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Protein misfolding and neurodegeneration; new approaches to combat toxic misfolded proteins

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ABSTRACT

Important molecular pathways implicated in diverse neurodegenerative diseases are the misfolding, aggregation, and accumulation of the proteins in the brain. Accumulation and misfolding of proteins leads to synaptic dysfunction, neuronal apoptosis, brain damage and disease. The mechanisms which lead to protein misfolding and subsequent neurodegeneration are not clearly understood. The techniques for combating neurodegeneration due to toxic proteins are being investigated. The approach can be two pronged i.e to either do the gene repair using novel techniques like the usage of peptide nucleic acid or to alter the sensitivity or conformation of the receptor proteins or enhancement of enzymes responsible for degradation of these misfolded proteins for e.g ubiquitin-proteosome.

Introduction

The proteins are manifestation of the genetic functions and are central to various biological processes. Misfolded proteins may lead to pre-fibrillar assemblies and and can cause destabilizing mutations on disease proteins. Protein folding has been studied in detail by both experimental and theoretical methods (Soto & Estrada, 2008). Human diseases characterized by insoluble deposits of proteins have been recognized for more than 180 years. The misfolding and aggregation of proteins implicated in neurodegenerative diseases has been modeled *in vitro*. There is no evident sequence or structural homology among the proteins involved in diverse neurodegenerative diseases. This diverse group of diseases includes common disorders such as Alzheimer's disease (AD) and Parkinson disease (PD) and rarer disorders such as Huntington's disease, spinocerebellar ataxia, transmissible spongiform encephalopathies, and amyotrophic lateral sclerosis. The toxic proteins responsible for the various neurodegenerative disorders have been nicely depicted in Table 1 (Bertolotti, 2007). Despite the significant differences in clinical manifestation, neurodegenerative disorders share some common features such as their appearance late in life, the extensive neuronal loss and synaptic abnormalities, and the presence of cerebral deposits of misfolded protein aggregates. Misfolded toxic proteins cause irreversible damage by prolonged ER stress triggers an apoptotic program that includes the induced C/EBP-homologous transcription factor (CHOP) (Kaufman, 1999), activation of c-Jun N-terminal kinases (JNK) (Urano *et al.*, 2000), and cleavage of the UPR specific cysteine protease, caspase-12 (Nakagawa and Yuan, 2000). Recent findings suggest that the intrinsically lower ubiquitin-proteasome system (UPS) activity in neurons is a major contributor to the preferential accumulation of misfolded proteins in neurons seen in various neurodegenerative diseases. Addressing this enigma could help explain the mechanisms behind the selective neuropathology in a variety of neurodegenerative disorders that are caused by misfolded proteins. This review covers these aspects of protein misfolding and neurodegeneration along with strategies to combat these issues for curative purpose.

Protein misfolding and chemical chaperones

There are some important examples of chemical chaperones for preventing damage by misfolded toxic proteins. Probably the best known example of protein misfolding that is responsible for a disease is the ΔF508 mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), which causes cystic fibrosis. The ΔF508 allele of CFTR has been confirmed as a trafficking mutation that blocks the maturation of the protein in the ER and targets it for premature proteolysis (Dalemans et al 1991). However, if the ΔF508 protein is redirected to the cell surface, cAMP-mediated transport can be restored. The clinical importance of this mutation becomes evident when considering that the ΔF508 mutation accounts for nearly 70% of patients diagnosed with cystic fibrosis (Brown *et al* 1996).

When overexpressed in heterologous systems, the ΔF508 mutation leads to the appearance of a small number of functional CFTR Cl2 channels in the plasma membrane. As a result of this observation, it has been proposed that some nascent ΔF508 molecules can fold correctly, thereby escaping degradation. Interestingly, Xenopus oocytes and mammalian cells incubated at reduced (20–30°C) temperatures, express more ΔF508 molecules at the cell surface than those incubated at standard temperatures. At these lower temperatures, a fivefold increase in cAMP-stimulated whole-cell currents was detected.

As relating to the Alzheimer's disease Aβ has emerged as the most promising target in the treatment or prevention of AD. Inhibition of fibril formation might be a reasonable therapeutic strategy because familial mutations that lead to an increase in Aβconcentration or to its aggregation increase neuropathology (Makin and Serpil, 2002). Unfortunately, no effective therapy

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using a chemical chaperone system has been successfully conducted so far. A previous study has shown that osmolytes such as glycerol and trimethylamine *N*-oxide (TMAO), acting as chemical chaperones, correct folding defects by preferentially hydrating partially denatured proteins and entropically stabilize native conformations (Zhao et al 2007).

