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Role of the unfolded protein response in the pathogenesis of Parkinson's disease

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Parkinson's disease is the second most common neurodegenerative disease which affects almost 1% of the population above the age of 60. It is is characterized by loss of dopaminergic neurons in the striatum and substantia nigra, coupled with the formation of intracellular Lewy bodies in degenerating neurons. Recent evidence suggests endoplasmic reticulum stress as a common and prominent occurrence in the progression of Parkinson's disease pathogenesis in the affected human brain. One of the cellular defense mechanism to combat endoplasmic reticulum stress due to excessive protein accumulation is through activation of the unfolded protein response pathway. In this review we focus on the impact and role of this unfolded protein response as a causative factor of Parkinson's disease leading to neurodegeneration.

Key words: Parkinson's disease, UPR, α-synuclein, ER stress, mitochondrial stress

INTRODUCTION

Parkinson's disease – prevalence and incidence

Parkinson's disease (PD) is a type of protein misfolding disorder (Soto 2003) which impairs movement and control and thus it is often termed as a 'movement disorder'. It is the second most common age-related neurodegenerative disorder after Alzheimer's and affects 0.6% of the population who are 65–69 years of age and 2.6% of the population between 85–89 years of age (De Lau and Breteler 2006). PD is a multifactorial disease involving a combination of genetic and environmental risk factors of which, aging is the major risk factor of the disease (Bekris et al. 2010). It is characterized by the loss of nigrostriatal dopaminergic neurons and deposits of fibrillary aggregated post-translationally modified (ubiquitinated, phosphorylated, and/or S-nitrosylated) α-synuclein (αSyn) in the brain termed as Lewy bodies (LBs) (Spillantini et al. 1997, Duda et al. 2000). These Lewy bodies are the pathological hallmark of this disease and their distribution in the brain varies from one individual to another. The anatomical distribution of the Lewy bodies is often directly related to the expression and degree of clinical symptoms of each individual. Neuronal loss is aggravated by activation of apoptotic pathways which is a feature of dying neurons and thus leads to the progression of disease symptoms. This neuronal loss results in a severe and gradual depletion of dopamine content in the striatum. Dopamine is a neurotransmitter which relays messages between the substantia nigra (SN) and other parts of the brain thus controlling voluntary movements of the body. High levels of dopamine promote motor activity, whereas lower levels demand greater effort for any given movement (Obeso et al. 2008). Thus, the degeneration of the dopaminergic neurons in the SN leads to the Parkinsonian symptoms.

Clinical manifestations and diagnosis

There are four motor symptoms which are cardinal to PD. They are: rest tremor, slowness of movement (Bradykinesia), rigidity, postural instability (Jankovic and Aguilar 2008). In addition to motor symptoms, PD patients also show some non-motor symptoms such as autonomic dysfunction, cognitive/neurobehavioral

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abnormalities, sensory and sleep disorders. There is no definitive lab test to diagnose PD so the diagnosis of PD is dependent on understanding the disease based on clinical criteria. The gold standard historically used to diagnose PD was the pathological confirmation of the hallmark Lewy body on autopsy. Thus, a thorough understanding of the different clinical manifestations of PD is mandatory to improve diagnosis and identify persons at risk. Ample research has documented that non-motor complications of PD are associated to varying degrees with excess disability and can be used to diagnose PD at the early stages, hence emerging as an independent area of clinical focus and research.

Treatments

The treatments for PD began with 'correcting' the dopaminergic deficit, thereby alleviating the cardinal motor symptoms of the disease. The discovery of dopamine precursor, Levodopa revolutionized the treatment of PD (Carlsson et al. 1957, Barbeau 1969). Several studies have demonstrated the challenges associated with the use of this drug, specifically the development of long-term motor complications, such as involuntary movements (dyskinesias) (Cotzias et al. 1969, Huot et al. 2013). These complications were appreciated and developments in levodopa therapy led to the use of dopamine receptor agonists which lowered the risk of troublesome dyskinesias (Calne et al. 1974, Debono et al. 1976). Modern treatments including drugs such as Levodopa, dopamine agonists, anticholinergics, amantadine and monoamine oxidase inhibitors are effective at managing the motor symptoms (Jankovic and Aguilar 2008, Yuan et al. 2010). Surgery and deep brain stimulation are used as a last resort in severe cases where drugs are ineffective (Hariz 2014). Despite all the investigations done, current treatments are palliative in nature and only aim to improve the quality of life by providing temporal relief from symptoms. This brings us to the point of finding a cure for PD which requires understanding its underlying mechanisms.

Genetics of PD

The genetic basis of PD has also been a widely studied area suggesting that mutations in 6 genes (SNCA, LRRK2, PRKN, DJ1, PINK1, and ATP13A2) have conclusively been shown to cause familial parkinsonism (Bekris et al. 2010). Mutations in the leucine-rich repeat serine/threonine kinase 2 gene (LRRK2/PARK8) occur in 1% to 2% of all PD cases, thereby being the most common known genetic cause of the disease. Autosomal dominant cases of PD has been shown to be caused by point mutations (Ala53Thr, Ala30Pro and Glu46Lys), as well as duplication and triplication in the most widely studied PD-related gene known as SNCA/PARK1(Martin et al. 2011).

Molecular mechanisms

Researchers have been trying to understand the pathogenic processes that are proposed to contribute to the dopaminergic neuron loss in PD. Some of the processes are oxidative stress, impaired calcium homeostasis, mitochondrial (MT) dysfunction, altered endoplasmic reticulum (ER)-to-Golgi trafficking, and altered mitophagy and proteasome function. Growing evidence indicates that oxidative damage and MT dysfunction contribute to the cascade of events leading to degeneration of dopaminergic neurons (Henchcliffe and Beal 2008, Zhu and Chu 2010, Schapira and Jenner 2011). Pathologically, PD is characterized by the accumulation of misfolded proteins (Rao and Bredesen 2004, Hoozemans et al. 2007). The PD neurons are marked by the presence of ubiquitinated protein deposits in the neuronal cytoplasm, called Lewy bodies. These Lewy bodies are largely composed of α-synuclein which is a key role player in this disease. The function of α-synuclein is still unclear but studies have indicated that it plays a role in PD pathogenesis and is likely to involve multiple mechanisms (Sugeno et al. 2008). Familial cases of PD that carry either a missense mutation in the α-synuclein gene or have a duplication of the α-synuclein locus are some of the proofs enough to show that α-synuclein is involved in PD pathogenesis (Chartier-Harlin et al. 2004, Ibanez et al. 2004). The presence of misfolded proteins triggers a cellular stress response in the (ER) called unfolded protein response (UPR) (Xu et al. 2005, Kim et al. 2008, Maly and Papa 2014). Several studies in PD have shown that ER is subject to unfolded protein stress and suggest the involvement of ER-UPR signalling in neuronal death. Sugeno and colleagues (2008) demonstrated that activation of UPR by α-synuclein likely involves multiple mechanisms.

This review highlights the importance of studying ER stress in PD hypothesising that UPRs could possi-

bly be a therapeutic strategy to attenuate ER stress levels which is a common and salient feature of this disease.

UPR – THE ARSENAL OF DEFENCE

The common theme of neurodegenerative diseases such as PD, Alzheimer's disease (AD), Huntington's Disease (HD), amyotrophic lateral sclerosis (ALS) and prion protein diseases is abnormal or misfolded protein aggregation (Kopito and Ron 2000, Taylor et al. 2002, Selkoe 2003).

Protein folding

Proper protein folding is a crucial process which decides the survival of the cell. Romisch, 2004 highlighted that protein folding in general is an inefficient process where 30% of the proteins never acquire their fully folded conformation. The ER is the designated organelle in the cell that folds and modifies secretory and integral membrane proteins. It is also engaged in lipid biosynthesis, calcium storage and protein sorting, thus serving as a 'multifunctional organelle'. The ER environment is highly oxidizing making it suitable for protein folding and maturation. Polypeptides in the cytoplasm are recognized by an N-terminal signal peptide and are co-translationally imported into the ER through the translocon pore, the SEC61 (secretory 61 complex) (Rapoport 2007). Molecular chaperones on the luminal side of the ER meet with the proteins passing through the ER and protein modifying enzymes that fold polypeptides modified by disulfide bond formation and append chemical groups such as glycans to produce fully mature proteins (Braakman and Bulleid 2011). The major chaperones and folding sensors in the ER are: Glucose regulated protein 78 (GRP78, also known as Immunoglobulin binding protein-BiP) and 94 (GRP94), the lectins, calnexin and calreticulin and the thiol-disulfide oxidoreductases, protein disulfide isomerase (PDI) and ERp57 (Schroder and Kaufman 2005, Braakman and Bulleid 2011). GRP78, a 78 KDa ER-resident protein is a member of the Heat shock protein 70 family (HSP70) and interacts with most nascent polypeptides as they enter the ER. It is a misfolded protein sensor/UPR activator. BiP does not actively fold proteins but it maintains the peptides in a folding competent state by binding and preventing aggregation during the folding process (Gething 1999, Schroder and Kaufman 2005). The ER lectin-like chaperone system includes Calnexin and Calreticulin. They promote folding of glycosylated proteins (Braakman and Bulleid 2011, Rutkevich and Williams 2011). PDI catalyzes and aids in disulfide bond formation. These components collectively form the Endoplasmic Reticulum Quality Control system. This system ensures good protein quality and plays a role in converting the protein from its nascent state to native state (Bukau et al. 2006, Araki and Nagata 2011). There are several mechanisms by which cells alleviate aberrant folding. One such mechanism is the degradation pathway called endoplasmic reticulum associated degradation pathway (ERAD). The misfolded proteins are degraded *via* the ERAD pathway but the exact mechanisms by which the proteins are targeted to the ERAD pathway remain unclear. The ERAD targets are then degraded by the ubiquitin proteasome machinery or eliminated by autophagic degradation (Smith et al. 2011).

