The Role of Mutant Glucocerebrosidase and α-synuclein Oligomerization in Neurodegeneration: Linking Gaucher’s Disease and Parkinson’s Disease

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Gaucher’s disease is a pathology associated with intracellular accumulation of glucosylceramide due to glucocerebrosidase dysfunction. Gaucher’s disease, type I in particular, does not usually present with neurologic components; however, researchers and physicians have recently noted an increased incidence of Parkinsonism in patients with type I Gaucher’s. Parkinson’s disease is a pathology affecting dopaminergic nerve tracts in motor cortices, most notably the substantia nigra. The disease is microscopically characterized by the presence of α-synuclein inclusions (Lewy bodies) in dopaminergic neurons. In an attempt to link the pathologies at a biomolecular level, researchers utilized numerous strategies, all culminating in several common observations: mutations of glucocerebrosidase, mutations of α-synuclein, and lysosomal dysfunction. Accumulated mutations and lysosomal dysfunction result in a complex feed-forward mechanism leading to aberrant ER-Golgi function. This review aims to highlight the previously unknown mechanism of Gaucher-linked Parkinsonism and to shed light on the future direction of linking and treating similar pathologies.

INTRODUCTION

Observing an increased incidence of Parkinsonism in patients with type I Gaucher’s disease

Researchers have spent considerable amounts of energy in an attempt to link two pathophysiological events, α-synuclein aggregation and glucocerebrosidase dysfunction. α-Synuclein is a highly acidic, ubiquitous protein that serves numerous functions in the central nervous system, including acting as a chaperone protein in the synthesis of soluble NSF attachment protein complexes and mediating vesicle trafficking in the neuronal Golgi apparatus (Burré et al. 2010; Cooper et al. 2006). Due to the prevalence of α-synuclein, any pathology affecting α-synuclein metabolism or distribution could have dire pathophysiological consequences. The intracellular accumulation of normal and misfolded α-synuclein is implicated in Parkinson's disease, dementia with Lewy bodies, and other synucleinopathies (Auluck et al. 2010; Brockmann et al. 2011; Choi et al. 2011).

Hypoglucocerebrosidemia or absence of functional glucocerebrosidase is implicated in the development of Gaucher’s disease (Mazzulli et al. 2011). Gaucher's disease is the most common autosomal recessive lysosomal storage disorder (Dawson and Dawson 2011). It is pathologically characterized by intralysosomal and leukocytic accumulation of glucosylceramide and other glycosphingolipids (Guschina et al. 2011; Latsoudis and Papapetropoulos 2010; Leverenz et al. 2009; Lopez and Sidransky 2010; Manning-Boğ et al. 2009; Neumann et al. 2009; Nishioka et al. 2011). Three variants of Gaucher's disease exist as a result of over 200 characterized mutations in GBA1 on chromosome 1q22 (Cullen et al. 2011). Each variant of the disease manifests in a slightly different manner. This paper will focus on type I Gaucher's disease, common among the Ashkenazi Jews. Type I Gaucher’s disease is the non-neuropathic variant that results in hepatomegaly, splenomegaly, thrombocytopenia, and leukopenia, although most patients remain asymptomatic (Rosenbloom et al. 2011). Current research is focused on elucidating a common biomolecular link between Parkinson's disease and type I Gaucher's disease. Patients with the type 1 variant of Gaucher's disease preferentially develop an atypical, severe form of early onset Parkinson's disease (Rosenbloom et al. 2011).