The Ubiquitin-proteosome system:

Ubiquitin-proteosomes are responsible for degradation of toxic proteins. Decreased proteasome activity is not found in brain lysates of Huntington's disease (HD) mice (Díaz-Herna´ndez *et al*., 2003; Zhou *et al*., 2003) The ubiquitin-proteasome system (UPS) removes damaged or misfolded proteins by ubiquitinating them via ubiquitin ligases and then targeting these ubiquitinated proteins to the proteasome for degradation (Ciechanover, 2005; Demartino and Gillette, 2007). Normal UPS function is particularly important for preventing diseases that are caused by misfolded proteins (Rosenbaum & Gaardenar, 2011). It has been reported by Tydlacka *et al* (2008) that differential activation of UPS may account for accumulation of toxic proteins. In Huntington's disease (HD), selective neurodegeneration preferentially occurs in the striatum and extends to various brain regions as the disease progresses (Difiglia *et al* 1997, Martin and Gusella, 1986). HD is caused by the expansion of a polyQ tract in the N-terminal region of huntingtin (Gusella and Macdonald, 2006), a large protein of 350 kDa that is ubiquitously expressed and interacts with a number of proteins (Li and Li, 2004). Like other polyQ disease proteins, mutant htt also induces selective neurodegeneration. Understanding the mechanism underlying this selective neurodegeneration will help elucidate the pathogeneses of polyQ diseases and other neurological disorders, such as Alzheimer's and Parkinson's diseases, which also show the selective accumulation of toxic proteins in neuronal cells. In the human brain, glia make up the major population (90%) of cells and provide neurons with nutrients, growth factors, and other support. Although mutant htt is also expressed in glial cells (Shin *et al.*, 2005), significantly more neurons than glia contain htt aggregates (Shin *et al*., 2005). As such, neuronal htt toxicity in HD has been better characterized than glial pathology (Li and Li, 2006). Because neurons are postmitotic cells, their ability to cope with misfolded proteins may be different from that of other cell types, such as glial cells, which can proliferate and regenerate (Lee and Yu, 2005). Despite the critical role the UPS plays in clearing misfolded proteins in different cell types, little is known about potential differences in UPS activity in neurons versus glia in the brain. Addressing this issue could help explain the mechanisms behind the selective neuropathology in a variety of neurodegenerative disorders that are caused by misfolded proteins.

Mechanisms of Gene Repair:

DNA-binding proteins play an essential role in many fundamental biological activities, including DNA transcription, packaging and repair (Luscombe et al 2000). Given only the structure of a DNA binding protein, it is of interest to determine the DNA binding protein residues without the knowledge of the associated specific DNA sequence and structure with which the protein interacts and some novel strategies like the Fly casting in protein DNA interaction can help in not just gene repair but also modulating the toxic protein (Gao and Skolnick, 2009; Levy *et al* 2007). On the other hand the single strand breaks (SSB) repair proteins excise terminal blocking groups, permitting gap-filling synthesis and sealing of the final nick in DNA. Zinc-finger nucleases (ZFN) link a DNA binding domain of the zinc-finger type to the nuclease domain of Fok I and enable the induction of doublestrand breaks (DSBs) at preselected genomic sites (Rolig and McKinnon,2000; Tyagi and Tyagi, 2010). DSBs closed by the error-prone, nonhomologous endjoining (NHEJ) DNA repair pathway frequently exhibit nucleotide deletions and insertions at the cleavage site. This technology has been introduced to knockout mutations into the germ line of rats and zebrafish by the expression of ZFPs in early embryos that target coding sequences (Davis and Stokoe, 2010). This technology can be used for possible treatment of neurodegenerative disorders. On the other hand PNAs are DNA mimics with a pseudopeptide backbone able to form stable duplex structures with Watson–Crick complementary DNA, RNA (or PNA) oligomers to promote helix invasion in duplex DNA (Tyagi, 2011). Furthermore, the binding affinity of PNAs to DNA targets is stronger than that of DNA/DNA interaction. This enhanced binding affinity is partially due to the uncharged property of the PNAs that diminishes the electrostatic repulsion formed from DNA/ DNA duplexes. As a result, the length of oligonucleotides necessary to achieve maximal effects is also significantly reduced. In most of the hybridization studies, PNAs with lengths of 12–18 nucleotides are sufficient to form strong duplex formation and to distinguish single base mutations. In this report, they show that PNA–ssODNs can induce stable single base pair alterations in the dystrophin gene and result in a significant increase in the level of dystrophin being expressed when are sufficient to form strong duplex formation and to distinguish single base mutations (Kayali *et al* 2010).

In this report, it was shown that PNA–ssODNs can induce stable single base pair alterations in the dystrophin gene and result in a significant increase in the level of dystrophin being expressed when compared with oligonucleotides made of unmodified bases.