ER Stress

Various stresses to the ER such as accumulation of unfolded/misfolded/mutated proteins (Hetz 2012, Viana et al. 2012), disturbances in redox status and endogenous reactive oxygen species (ROS) production (Fedoroff 2006), hypoxia (Sawada et al. 2008), hyperglycemia, and hyperlipidemia (Fonseca et al. 2011, Back et al. 2012), disturbances in calcium regulation (Gorlach et al. 2006) and viral infections (Zhang and Wang 2012, Sen et al. 2014) disrupt the ER homeostasis and make it dysfunctional. Under these conditions the ER is said to be under "stress". The toxic build up of unfolded proteins in cells activates the rescuer response known as the UPR (Mori 2000, Kim et al. 2008). Activation of UPR is necessary to combat the toxicity in the cells due to misfolded protein aggregation.

PD is characterized by the accumulation of misfolded proteins. It remains unknown whether these protein aggregates are the cause of cytotoxicity and disease or the by-product of a disease state. There is evidence, however, that the UPR pathways in the ER and in MT are either upregulated or abrogated in many of these diseases. This raises the possibility that the accumulation of unfolded proteins triggers stress-response pathways that induce the neurotoxicity and cell death exhibited in brains of afflicted patients.

ER-UPR

The goal of UPR is to return the ER to its normal functioning state. On activation of UPR, there is a shutdown of global protein synthesis and mechanisms that allow the cell to deal with the accumulation of unfolded proteins get activated.

The cellular responses triggered by UPR include: (1) attenuation of protein translation which is mediated by the serine-threonine kinase PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase (PERK) which phosphorylates the eukaryotic initiation factor 2α (eIF2 α) thereby reducing translation; (2) up-regulation of ER chaperones such as Glucose regulated protein 78 – GRP78/ immunoglobulin heavy chain binding protein – BiP to assist in the refolding of proteins; (3) degradation of misfolded proteins by the proteasome by a process called ER associated degradation (ERAD).

Under chronic stress, (i.e, when the UPR is unable to rescue the cells), the ER stress will lead to apoptosis (Tabas and Ron 2011) *via* an increase in the expression of CHOP (CCAAT/enhancer-binding protein-homologous protein), an apoptotic transcription factor (Wang et al. 1996, Zinszner et al. 1998) or the activation of ER-specific caspases.

The ER stress sensors

Three ER transmembrane proteins have been identified till date in mammalian cells which act as stress sensors and comprise the UPR machinery (Fig. 1) (Schroder and Kaufman 2005, Kohno 2010): (1) PERK; (2) ATF6 (activating transcription factor 6); and (3) IRE1 (Inositol requiring element-1).

The UPR is initiated by the binding of the ER chaperone, GRP78/BiP to misfolded proteins (Harding et al. 1999, Rutkowski and Kaufman 2004). The binding of GRP78/BiP to the misfolded proteins causes dissociation of GRP78/BiP from the three transmembrane proteins (PERK, ATF6 and IRE-1) which consequently are activated (Lai et al. 2007).

PERK pathway activation leads to translational attenuation

PERK pathway is the translational arm of UPR. Upon ER stress, the PERK pathway leads to attenuated rates of translation. PERK is a type I transmembrane serine threonine kinase that appears to be present in most cells (Harding et al. 1999). It exists in an inactive monomeric state by binding to BiP and when the binding is disrupted, PERK homodimerizes and phosphorylates itself (Ma et al. 2002, He 2006). The autophosphorylation of PERK initiates translational arrest by phosphorylation at serine residue 51 of α subunit and inactivating eukaryotic initiation factor 2α (eIF2α) (Harding et al. 1999, Raven et al. 2008) (Fig. 1).

eIF2α

eIF2α is a translation initiation factor and also the most studied and well known substrate of PERK. Phosphorylation of eIF2α results in the formation of a stalled 43S ternary complex that causes general decrease in translation of most proteins, thereby reducing protein load on ER and causing cell cycle arrest (Brewer and Diehl 2000). In 2001, Krishnamoorthy and coworkers (2001), highlighted that eIF2α plays an essential role in translation initiation in its GTP-bound form and its α subunit phosphorylation inhibits the exchange of GDP for GTP thus inhibiting protein synthesis. eIF2α phosphorylation is also known to affect gene transcription (Harding et al. 2000). This is achieved by the translational upregulation of transcription factors such as ATF4 and ATF5 under conditions of high levels of phosphorylated eIF2α, abbreviated eIF2(αP) (Vattem and Wek 2004, Zhou et al. 2008). Specific translation of ATF4 upregulates many important genes that play a role in amino acid metabolism and transport, oxidation-reduction reactions, and ER stress-induced apoptosis (Zhang and Kaufman 2006). Under severe ER stress, ATF4 induces cell death by controlling the transcription of pro-apoptotic BCL-2 family members including PUMA and BIM, in addition to GADD34 and CHOP.

Apart from PERK, the cells have the following three kinases that affect eIF2α phosphorylation under other conditions of stress (Harding et al. 2003): (1) GCN2 responds to amino acid starvation; (2) PKR to viral infection; and (3) HRI to heme depletion.

Thus eIF2(αP) plays a key signalling role in several stress responses and forms part of the gene expression program referred to as the Integrated stress response (ISR). ISR contributes to the overall gene expression program activated by ER stress (Harding et al. 2003).

