used to alleviate microglia-mediated dopaminergic neuronal injury in PD.

**Hsp70**

Auluck et al. [37] reported that application of Hsp70 can prevent dopaminergic neuronal loss in αSN transgenic Drosophila and interference with endogenous chaperone activity can accelerate αSN toxicity. Furthermore, Lewy bodies in human postmortem tissues were usually immunostained positive for molecular chaperones, suggesting that chaperones may play a role in PD progression [37].

It has been reported that Hsp70 can enhance parkin binding and ubiquitinating of expanded polyglutamine protein in vitro, suggesting that Hsp70 may help recruit misfolded proteins as substrates for parkin E3 ubiquitin ligase activity [38]. This finding provides a direct evidence to show the Hsp70 can promote the activity of E3 ligase to degrade aberrant αSN.

It is postulated that Hsp70 itself or cooperating with other factors can protect the neurons from cytotoxicity caused by aberrant proteins. The crosstalk between the Hsp70 and UPS may provide a clue for the intrinsic mechanism of protein aggregation and degradation. Moreover, Hsp70 exerts anti-apoptotic activity by blocking the function of several key proapoptotic factors [3]. Recently, several studies have demonstrated that Hsp70 may play a role in neuroprotection against rotenone-mediated apoptosis in human dopaminergic cell line SH-SY5Y in vitro and against MPTP-induced nigral injury in vivo by inhibiting the proapoptotic factors as well as activating the survival pathway [39, 40].

**Small HSPs**

Chaperone Hsp25/27 (Hsp25 in mice and Hsp27 in humans), is an inhibitor of actin polymerization [41], which has been demonstrated to play a major role in actin filament dynamics in diverse cell types [3].

In human endothelial cells, inhibition of p38-MAPK activation can abolish Hsp27 phosphorylation, actin polymerization, and cell migration [42]. p38-MAPK may act as an upstream activator of stress-inducible Hsp25/27 phosphorylation. It has been demonstrated that Hsp27 could bind to the microtubule associated protein tau and lead to decreased level of hyperphosphorylated tau and therefore enhance cell survival in AD [43]. Another important function of Hsp27 is its protective effects on mitochondria pathway leading to inhibition of apoptosis [44]. It has been found that Hsp27 can block the TID entering the mitochondria and reduce SMAC and Cytochrome C releasing from mitochondria so as to block the apoptotic process [3].

αB-crystallin Chaperone (Hsp22): Increased expression and abnormal aggregation of small HSPs αB-crystallin has been detected in Lewy bodies and reactive astrocytes in various neurodegenerative diseases [45]. Rekas et al. demonstrated that αB-crystallin was a potent inhibitor of αSN fibrillization in vitro [46]. αB-crystallin may redirect αSN from a fibril-formation pathway towards an amorphous aggregation pathway, thus reducing the amount of physiologically stable amyloid deposits in favor of easily degradable amorphous aggregates [46]. It has been reported that treatment with proteasomal inhibitors MG-132 or lactacystin in cultured rat brain oligodendrocytes can cause apoptotic cell death and induction of heat shock proteins in a time- and concentration-dependent manner [47]. Specifically in this study, αB-crystallin was up-regulated, and ubiquitinated proteins were accumulated. Meanwhile, the tau was dephosphorylated, which enhanced its microtubule-binding capacity [47]. These findings imply that αB-crystallin may work together with other HSPs, ubiquitin and microtubule associated proteins (MAPs) to cope with stressed conditions.

5. Potential Target for the Treatment of PD

Dong et al. [48] reported that Hsp70 gene transferred to dopaminergic neurons by a recombinant adeno-associated virus significantly protected the mouse against MPTP-induced nigral dopaminergic neuronal loss and striatal dopamine levels decline [48]. Hsp70 attenuated MPTP induced apoptosis in the SNpc, and increased amphetamine-induced rotation [48]. Collectively, these results demonstrate that increasing chaperone activity may be beneficial for the treatment of PD.

HSPs may exert protective function through two major pathways besides their own chaperon activity: reducing mitochondrial dysfunction and oxidative stress, and preventing UPS impairment.

**Anti-apoptotic effects of HSPs in PD**

Mitochondrial dysfunction is probably the leading cause of increased oxidative stress and apoptosis in PD. Dopaminergic neurons are more vulnerable to oxidative stress than other neurons because of the special substrate dopamine [49]. In general, apoptotic process can be divided into the three phases: induction (or triggering), transduction of signal, and execution. Theoretically, HSPs may modulate any of these apoptotic phases to rescue the cells [3, 50]. In addition, it has been reported that stable expression of wild-type αB-crystallin protects cancer cells from caspase-3 activation in vitro, indicating that small HSPs αB-crystallin is a novel inhibitor of the activation of apoptosis [51]. (Figure 1) Other gene products linked to monogenic forms of PD also appear to be implicated in mitochondrial dysfunction. Parkin can interact with leucine-rich repeat kinase 2 (Lrrk2) which is part of the mitochondrial outer membrane [52, 53]. Thus Parkin may have an unexpected role in the regulation of normal mitochondrial function in an indirect way [54, 55].