Rescue of receptor proteins:

Several strategies, including genetic, chemical and pharmacological approaches have been shown to rescue function of trafficking-defective misfolded G protein-coupled receptors. Among these, pharmacological strategies offer the most promising therapeutic tool to promote proper trafficking of misfolded proteins to the plasma membrane. As with other protein molecules, structural alterations induced by mutations or genetic variations in the gene sequence of GPCRs may lead to abnormal function of the receptor and, subsequently, to disease, depending on the location and the nature of the substitution or modification. Mutations in these receptors are known to be responsible for a large number of disorders, including cancers, heritable obesity and endocrine disease, which underlines their importance as therapeutic targets. These structural alterations may provoke either gain- or loss-of-function of the affected receptor (Milligan, 2007; Ulloa-Aguirre & Conn, 2004). The GnRHR is one of the smallest GPCRs (328 amino acids in the human and most non-rodent mammals; 327 in rat and mouse sequences); it may be close to the "limit" size, containing only the bare essentials required for ligand binding and signal transduction. There are technical advantages for working with such small proteins, since these require fewer primers for synthesis and for sequencing than do larger GPCRs (typically twice the size of the GnRHR). In fact, over 20 years, scientists have successfully created and characterized a library of hundreds of useful naturally occurring or laboratory manufactured GnRHR mutants and epitope and fluorescently

tagged chimeras that have been extremely useful in studying receptor routing (Jardon-Valadez *et al.*, 2009;Ulloa-Aguirre, Janovick, 2009) In addition, naturally-occurring mutants of the GnRHR system are frequently located in similar regions *(i.e.*associated with export motifs) as those reported for other GPCRs (Ulloa-Aguirre & Conn, 2004). The relatively small size of the GnRHR also presents fewer domains to consider in identification of important structural motifs and because the size of hydrophobic domains is relatively constant, the ratio of these to nonhydrophobic regions is relatively high in the GnRHR due to the short amino and carboxyl tails (Jardon-Valadez *et al*., 2009). As in the case of the V2R and rhodopsin, the relatively small size of the GnRHR has allowed us to understand a great deal of its structure, including the mechanism of action of several mutants (P. M. Conn et al., 2007). The size of these receptors, might explain why they are the most frequenly affected among the GPCRs superfamily by mutations leading to ER trapping and disease (Tan, Brady, Nickols, Wang, & Limbird, 2004).

Pharmacoperone approach to tackle toxic proteins?

Pharmacological chaperones or "pharmacoperones," are small, often lipophilic compounds that enter cells, bind selectively to biosynthetic intermediates or conformationally defective proteins to influence folding and allow correct routing to their final destination in the cell (Welch and Brown, 1996). This novel approach can also be utilized to tackle the toxic misfolded proteins and make them inert after receptor binding to the rescued receptor. This concept although a novel proposition needs to be experimented and tried out by intensive research at molecular level. Frequently, such molecules were initially identified as peptidomimetics from high throughput screens for antagonists or agonists and may come from different chemical classes. Because such peptidomimetics interact with proteins to which they are selectively targeted, it has been the first place where many investigators have started in the search for agents that bind to and stabilize G protein-coupled receptors (GPCRs) (Bolen and Baskakov, 2001). *In vitro* and *in vivo* studies have demonstrated that pharmacoperone rescue may apply to a number of diseases, including inherited metabolic disorders (e.g. Pompe disease), cystic fibrosis, hypercholesterolemia, cataracts, phenylketonuria, neurodegenerative diseases (e.g. Alzheimer's, Parkinson's, and Huntington's), and cancer as well as to GPCRs-related diseases such as retinitis pigmentosa, nephrogenic diabetes insipidus, and hypogonadotropic hypogonadism, among others (Yang et al 1999; Zhao *et al* 2007).

Conclusion : In conclusion it can be stated that the common feature of several neurodegenerative diseases is the presence of toxic protein aggregates or inclusions attributable to the accumulation of misfolded proteins in selective brain regions. These misfolded proteins predominantly accumulate in neurons, despite the fact that neurodegenerative disease proteins are widely expressed throughout the brain and body. By monitoring the structural correctness of newly synthesized proteins, and prevent accumulation of defective (misfolded) proteins that may potentially accumulate, aggregate and interfere with normal cell function newer strategies can be evolved. The scrutiny by the ER relies on conformational features of the protein rather than on functional criteria, so even minor alterations in the secondary or tertiary structure of a protein may lead to intracellular retention and degradation. A variety of mechanisms operate at the ER to identify and sort proteins according to their maturation status. These mechanisms include specialized folding factors, escort proteins, retention factors, enzymes, and members of major molecular chaperone families. Newer strategies to combat the fallout of these misfolded proteins are discussed.

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Table 1 : Toxic proteins and neurodegenerative disease

Disease	Protein	Toxic protein	Disease genes	Risk factor
Parkinson's linkage	Lewy proteins	α-synuclein	α-synuclein	tau disease
Alzhemimer's disease	extracelluar plaques	Aβ	APP Presenilin 1 Presnilin 2	ApoE4 allele
	intracellular tangles			
Prion plaque	PrPsc	PRNP	Homozygosity	Prion disease at prion codon 129
tauopathy	cytoplasmic tangles	tau	tau	tau linkage
polyglutamine	nuclear and cytoplasmic inclusions	polyglutamine containing proteins	9 different genes with CAG repeat expansion	
Familial amyotrophic lateral sclerosis	Bunina bodies	SOD1	SOD1	

*Misfolded toxic proteins and neurodegenerative disease (Courtesy, Anne Bertoloti, EMBO, 2007)