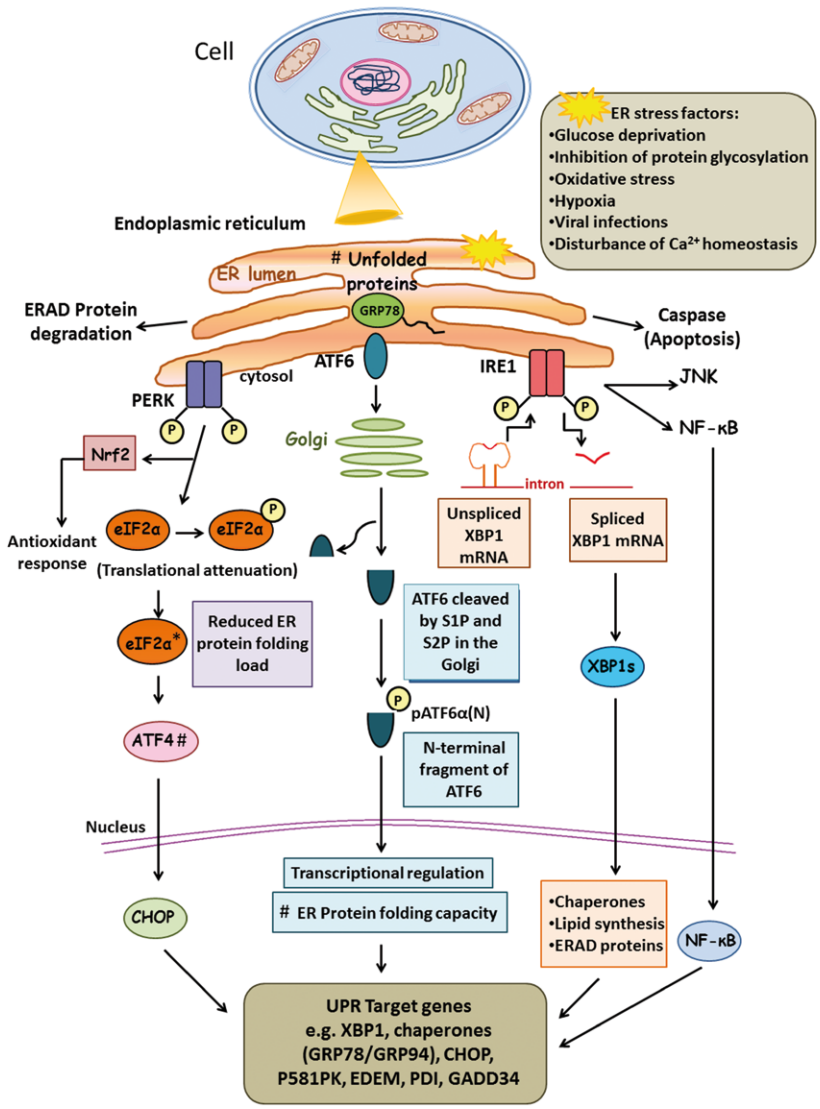

Fig. 1. The UPR. Several factors affect the protein folding in the ER. These include: Energy (Glucose) deprivation, inhibition of protein glycosylation, oxidative stress, disturbances in ER calcium concentration, hypoxia and viral infections. These factors contribute to the accumulation of misfolded or unfolded proteins in the ER leading to the dissociation of the misfolded protein sensor, GRP78/BiP from the three ER stress sensors- PERK, ATF6 and IRE-1. Activation of PERK pathway (left) decreases the general protein synthesis rate *via* phosphorylation of eIF2α. Phosphorylation of eIF2α reduces the protein folding load in the ER and also increases translation of the potent transcription factor, ATF4 which can lead to both protective and apoptotic signalling (*via* activation of CHOP). PERK also regulates other transcription factors like Nrf2 to upregulate the antioxidant response. BiP dissociates from ATF6 (center) and facilitates its translocation to the Golgi by a Golgi Localization signal (GLS) (not shown here). In the Golgi, ATF6 undergoes regulated intramembrane proteolysis (RIP) and is cleaved by the Golgi-resident serine proteases S1P and S2P (site 1 and site 2 proteases respectively). The Cleaved ATF6 (N-terminal fragment of ATF6) acts as a transcription factor, travels to the nucleus and induces transcription of UPR target genes including X-box binding protein 1 (XBP1), CHOP (CCAAT/enhancer-binding protein homologous protein) and molecular chaperones such as GRP78 and GRP94. Thus this arm of the UPR is associated with increase in the protein folding capacity of the ER to cope with unfolded proteins. IRE1 (right) activation is associated with unconventional splicing of XBP1mRNA. The endoribonucelase activity of IRE1converts them from their inactive unspliced form (XBP1u) to the active XBP1 form which then translocates to the nucleus and regulates genes involved in UPR and ER-associated degradation (ERAD). IRE1 signalling also involves JNK signalling (Jun-N- terminal kinase) linking ER stress to cell death and dysfunction and NF-κB signalling pathways. (#) Upregulation; (*) downregulation.

The IRE-1α pathway – transcriptional arm of UPR

IRE1 is a type I transmembrane Serine/threonine receptor protein kinase. Like PERK, IRE1 also functions as a sensor for misfolded/unfolded proteins in the ER lumen. It is the oldest and the most conserved arm of the UPR (Cox et al. 1993). When there is an ER stress, IRE1α gets activated by homo-oligomerization which opens Ser/Thr kinase domain at the cytosolic carboxyl terminal aligning it for trans-autophosphorylation thereby activating the endoribonuclease domain (Shamu and Walter 1996, Liu et al. 2003). This RNAse domain is used to cleave a pre-existing mRNA, HAC1 in yeast (Cox and Walter 1996) and XBP1(X-box binding protein 1) in animals (Yoshida et al. 2001) by an unconventional splicing event which results in a frameshift liberating a 26 nucleotide intron. This produces a spliced mRNA that codes for bZIP-family transcription factor sXBP1 (spliced XBP1) that activates UPR target genes such as the GRP78 gene (HSPA5) and ERAD (Lee et al. 2002, Hetz 2012). Phosphorylated mammalian IRE1 collaborates with modulators and adaptors in the cytosol thus initiating signalling events in response to the intensity and duration of stress. It recruits adaptors like tumor necrosis factor receptor (TNFR)-associated factor 2 (TRAF2) thus activating Jun N-terminal kinase (JNK) (Urano et al. 2000). This activity has been proposed to link ER stress to cell death and dysfunction but this area needs to be further explored.

Regulated IRE1-dependent degradation (RIDD) is a mechanism which works along with PERK in attenuating the load of unfolded proteins that enter the ER. This process makes use of the RNase activity of IRE1 and helps in degrading the mRNAs which encode for the misfolded proteins (Hollien et al. 2009). IRE1α also triggers the activation of other kinases such as extracellular signal-regulated kinases (ERKs) as well as nuclear factor κB (NF- κB) pathways (Fig.1) (Hu et al. 2006).

ATF6 pathway

ATF6 is a type 2 transmembrane protein localized to the ER. On ER stress, ATF6 is activated and is on an expedition from the ER to the Golgi complex, where they undergo regulated intramembrane proteolysis (RIP) by Golgi-resident serine proteases S1P and S2P (site 1 and site 2 proteases respectively) (Fig. 1) (Haze et al. 1999, Ye et al. 2000). The cleaved product is a 50-kDa cytoplasmic b-ZIP (basic leucine zipper)-containing fragment. It moves to the nucleus and activates the transcription of UPR targets such as GRP78, CHOP and XBP1 (Haze et al. 1999, Yoshida et al. 2001). New membrane-bound bZIP transcription factors which had similar proteolysis patterns as ATF6 were identified. OASIS (Old astrocyte specifically induced substance) which is similar to ATF6 has been identified as a transducer of ER stress in astrocytes (Kondo et al. 2005). Similarly, CREB-H (c-AMP response-element-binding protein H) is a liver-specific transcription factor that is cleaved upon ER stress thereby activating expression of acute phase response (APR) genes like those encoding serum amyloid P-component (SAP) and C-reactive protein (CRP) thus correlating between ER stress and acute inflammatory responses (Zhang and Kaufman 2006).

Thus all the three UPR stress sensors are master regulated by GRP78/BiP (Xu et al. 2005). The UPR behaves as a global stress network and when the UPR is unable to regulate the stress, it decides the fate of the cell through a variety of mechanisms which can be apoptotic (Tabas and Ron 2011), non-apoptotic (Ullman et al. 2008), or autophagic (Yorimitsu et al. 2006, Ullman et al. 2008) thereby resulting in cellular demise.

PD AND UPR

PD is a neurodegenerative disorder involving selective loss of dopaminergic neurons in the substantia nigra pars compacta as well other regions of the brain. Neuronal loss is aggravated by activation of apoptotic pathways which is a feature of dying neurons and thus leads to the progression of disease symptoms. This neuronal loss results in a severe and gradual depletion of dopamine content in the striatum. Dopamine is a neurotransmitter which relays messages between the substantia nigra and other parts of the brain thus controlling voluntary movements of the body. High levels of dopamine promote motor activity, whereas lower levels demand greater effort for any given movement (Obeso et al. 2008). Thus, the degeneration of the dopaminergic neurons in the substsantia nigra leads to the Parkinsonian symptoms.

Evidences that UPR plays a role in PD

UPR activation in cellular models of PD

An analysis using *post mortem* samples of brain tissue from patients with Parkinson's disease revealed the activation of the UPR, suggesting the involvement of ER stress (Hoozemans et al. 2007).

The UPR has been implicated in many neurodegenerative diseases such as AD, HD, PD, ALS, progressive supranuclear palsy (PSP) as well as several cancers and a host of inflammatory diseases including diabetes, atherosclerosis, inflammatory bowel disease and arthritis (Table I). Like PD, several other neurodegenerative disorders are characterized by pathological aggregates of misfolded proteins in the brain. Previous work showed that the UPR is activated in post-mortem AD brains (Hoozemans et al. 2009, Stutzbach et al. 2013), as well as in the brains of patients with frontotemporal lobar degeneration with tau inclusions (FTLD-tau) (Nijholt et al. 2012), amyotrophic lateral sclerosis (ALS) (Wang et al. 2010), and multiple system atrophy (MSA) (Makioka et al. 2010). Several of the UPR components are commonly overexpressed in majority of these diseases (Table I). For example, in one study the authors investigated activation of PERK and eIF2α in postmortem brains from subjects with PSP and AD, as well as from normal elderly subjects (Stutzbach et al. 2013) (Table I). The study revealed activation of PERK and its downstream effector eIF2α in disease affected brain regions (midbain, medulla, pons and the brain stem areas) in PSP. In case of AD, strong immunoreactivity was found for phosphorylated PERK and eIF2α in the frontal cortex and the hippocampus. Such expression was absent in most of the normal aged brain controls (Stutzbach et al. 2013). Overexpression of PERK and eIF2α was also found at a higher level in neuromelanin containing dopaminergic neurons in the substantia nigra of PD cases but not in control cases (Hoozemans et al. 2007). The role of UPR in neurodegenerative diseases is initially cytoprotective, however when activation of UPR is sustained over a period of time, apoptotic pathways are upregulated. For example, the UPR has been shown to be involved in cellular models of PD (Fig. 2). The study was conducted using drugs to mimic certain aspects of PD (Ryu et al. 2002, Smith et al. 2005). In order to understand the causes of sporadic PD, Ryu's group was a pioneer in using drugs that mimic the selective dopaminergic neuron degeneration that occurs in this disorder. The agents used by them included 6-hydrozydopamine (6-OHDA), 1-methyl-4-phenyl-pyridinium (MPP^+), and the pesticide rotenone. Several studies have shown that these neurotoxins not only selectively destroy dopaminergic neurons but appear to do so by accessing cellular processes relevant to the naturally occurring disease (Ungerstedt et al. 1974, Langston et al. 1983, Betarbet et al. 2000). Thus understanding the mechanisms by which they act is vital for uncovering pathophysiological events in PD. These agents inhibit the MT electron transport chain resulting in the production of reactive ROS. Cumulatively, they are believed to contribute to neuronal death. They also activate the PERK and IRE1α pathway (Ryu et al. 2002, Holtz and O'Malley 2003). The underlying mechanism used to induce death by these agents requires transcription (Itano et al. 1994, Walkinshaw and Waters 1994, Grunblatt et al. 2000). Gene expression profile analysis using SAGE identified UPR as the major signature engaged by PD-inducing neurotoxins in culture. Dopaminergic cells treated with these drugs triggered the induction of a large number of genes involved in ER stress and the unfolded protein response, such as ER chaperones and elements of the ubiquitin-proteasome system (Ryu et al. 2002, Holtz and O'Malley 2003). These findings confirm the link between PD, the UPR and ER stress.