**HSPs may promote the UPS in protein degradation**

The UPS plays a pivotal role in the degradation of short-lived regulatory proteins which are components of cell cycle regulation, cell surface receptors, ion channels modulation, and antigen presentation [56]. (Figure 2) It is believed that once the disposal system fails to work, the substances, such as regulatory molecules p53, NfκB, and Bax that promote apoptosis, may accumulate to a high level that is harmful to the cell [57]. A hypothesis for the
etiology of PD is that subsets of neurons are vulnerable to a failure in proteasome-mediated protein turnover [56]. Accumulation of ubiquitin conjugates has been reported in the pathologic lesions of many chronic neurodegenerative diseases, such as the neurofibrillary tangles in AD and brainstem Lewy bodies in PD [55, 56]. Inhibition of proteasome activity will sensitize dopaminergic neurons to protein alterations and oxidative stress [58].

Hsp90, together with Hsc70, Hsp40, and 20S proteasome subunit are the effective components to capture firefly luciferase during thermal inactiveness and to prevent it from undergoing an irreversible off-pathway [59]. The 20S proteasome has been found in tight association with the molecular chaperone Hsp90 [60]. Composed within 26S proteasome subunit, they form a complex involved in a multitude of intracellular processes [56]. In addition, Kim et al. has demonstrated that the inhibition of proteasome can increase the expression of Hsp27 and Hsp70 [61], implying that HSPs may act as compensation of UPS or work together to regulate the intracellular protein level. Robertson et al. supported the hypothesis by demonstrating that Hsp70 antisense oligomers enhanced proteasome inhibitor-induced apoptosis [62].

All evidences above implicate that HSPs and UPS are participants in keeping proteins folding correctly. They provide an effective protein quality control system that is essential for cellular functions and survival in many tissues. Dysfunction of these systems leads to protein aggregation and inclusion body formation in dopaminergic neurons.

Figure 1: The role of anti-apoptosis of families of HSPs. Most of them function as the inhibitor of the crucial molecule in the apoptosis pathway such as JNK, BAK, Cyto C, caspase-3 and et al. Meanwhile, Hsp27 and Hsp90 can also promote function of AKT to maintain the cell survival.
Figure 2: The Ubiquitin-Proteasome System and the functions of PD associated genes. Parkin works as E3 ligase to help the aberrant protein be poly-ubiquitinated. And UCH-L1 helps hydrolyze the poly-Ub to Ub which can be reused in the next cycle. And when the 26S proteasome is inhibited, HSPs will be induced to compensate the function of protein degradation.

**HSPs inducers and their potential application in PD**

It is proposed that up-regulation of protective factors may benefit our cells, but overload of some proteins may be a burden for cells or even cause cancer. So we need to find better way to keep cells in delicate balance with maximal protective effects and minimal side effects.

Cyclopentenone prostaglandin A1 (PGA1), an inducer of HSPs, has been shown to inhibit SH-SY5Y neuron apoptosis. PGA1 can protect against rotenone-induced neuronal degeneration by promoting the expression of HSPs as well as attenuating nuclear translocation of NF-kappaB and caspase-3 activation [63].

Geldanamycin (GA) binds to an ATP site on HSP90 and blocks its interaction with HSF1 to promote HSF1 activation [64]. GA also sensitizes the stress response within normal physiological parameters to enhance chaperone activity and offer protection against αSN neurotoxicity [65]. Furthermore, GA uncouples neuronal toxicity from Lewy body and Lewy neurite formation so that dopaminergic neurons are protected from the effects of αSN expression despite the continued presence of or even increased inclusion pathology [65].

Significantly, GA does not alter the basal level of HSP70, suggesting that GA acts only to elevate chaperone levels in stressed cells and does not alter chaperone activity in neighboring, healthier cells [65]. Because αSN expression leads to a local elevation of inducible HSP70 in dopaminergic neurons [37], these neurons should be preferentially targeted by GA treatment [65]. Its new derivative 17-Allylamino-17-demethoxygeldanamycin 17-AAG shares its important biological activities with less toxicity [28], which gives us a much bright perspective to use GA to induce specific HSPs expression and to attenuate the side effect.

There is feasibility to use Hsp70 as a pretreatment therapy because there are many nontoxic or low toxic Hsp70 inducers available, such as paeoniflorin [66], bimoclomol (co-inducer to increase the activity) [67, 68], radicicol, and valproic acid (VPA) [39]. These Hsp70 inducers can up-regulate Hsp70 effectively for reconfirmation of the cellular homeostasis. Thus, it is hope that modulates the stress response by inducers can be a promising target for treatment of PD.
6. Conclusion

HSPs have two main cellular functions aimed at promoting the UPS function and inhibiting the apoptotic activity. However, the detailed molecular mechanisms underlying their biological functions are still unclear. It is believed that HSPs, UPS, mitochondria and other organelles may work coordinately to keep the cell in a stable and well-operated state. HSPs are particularly important in PD and other neurodegenerative disorders because aberrant protein aggregation and neuron degeneration are the common pathophysiology of these disorders. Numerous studies in vitro and in vivo model of PD have demonstrated that increase in the expression of HSPs especially Hsp70 by gene transfer or HSPs inducers can reduce the aberrant protein misfolding and inhibit the proapoptotic pathway to attenuate dopaminergic neuron degeneration. Thus such intervention provides a promising therapy for PD. Advance in the research of HSPs targets will shed light on the feasibility of clinical application of HSPs in PD. The future study will focus on finding the mechanisms of aberrant protein aggregation and searching for the selective HSPs inducers for the treatment of PD.

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Conflict of interest

The author has declared that no conflict of interest exists.

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