Genetic models of PD

The pathogenesis of PD has also been linked to genetics. Several studies on the genetic aspects of PD in the past decade from different geographical regions worldwide have strengthened the hypothesis that PD has a substantial genetic component. Several gene loci have been demystified by either linkage analysis or genomewide association studies. A total of 18 PD loci have been nominated through linkage analysis (PARK1-15) or genomewide association studies (PARK16-18) (Gasser et al. 1998, Hicks et al. 2002, Pankratz et al. 2003, 2009, Strauss et al. 2005, Farrer 2006, Belin and Westerlund 2008, Lautier et al. 2008, Di Fonzo et al. 2009, Paisan-Ruiz et al. 2009, Satake et al. 2009, Hamza et al. 2010).

ER stress as a causative factor of PD

Mutations in several genes have been identified to play a role in causing familial parkinsonism. The

Table I

UPR deregulation in neurological (PD not included) and non-neurological disorders

S. No:	Disease	UPR components involved	Mechanism	References
1	Alzheimer's Disease	BiP	Overexpression of BiP	Hoozemans et al. 2005
		PERK, IRE1	Phosphorylated tau colocalizes with PERK and IRE1 in neurons	Hoozemans et al. 2009, Nijholt et al. 2012, Stutzbach et al. 2013
		pPERK, peIF2α	Amyloid β peptide accumulation trigger ER stress leading to phosphorylation of PERK and eIF2α	Katayama et al. 2004
2	Huntington's Disease	CHOP, GRP78 and herp	Mutant huntingtin related to elevated expression of CHOP, GRP78 and herp	Carnemolla et al. 2009, Varshney and Ehrlich 2003
3	Progressive supranuclear palsy (PSP)	pPERK, peIF2α	Increased phosphorylation of PERK and eIF2α	Stutzbach et al. 2013
4	Diabetes	XBP1	Deficiency in XBP1 induces development of insulin resistance	Ozcan et al. 2004
5	NAFLD (non-alcoholic fatty liver disease)	eIF2α, IRE1, ATF6 α	Genetic ablation	Rutkowski et al. 2008
6	Cancer	ATF4, XBP1, GRP78	VEGF, a pro-angiogenic factor has been shown to be up-regulated through ATF4 during ER stress, upregulation of BiP	Jamora et al. 1996, Romero-Ramirez et al. 2004, Roybal et al. 2004, Dong et al. 2008, Zhang and Zhang 2010
7	Retinitis pigmentosa	ATF6, peIF2 α, CHOP	Over expression seen in presence of rhodopsin-P23H mutation	Gorbatyuk et al. 2010
		XBP1 and HRD1	Expression of XBP1 and HRD1 increased in presence of mutant Rh1	Kang and Ryoo 2009
8	Prion disease	pPERK, peIF2α	Misfolded Prion protein (PrP) rises during the disease resulting in progressive increase of pPERK and peIF2 α	Andre and Tabrizi 2012, Moreno et al. 2012
9	Amyotrophic Lateral Sclerosis (ALS)	PDI	The protein level of ER chaperone, PDI was increased and shown to colocalize with aggregated mSOD1 protein	Atkin et al. 2006
10	Inflammatory bowel disease (IBD)	HSPA5, PDIA4, XBP1, PERK, GRP78	Higher protein levels of HSPA5, GRP78 and, PDIA4, increased XBP1 splicing, increased phosphorylation of EIF2α in colonic IBD	Bogaert et al. 2011, Kaser et al. 2013
11	Rheumatoid arthritis (RA)	BIP, synoviolin (ERAD pathway), IRE-1α	Overexpression of Bip and synoviolon in RA synovium, activated IRE-1α in RA synovial fluid	Blass et al. 2001, Yamasaki et al. 2005, Qiu et al. 2013

genes involved in autosomal recessive PD are Parkin (PARK 2), PTEN-induced putative kinase 1 (PINK1, PARK 6), DJ-1/PARK 7 and ATP13A2/PARK 9, whereas genes involved in autosomal dominant PD are α-synuclein (PARK1/4), Leucine-rich repeat kinase 2 (LRRK2/PARK 8) and Ubiquitin carboxy-terminal hydrolase L1 (UCHL-1/PARK 5) (Wider et al. 2010, Omura et al. 2013). Of the several genes being identified, ample research suggests that LRKK2 and Parkin may contribute to dopaminergic neurodegeneration, but the underlying molecular mechanisms remain unclear.

LRRK2 (PARK 8): The gene for PARK 8 was identified as Leucine-rich repeat kinase-2 (LRRK2) in 2004 (also called dardarin, from the Basque word for tremor) because it was identified in families from the Basque region of Spain, Britain, western Nebraska, and in an American kindred of German descent (Paisan-Ruiz et al. 2004, Zimprich et al. 2004). It plays a role in intracellular signalling pathways. Mutation in LRRK2 is known to be the leading genetic cause of autosomal dominant PD. The function of the encoded protein and the mechanism by which LRRK2 mutation contribute to neurodegeneration is largely unknown (Paisan-Ruiz et al. 2004, Zimprich et al. 2004, Dauer and Ho 2010). Currently, there are limited post-mortem data on pathogenic LRRK2 mutations, but the typical LB pathology is seen in most LRRK2-related patients. Over 40 missense or nonsense mutations have been reported in LRRK2 but the pathogenecity of these variants has not yet been determined (Funayama et al. 2002, Zimprich et al. 2004, Mata et al. 2006). Of these, 6 mutations have been evidenced to be disease causing in humans (R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T) (Healy et al. 2008), the most prevalent among these being the G2019S mutation (Fig. 2).

Studies have suggested multiple mechanisms underlying the LRRK2 pathology (Greggio and Cookson 2009, Gehrke et al. 2010). In order to understand these mechanisms several animal models with wild type and mutant forms of human LRRK2 have been generated in nematodes (Saha et al. 2009, Hsu et al. 2010), flies (Liu et al. 2008) and rodents (Tong et al. 2009) and it was found that LRRK2 interacts with components involved in the autophagy lysosomal pathway (Tong et al. 2009) or protein quality control (Ng et al. 2009), modulate oxidative stress (Ng et al. 2009, Saha et al. 2009), regulate protein synthesis (Kanao et al. 2010), and mediate the microRNA pathway (Gehrke et al. 2010). Interesting reports have been achieved from the nematode, *C.elegans* demonstrating that expression of wild-type LRRK2 protects dopaminergic neurons against neurotoxcity induced by either 6-OHDA or human α-synuclein (Yuan et al. 2011). The cytoprotective role exhibited by LRRK2 is attributed to the synthesis of GRP78/BiP, a chaperone playing a key role in promoting cell survival following ER stress, possibly signaling through the p38 pathway (Harding et al. 1999, Yuan et al. 2011). *C.elegans* lacking the LRRK2 homolog develop spontaneous neurodegeneration and hyper-susceptibility to experimental ER stress, a phenotype reverted in a background lacking the worm homolog of the MT serine/threonine kinase PINK1 (Samann et al. 2009). Thus, there is an unexpected functional link between LRRK2 signalling and ER stress response. As discussed earlier, improperly folded proteins are degraded by the ERAD pathway of the UPR. Proteins are thought to be degraded by the two ERAD models for protein degradation: ubiquitin-proteasome ERAD, designated as ERAD (I), and autophagy-lysosome ERAD, designated as ERAD (II) (Fujita et al. 2007, Korolchuk et al. 2010). In both the models, ERAD substrates are translocated to the cytoplasm from the ER with the aid of the Cdc48p–p97 complex. However, in the ERAD (II) pathway, misfolded proteins (both soluble and insoluble) are degraded in autolysosome by utilizing autophagy receptors and adaptors, called ALFY, p62/SQSTM1, HDAC6 and NBR1. These molecules bind to proteins with K63-specific monoubiquitination or polyubiquitin chains in the process of development of the autophagosomes (Behrends and Fulda 2012). Recently the UPR transcription factor, XBP1 was shown to control dopaminergic neuron survival by increasing the number of LC3-positive vacuoles and autophagy regulators like Beclin 1 in the ventral midbrain of $XBP1^{Nes-/-}$ mice which correlated with accumulation of protein aggregates and increase in cell death markers (Valdes et al. 2014). The pathogenic effects of mutant LRRK2 in PD could also be attributed to autophagy. It has been shown that mutation in LRRK2 results in neurons with shorter and less branched processes (MacLeod et al. 2006). LRRK2 mutations have been also shown to cause increased expression of autophagy markers in a cell culture system. The study revealed that neurite morphology was affected by increased autophagy as found by both genetic and pharmacological manipula-

tions (Plowey et al. 2008). However, the exact role of autophagy in LRRK2 associated neuronal death is not clear. Recently, in one study, LRRK2 was found to be degraded by chaperone mediated autophagy (CMA) in the lysosomes unlike its common pathogenic mutant form, G2019S (Orenstein et al. 2013). In the presence of CMA substrates the binding of both the wild type and other pathogenic mutants of LRRK2 proteins to the lysosomes were enhanced leading to disorganization of the CMA translocation complex. In induced pluripotent stem cell-derived dopaminergic neurons, brains of Parkinson's disease patients with LRRK2 mutations, and brains of LRRK2 transgenic mice, increased levels of CMA lysosomal receptor was seen as a response to LRRK2 mediated CMA compromise. The authors argued that such an inhibitory effect on CMA by LRRK2 could result in reduced degradation of α-synuclein leading to toxicity (Orenstein et al. 2013). Apart from LRRK2 gene, a few other genes have been found relating PD to ER stress such as Parkin/PARK 2, the ubiquitin carboxyl-terminal hydrolase UCHL-1/PARK 5, DJ-1/PARK 7. Disruption of ER homeostasis is a common pathological event triggered by the genes linked to PD.

Parkin (PARK 2): Parkin is an E3 ubiquitin ligase (Shimura et al. 2000) expressed in many tissues,

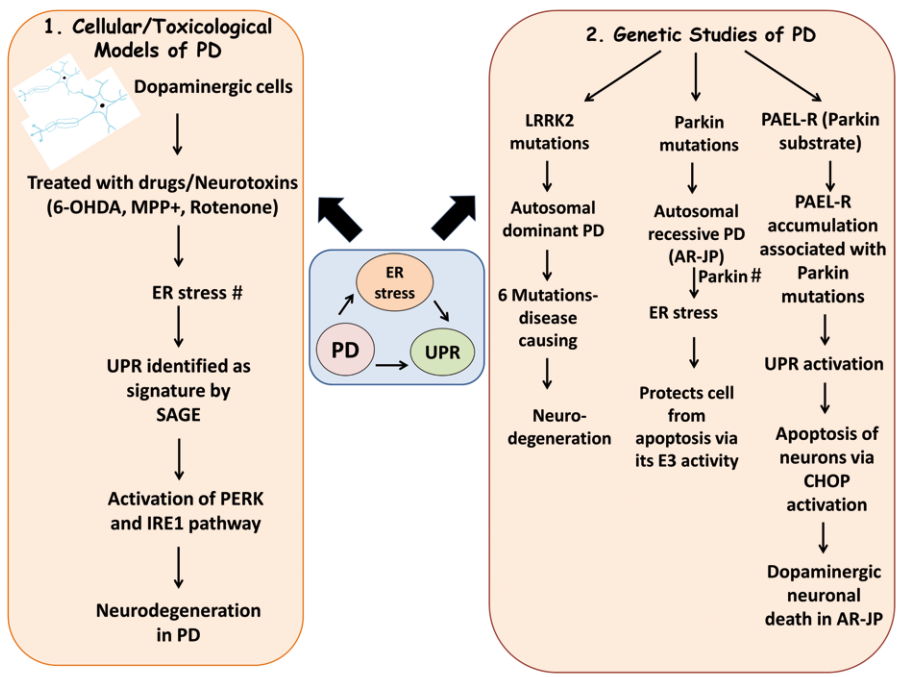

Fig. 2. UPR activation in cellular and genetic models of PD. The figure illustrates the involvement of UPR in cellular/ toxicological and genetic models of PD. (1) Cellular models of PD (Left) were used to show that UPR is involved in PD. Dopaminergic cells treated with neurotoxins such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-pyridinium (MPP^+), and the pesticide rotenone were shown to elevate ER stress and serial analysis of SAGE identified UPR to be the major factor involved in the selective dopaminergic neuron degeneration which is seen in PD. The PERK and IRE1 pathway were involved. (2) Genetic Models of PD (Right) illustrate the fact that PD pathogenesis has a genetic component with evidence that ER stress is a causative factor and also highlight the role of LRRK2 and Parkin mutations and their contribution to neurodegeneration seen in PD. LRRK2 mutations result in autosomal Dominant PD with the mechanism remaining unknown. 6 mutations have been evidenced to be disease causing (R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T). Parkin mutations are associated with Autosomal recessive Juvenile Parkinsonism (AR-JP) and plays a role in tagging proteins for destruction *via* the Ubiquitin Proteasome System (UPS) and thus the mutations generate ER stress during which Parkin is overexpressed. Parkin overexpression can alleviate ER stress by protecting the cell from apoptosis *via* its E3 activity. Studies on PAEL-R are used to understand the link between Parkin and ER stress. Parkin-associated endothelin receptor-like receptor (PAEL-R) is a substrate of Parkin and so mutations in Parkin cause the accumulation and misfolding of PAEL-R resulting in UPR activation and eventually apoptosis of neurons through CHOP activation. This process is responsible for the dopaminergic neuronal death in AR-JP. (#) Upregulation.

including various regions in the brain and the substantia nigra. Familial type of Parkinson's disease called Autosomal recessive Juvenile Parkinsonism (AR-JP) results from mutations of PARK 2 (encoding Parkin) (Kitada et al. 1998). Parkin plays a role in the Ubiquitin proteasome system (UPS) tagging proteins for destruction by the proteasome. Dysfunctional UPS and protein misfolding are some of the key pathological features of PD and activation of UPR seems to be an early event in PD (Hoozemans et al. 2012). Also, since UPS is an essential component of the ERAD pathway it is possible that Parkin or UCHL-1 mutations could also generate ER stress. But most of these observations require *in vivo* validation and remain to be confirmed experimentally. Parkin is usually upregulated in response to ER stress/ unfolded protein stress and protects the cell from undergoing apoptosis *via* its E3 activity (Fig. 2). Parkin overexpression has been shown to reduce ER stress (Imai et al. 2000, Dawson and Dawson 2003) caused by the expression of a polyglutamine peptide. Recent studies have demonstrated that ATF4 plays a protective role in PD by regulating Parkin (Sun et al. 2013). In order to understand the connection between Parkin and ER stress, an under-

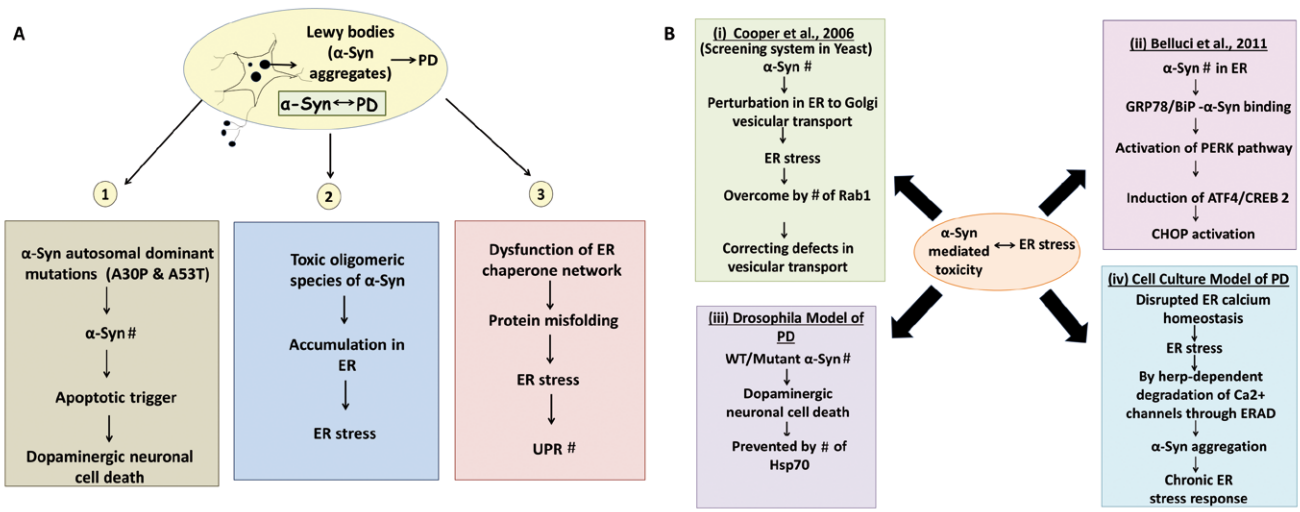

Fig. 3. (A) α-synuclein (α-Syn) and PD. The figure depicts the role of (α-Syn) and other abnormalities linked to it in causing ER stress in PD. (1) Mutations in α-Syn gene particularly the A53T and A30P cause rare forms of autosomal dominant PD. When α-Syn is overexpressed it triggers apoptotic mechanisms thus inducing death of dopaminergic neurons observed in PD pathogenesis. (2) Another factor known to trigger ER stress and UPR activation in PD is toxic oligomeric species of α-Syn. α-Syn oligomers accumulate within the ER leading to the occurrence of ER stress. These were seen in transgenic mice models as well as in the post-mortem human brain tissue from PD patients. (3) Age-related decline in the ER chaperone network which regulates protein folding and monitors refolding of misfolded proteins causes protein to be misfolded and ER to be stressed thereby producing an adaptive stress response known as UPR. (B) Studies linking α-Syn mediated toxicity and PD. The figure summarizes the different studies carried out in PD models that link α-Syn mediated toxicity and PD: Studies using a screening system in Yeast by Susan Linquist's group (Cooper et al., 2006), Investigations by Belluci's group regarding the binding of α-Syn to GRP78/BiP, effects of over-expression of wild type or mutant α-Syn in drosophila model of PD, mechanism contributing to ER stress induced by mutant α-Syn expression in a cell culture model of PD. (i) Studies in Yeast showed that over expression of α-Syn interferes with ER to Golgi transport of vesicles. Any perturbation in this transport imposes ER stress. Rab-1, a small GTPase comes to the rescue and over-expression of Rab-1 corrects the defects in vesicular transport. It also attenuates the dysfunction of dopaminergic neurons over-expressing α-Syn. (ii) Accumulation of α-Syn within the ER results in GRP78/BiP-α-syn binding resulting in the activation of the PERK dependent pathway of the UPR, leading to the induction of ATF4/CREB-2. This drives the cell towards apoptosis *via* CHOP activation. (iii) In a drosophila model of PD, over-expression of wild type or mutant α-Syn triggers dopaminergic neuron cell loss that is prevented by over-expression of Hsp70. (iv) Disruption of the ER Calcium homeostasis is a factor known to cause ER stress. The underlying mechanism is the Herp-dependent degradation of Calcium channels (IP3R and RyR) through ERAD. The imbalance in the ER calcium level leads to aggregation of α-Syn leading to the chronic ER stress response. Such patterns of upregulated stress markers were noted in brain of α-Syn transgenic mice and individuals with PD. (#) Upregulation.

standing of the role of PAEL-R is required. Parkin-associated endothelin receptor-like receptor (PAEL-R) is a putative G-protein-coupled integral membrane polypeptide and also a substrate of ubiquitin ligase Parkin. Parkin mutations are associated with the accumulation and misfolding of PAEL-R causing UPR activation and eventually apoptosis of neurons through CHOP activation (Kitada et al. 1998, Silva et al. 2005). This process is responsible for the dopaminergic neuronal death in AR-JP (Fig. 2) (Imai et al. 2001). Thus the genetic studies mentioned above implicate a role for ER stress contributing to the dopaminergic neuronal death in cellular and toxicological models of PD in mice.

PD and α-synuclein

α-synuclein

α-synuclein is a 14 KDa neuronal protein from a family of structurally related proteins that are highly expressed in the brain (Jakes et al. 1994). It is closely associated with cell membranes on nervous tissue and concentrated at pre-synaptic vesicles and lipid rafts (Fortin et al. 2004). At the pre-synaptic terminals it promotes the assembly of the SNARE complex (Wang et al. 2011). While the function of wild type α-synuclein still needs to be resolved, it is proposed to play a role in neurotransmitter release (Bartels et al. 2011), pre-synaptic vesicle formation (Chandra et al. 2005) as well as protection of nerve terminals against injury (Chandra et al. 2005).

α-synuclein aggregation (A Tale of two conformations-monomers to oligomers)

Growing evidence suggests that α-synuclein aggregates are involved in neurodegenerative diseases such as PD in the form of inclusions called Lewy bodies as well as in other synucleopathies. Universally, α-synuclein is accepted to have a natively unfolded tertiary structure but recent studies have suggested it exists as an α-helical folded tetramer in its native state (Bartels et al. 2011, Wang et al. 2011). But these findings needs to be validated and as of now the main physiological form of α-synuclein in the brain appears to be an unfolded monomer (Fauvet et al. 2012). Aggregation of α-synuclein from monomers to inclusions appears to be a multi-step process. Two or more monomers aggregate to form oligomeric species which are termed as protofibrils (Kalia et al. 2013). The first evidence for the presence of α-synuclein oligomers in PD patients came from their detection in post-mortem samples of the brain (Sharon et al. 2003). More recent studies in PD patients have identified their presence in plasma (El-Agnaf et al. 2006) and cerebrospinal fluid (CSF) (Tokuda et al. 2010). Post translational modification of α-synuclein such as phosphorylation and nitrosylation cause the subsequent misfolding and deposition of this protein (Fig. 3A) (Brundin et al. 2008).

Factors promoting α-synuclein aggregation

Missense mutations in the α-synuclein gene (SNCA) – A53T, A30P, E46K (Polymeropoulos et al. 1997, Conway et al. 1998, Kruger et al. 1998, Zarranz et al. 2004), mutations in which SNCA is duplicated or triplicated (Singleton et al. 2003, Chartier-Harlin et al. 2004), some polymorphisms in SNCA increase α-synuclein protein expression (Mata et al. 2010). Post-translational modifications also influences α-synuclein protein aggregation, especially phosphorylation at Ser 129 promotes aggregation (Fujiwara et al. 2002), whereas ubiquitination or nitration of α-synuclein is associated with reduced aggregation (Kalia et al. 2011), dysfunction of chaperone networks which regulate protein folding and handle detrimental proteins as well as the Ubiquitin-proteasomal system (UPS) and autophagy lysosomal pathway (ALP) responsible for elimination of misfolded proteins (Tyedmers et al. 2010).

α-synuclein and ER stress

Mutation in the α-synuclein gene results in Autosomal dominant form of familial Parkinsonism (Polymeropoulos et al. 1997). Thus, any abnormalities in α-synuclein are mechanistically linked to pathogenesis of PD and other α-synucleopathies such as multiple system atrophy, dementia with Lewy bodies, and pure autonomic failure (Obeso et al. 2010). Aggregation of α-synuclein within dopaminergic cells induces their cell death (Sidhu et al. 2004) and when α-synuclein is overexpressed it triggers apoptotic mechanisms. The mutant form of α-synuclein is known to decrease proteasome activity which leads to cell death (Stefanis et al. 2001, Tanaka et al. 2001). Two mutations in

α-synuclein namely A30P and A53T, cause rare forms of autosomal dominant PD (Fig. 3A) (Polymeropoulos et al. 1997, Kruger et al. 1998).

α-synuclein also activates the UPR pathway (Sugeno et al. 2008, Bellucci et al. 2011). An association between α-synuclein-mediated toxicity and ER stress was demonstrated by studies in Yeast and other cells (Fig. 3B) (Smith et al. 2005, Cooper et al. 2006). Susan Linquist's group used a screening system in Yeast and showed that over expression of α-synuclein interferes with ER to Golgi transport of vesicles. ER to Golgi transport is crucial for secretion and any perturbation caused to this transport imposes ER stress. Rab-1, a small GTPase comes to the rescue and over-expression of Rab-1 corrects the defects in vesicular transport. It also attenuates the dysfunction of dopaminergic neurons over-expressing α-synuclein (Cooper et al. 2006). However, the question whether the ER stress accompanying a mutant α-synuclein expression-mediated block to vesicular transport contributes to the pathophysiology of PD remains unanswered. For this reason, Belluci's group in 2011 investigated whether α-synuclein directly binds to the UPR activator GRP78/BiP within the ER of cells showing α-synuclein aggregates as experimental models of PD. This consequently led to the study of UPR-associated transcription factor ATF4/CREB-2. The research further spearheaded into understanding whether the activation of the UPR pathway, in cells showing α-synuclein accumulation, coincides with one of the central proapoptotic changes: cytochrome c release from the MT (Fig. 3B) (Bellucci et al. 2011). The conclusions drawn from those findings include: (1) Accumulation of α-synuclein within the ER results in GRP78/BiP-α-syn binding resulting in the activation of the PERK dependent pathway of the UPR, leading to the induction of ATF4/CREB-2; (2) The studies also pointed out a novel role for α-synuclein, which may act as a neuronal sensor whose aggregation as a result of ER stress, may in turn activate the ER-stress related response pathways; (3) Activation of UPR by α-synuclein initially displays a beneficial effect but on exposure to stressful conditions such as oxidative damage, MT dysfunction, impaired energy metabolism it drives the neurons to cell death (Bowling and Beal 1995).

In a drosophila model of PD, over-expression of wild type or mutant α-synuclein triggers dopaminergic neuron cell loss that is prevented by over-expression of Hsp70 (Fig. 3B) (Auluck et al. 2002). In some models such as the brain of α-synuclein transgenic mice, ER stress markers are upregulated including the expression of the master regulator BiP, XBP1, CHOP, and ATF4 (Bellucci et al. 2011, Belal et al. 2012, Colla et al. 2012). Another mechanism contributing to ER stress induced by mutant α-synuclein expression was studied using a cell culture model of PD. In this study, the ER stress marker gene, Herp (homocysteine-induced ER protein) was monitored to see if it is functionally upregulated upon α-synuclein over-expression (Belal et al. 2012). Disrupted ER calcium homeostasis led to ER stress which was explained by the Herp-dependent degradation of Calcium channels (IP3R and RyR) through ERAD (Belal et al. 2012). Thus, an imbalance of ER calcium level leads to aggregation of α-synuclein leading to the chronic ER stress response (Fig. 3B). Such patterns of upregulated stress markers were noted in brain of α-synuclein transgenic mice and individuals with PD (Selvaraj et al. 2012).

To summarize, the mechanisms suggesting the involvement of α-synuclein and ER stress include: (1) Certain toxic species of α-synuclein oligomers accumulate within the ER leading to the occurrence of ER stress –the mutant α-synuclein accumulation were seen in transgenic mice models as well as in the post-mortem human brain tissue from PD patients; (2) age-related decline in the ER chaperone network which regulate protein folding and monitor refolding of misfolded proteins; (3) defects in ER-to-Golgi trafficking – impaired vesicle transport from the ER triggers the accumulation of immature proteins; (4) herp-dependent degradation of calcium channels through ERAD.

Thus, the protein α-synuclein is well recognized to contribute to the pathogenesis of PD and with the recent research throwing light on the toxicity of α-synuclein oligomers being toxic to cells, it is paving way for research into mechanisms by which these α-synuclein oligomers cause cell death.

UPR naturally declines in normal healthy brain, but not in PD affected brain

During the normal process of aging, there are evidences to suggest a change in dynamics of the UPR response. Key ER resident enzymes and chaperones which are necessary for correct protein folding has been shown to be impaired during the aging process. For example, Bip expression levels were significantly less in the cerebral cortex of older (22–24-month old)

C57/B6 mice compared with that in young (3-month old) mice (Naidoo et al. 2008). In another study BiP expression was found to be higher in the tissues (brain, lung, kidney, spleen and heart) of young Wistar rats when compared to that of aged animals (Hussain and Ramaiah 2007). The key ER stress respondent PERK was also found to be expressed at a reduced level in the hippocampus of aged rats (Paz Gavilan et al. 2006). Decline in PERK signaling induced GADD34 expression which abolished the translational block imposed by eIF2α phosphorylation leading to synthesis of proapoptotic proteins like CHOP (Hussain and Ramaiah 2007). There is evidence of the induction of CHOP and another proapoptotic protein, caspase-12, in aged rats under stress as compared to young stressed animals, indicating that aged animals are more vulnerable to apoptosis (Paz Gavilan et al. 2006). Several studies further show increased CHOP expression in aged mouse cortex, rat hippocampus and rat cortex (Paz Gavilan et al. 2006, Hussain and Ramaiah 2007, Naidoo et al. 2008). Thus, it seems that the protective adaptive response of the UPR arm shifts towards the more pro-apoptotic signaling pathway due to aging. Not only ER enzymes, but chaperones also get oxidized with age leading to the loss of their function and activity (Nuss et al. 2008). However in one study with human samples, it was observed that immunoreactivity for the UPR activation markers phosphorylated PERK and phosphorylated eIF2aα to be present at a higher level in neuromelanin containing dopaminergic neurons in the substantia nigra of PD cases but not in control cases (Hoozemans et al. 2007). In addition, phosphorylated PERK was also found to colocalize with increased α-synuclein deposits in dopaminergic neurons suggesting a strong association of UPR activation with increased immunoreactivity for α-synuclein (Hoozemans et al. 2007). In another study the authors investigated activation of PERK and eIF2α in postmortem brains from subjects with PSP and AD, as well as from normal elderly subjects (Stutzbach et al. 2013). The study revealed activation of PERK and its downstream effector eIF2α in disease affected brain regions in PSP like the midbain, medulla, pons and the brain stem areas. In case of AD, strong immunoreactivity was found for phosphorylated PERK and eIF2α in the frontal cortex and the hippocampus. Such expression was absent in most of the normal aged brain controls (Stutzbach et al. 2013). Thus it seems that although in healthy aged human individuals the UPR is not active, but in the brains of individuals affected with neurodegenerative protein folding disorders like PD, PSP and AD components of UPR are highly expressed.

PD AND MITOCHONDRIAL UPR

Mitochondrial UPR (MT-UPR) is known to be initiated by MT damage and stress caused by accumulation of misfolded proteins in MT. They have been widely accepted to contribute to the pathogenesis of this disease. Misfolded proteins such as α-synuclein have been demonstrated to localize to and accumulate in MT both in cell models of PD and in post-term brain samples of PD patients (Devi et al. 2008, Shavali et al. 2008). However, it remains to be understood whether the localization of α-synuclein to MT implicates its wild-type function or if it is aberrant and indicates cellular damage. The accumulation of α-synuclein in MT can induce MT dysfunction and apoptosis (Parihar et al. 2008). When the MT is defective, it exerts deleterious effect on cells. Several molecules comes to the rescue of the defective MT. The first line of defense is composed of molecular chaperones and proteases which maintain the correct folding and the number of proteins in MT. The chaperones which aid in protein folding are HSPA9, HSPD1/HSP60 and HSPE1/CNP10 (Horwich et al. 1999). The proteases such as LONP1 and CLPP degrade the proteins that cannot fold into proper tertiary structures. MT-UPR is triggered when the MT chaperones and proteases which regulate protein folding are unable to bear the load of misfolded or unfolded proteins thereby sending a retrograde signal to the nucleus to increase the expression of chaperones and proteases (Fig. 4) (Martinus et al. 1996, Zhao et al. 2002).

The following studies suggest a link between MT-UPR and PD.

Misregulation of PINK1 could affect protein folding in MT (Fig. 4). The accumulation of misfolded proteins is sensed by PINK1 (Jin and Youle 2013). PINK1 interacts with several proteins involved with the MT-UPR and MT quality control including TRAP1 (Pridgeon et al. 2007), HSP60 (Rakovic et al. 2011) and HTRA2/OMI (Plun-Favreau et al. 2007).

The MT protease, HTRA2/OMI has been reported to be involved in clearing unfolded proteins from the intermembrane space of MT in mammalian cells

(Radke et al. 2008, Moisoi et al. 2009). Mice knockouts of HTRA2/OMI leads to the aggregation of misfolded electron transport chain proteins in the MT membrane (Fig. 4) (Moisoi et al. 2009). The absence of HTRA2/OMI results in increased CHOP expression (apoptotic regulator) and MT dysfunction suggesting that it is required for cell survival (Moisoi et al. 2009). The activity of HTRA2/OMI is regulated by phosphorylation by PINK1 (Plun-Favreau et al. 2007). Finally, loss-of-function mutations in OMI/HTRA2 have been associated with PD incidence in a human population (Strauss et al. 2005). Interestingly mutations in the MT folding chaperone HSP70 also associate with PD and expression of these mutant alleles affect MT function in cell models (Fig. 4) (Burbulla et al. 2010).

The data described above suggests a crucial link between MT-UPR and neurodegeneration in PD. However, the exact mechanism or involvement of MT-UPR in PD still needs further investigation. This calls for an intense genetic study on the MT-UPR components and whether mutations in MT-UPR genes correlate with PD.

THERAPEUTIC APPROACHES FOR PD INVOLVING ER STRESS

The main focus of this review is to throw light on the involvement of ER stress in causing neurodegeneration in PD. Therefore, the therapeutic strategies proposed hereby are with reference to UPR and how protein misfolding could be resolved. The potential to target the UPR pathway in neurodegeneration as an alternate treatment strategy has recently been extensively reviewed (Halliday and Mallucci 2014).

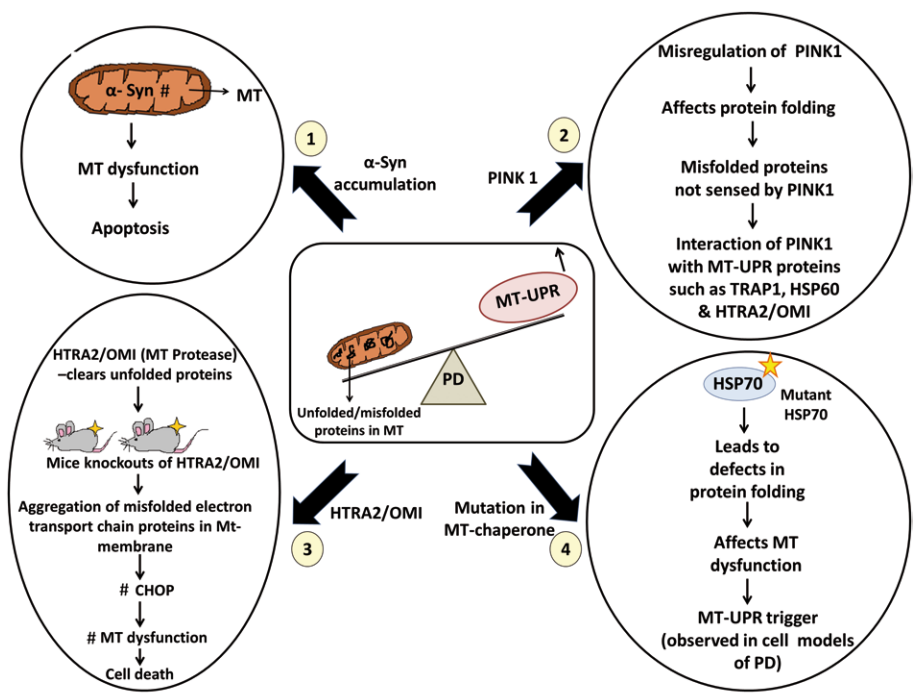

Fig. 4. MT-UPR and PD. The figure represents the various factors that trigger MT UPR as observed in models of PD. These factors are: Accumulation of α-synuclein, Misregulation of PINK1, HTRA Serine peptidase 2 (HTRA2/OMI), Mutation in MT chaperone (HSP70). (1) Accumulation of misfolded α-synuclein in MT can induce MT dysfunction and apoptosis. (2) PINK1 is associated with sensing misfolded proteins and interacting with several proteins involved with the MT-UPR and MT quality control including TRAP1, HSP60, HTRA2/OMI. Misregulation of PINK1 affects this function resulting in MT-UPR consequently leading to cell death. (3) HTRA2/OMI, a MT serine protease is reported to be involved in clearing unfolded proteins from the intermembrane space (IMS) of MT in mammalian cells. Mice knockouts of HTRA2/OMI showed aggregation of misfolded electron transport chain proteins in the MT membrane. The absence of HTRA2/OMI results in increased CHOP expression (apoptotic regulator) and MT dysfunction suggesting that it is required for cell survival. (4) Mutant HSP70 (a MT chaperone) is unable to correct defects in protein folding which leads to MT dysfunction and expression of these mutant alleles affect MT function in cell models. (#) Upregulation.

The proposed therapeutic strategies known are:

(1) Ensuring appropriate protein folding to avoid ER stress. This requires an efficient chemical or molecular chaperone network to promote the appropriate folding of proteins (Rajan et al. 2011). Mimori et al., reported that the chemical chaperone 4-phenyl butyrate (4-PBA) or its derivatives suppress the cell death caused by the accumulation of PAEL-R by ensuring the correct folding of unfolded PAEL-R (Mimori et al. 2012). Studies with 4-PBA also demonstrated that they improve motor deterioration in human α-synuclein A30P/A53T double-transgenic mice (Ono et al. 2009). ER-stress induced neuronal death can also be prevented by the molecular chaperone inducer BiP inducer X (BIX) (Kudo et al. 2008). Thus based on the above findings, it stands clear that chemical chaperones or BIX can be considered useful for the treatment of PD and other neurodegenerative disorders caused by ER stress (Fig. 5A).

(2) This treatment brings to limelight the molecule HRD1 and its role in averting apoptosis in neurodegenerative disease (Omura et al. 2013). HRD1 is a human homolog of yeast Hrd1p/Der3p (Kaneko et al. 2003). It is a RING finger domain containing E3 localized to the ER and is involved in ERAD. It has been noted that the mRNA and protein levels of HRD1 are upregulated in response to ER stress, and HRD1 inhibits its ER stress-induced cell death (Kaneko et al. 2003). Growing research also led to identifying chemicals that promote the expression of HRD1 proteins. The research met with success on identifying the antiepileptic drug, Zonisamide as an upregulator of HRD1 (Omura et al. 2012). The drug has been found to be effective in alleviating the cardinal symptoms of PD and is approved in Japan for use as a low-dose adjunctive therapy for PD patients (Fig. 5B) (Murata et al. 2001, Murata et al. 2007). But the molecular mechanism through which Zonisamide suppresses the progression of PD remains unclear. Similar to PD, in other neurological disorders like Prion and AD, there are enough evidences to suggest the involvement of UPR in disease progression thereby making it a potential therapeutic target. For example phosphorylation of PERK and eIF2α are widely reported to be associated with AD post mortem brains (O'Connor et al. 2008, Nijholt et al. 2011). Recently, Moreno and coauthors (2012) showed that the potential of the UPR as a thera-

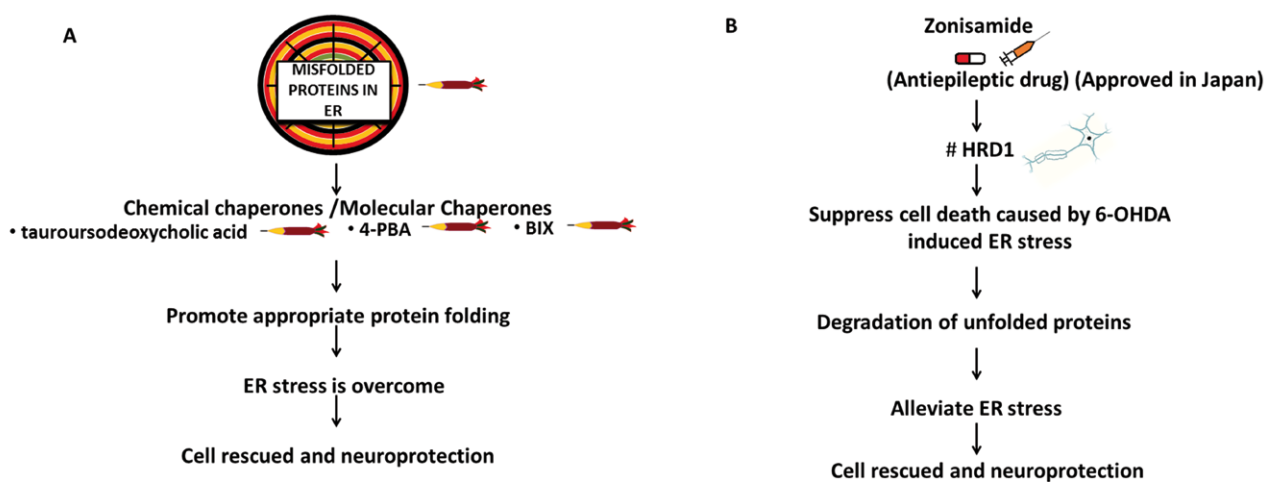

Fig. 5 (A) Targeting Protein misfolding. Misfolded proteins in ER is a major cause of ER stress. An efficient chemical/molecular chaperone network is required to ensure appropriate protein folding in order to overcome the ER stress. The chemical chaperone 4-phenyl butyrate (4-PBA) or its derivatives suppress the cell death caused by the accumulation of PAEL-R by ensuring the correct folding of unfolded PAEL-R (not shown here). ER-stress induced neuronal death can also be prevented by the molecular chaperone inducer BiP inducer X (BIX). (B) HRD1 and apoptosis. This treatment strategy highlights the role of HRD1 in averting apoptosis. Upregulation of HRD1 by the antiepileptic drug, Zonisamide has been found to be effective in alleviating the cardinal symptoms of PD and is approved in Japan for use as a low-dose adjunctive therapy for PD patients. The mRNA and protein levels of HRD1 are upregulated in response to ER stress, and HRD1 plays a role in degrading unfolded proteins by ERAD thus alleviating ER stress, suppressing cell death and leading to neuroprotection. (#) Upregulation.

peutic target in prion disease. In a murine model of Prion's disease, the authors established an increase in the phosphorylation of PERK and eIF2α as the disease progressed (Moreno et al. 2012). Levels of GADD34 were found to be insufficient to dephosphorylate the increased amounts of phosphorylated eIF2α indicating the activation of PERK/eIF2α which in turn might be causing inhibition of protein translation leading to reduction of the synaptic proteins (Moreno et al. 2012). Thus targeting UPR proteins like PERK could be a promising target for drug discovery in neurodegenerative disorders like Prion, PD and AD. Already, pharmacological agents for inhibition of PERK have been developed for use as anti-tumor agents (Atkins et al. 2013). Developing these or any other novel compounds for efficient penetration of the blood brain barrier could pave the way for their use as potential therapeutic agents to these diseases by inhibiting the PERK arm of UPR (Halliday and Mallucci 2014).

CONCLUSIONS

In this review we discuss in detail the most recent evidence linking disturbances of the UPR function to PD pathogenesis and note many interesting, complementary aspects underlying the impact of ER stress on the disease process. Misfolded α-synuclein play a central role in neuronal cell death in PD. Overexpression of mutant forms proteins may for example form cytoplasmic aggregates which can inhibit synaptic function. It could also cause cell death by deregulating key signal transduction pathways and interfering with normal protein degradation by the ubiquitin proteasome complex. Although several studies have established that the UPR is affected in PD, a firm linkage between cell death and UPR cannot be still made. UPR is critical in maintaining normal cell homeostasis and is involved in both cell survival and death. The initial activation of the UPR in PD pathogenesis might have a neuroprotective role, in an effort to clear the neurotoxic unfolded proteins. But prolonged ER stress and UPR activation can overwhelm the protective machinery of the cell, ultimately causing cell death. There are also evidences of direct involvement of the UPR in neuronal death in PD. For example, a recent study elucidated that the initiation of apoptosis coincides with α-synuclein overexpression in PD models (Bellucci et al. 2011). Thus it suggests that the ER-UPR could play an important role in neuronal degeneration as seen in PD. Therefore, dissecting the ER-UPR pathways should be valuable in understanding the pathogenesis of, and ultimately in designing therapy for, neurodegenerative diseases like PD that feature misfolded proteins.

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