The focus has been placed on linking mutations in the glucocerebrosidase gene GBA1 with accumulation of α-synuclein in neuronal tissue, particularly dopaminergic neurons in the substantia nigra pars compacta, in hippocampal layers CA2–4, in the locus ceruleus, in glutaminergic neurons, and in other motor cortices (Auluck et al. 2010; Goker-Alpan et al. 2010). Prevailing hypotheses put forward by the leading research groups are that an etiological toxic gain-of-function mutation in GBA1 results in a complex feed-forward mechanism in which accumulation of α-synuclein and complex lipids block ER-Golgi glucocerebrosidase transport and contributes to lysosomal dysfunction (Dawson and Dawson 2011; Gasser 2009; Parnetti et al. 2009; Sardi 2011; Velayati et al. 2010; Yap et al. 2011). The pathophysiologic mechanism eventually results in neuronal death with Parkinson-like sequelae (Mazzulli et al. 2011). The goal of this review is to highlight the progress of research linking Gaucher’s disease. Novel methodologies coupled with commonly utilized technologies have shed new light on linking Gaucher’s disease and Parkinson’s disease in a way other researchers examining similar pathologies could benefit from. After understanding the complex biochemical aetiology, researchers can redirect their focus to developing novel therapeutics targeting

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the various pathways leading to Parkinsonism secondary to Gaucher's disease.

**Glucocerebrosidase**

Glucocerebrosidase is an important enzyme present at different sites throughout the body. There are three glucocerebrosidase isozymes that are found within *Homo sapiens*: GBA1 is located in lysosomes, GBA2 is an isoform found in bile, and GBA3 in the cytosol (Nagase et al. 2000). Due to the glucocerebrosidase gene's proximity to two pseudogenes and the metatexin gene on chromosome 1q22, recombinant alleles occur (Díaz-Font et al. 2003). Genetic studies have demonstrated the polymorphisms causing Gaucher's disease arise as a result of gene conversion or irregular crossing-over between *GBA* and *GBAP*, the glucocerebrosidase gene and pseudogene respectively (Díaz-Font et al. 2003). The normal function of glucocerebrosidase in lysosomes is to catalyze the glycolipid glucocerebroside to glucose and ceramide as a final step in glycoshingolipid catabolism(Goker-Alpan et al. 2010). Glucocerebrosidase dysfunction is implicated in lysosomal storage disorders, most notably Gaucher's disease (Aerts et al. 2011). Several glucocerebrosidase mutations have been identified and linked to premature development of Parkinson's disease. Mutations commonly occur in the Ashkenazi Jewish population and typically manifest as N370S and L444P (Brockmann et al. 2011).

**α-Synuclein**

α-Synuclein is an acidic 140 amino acid protein encoded by the SNCA gene (Auluck et al. 2010). α-Synuclein is typically found as a membrane-bound protein primarily in presynaptic junctions in an α-helical form (Auluck et al. 2010; Cookson 2009). α-Synuclein, when not bound to a membrane, exists in an unfolded state (Cookson 2009). Interactions with the negatively-charged phospholipid bilayer occur due to the numerous cationic lysine residues (Auluck et al. 2010). α-Synuclein serves a myriad of functions—dopamine release and transport, fibril formation of microtubule-associated protein tau, and inhibition of caspase 3, one of the canonical executioner caspases (Goers et al. 2003; Goker-Alpan et al. 2010). α-Synuclein contains three domains, each with a specific function (Waxman et al. 2009). The non A-β component of Alzheimer disease amyloid plaque domain (NAC domain) is responsible for fibril formation in various synucleinopathies (Waxman et al. 2009). The next proximal region is the hydrophobic domain (Waxman et al. 2009). The hydrophobic domain of α-synuclein (A71-82) is responsible for membrane binding (Waxman et al. 2009). The hydrophobic domain plays a role in fibrillization and toxicity in vitro and in vivo (Auluck et al. 2010). The C-terminal domain regulates oligomerization and filamentous diameter (Waxman et al. 2009). Post-translational modification of α-synuclein is altered in synucleinopathies and in Gaucher-linked Parkinson's disease (Figure 1); phosphorylation at Ser129, tyrosine residue nitration, and dityrosine cross-linking are implicated in formation of insoluble α-synuclein fibers (Okochi et al. 2000). Approximately 90% of α-synuclein involved in proteinaceous pathologies are phosphorylated at Ser129 (Okochi et al. 2000).

Pathophysiologically, α-synuclein fibrils are found in β-amyloid plaques, Lewy bodies, glial inclusions, and axonal spheroids (Auluck et al. 2010). Accumulation of α-synuclein leads to the development of a synucleinopathy (e.g., Alzheimer's disease and Gaucher's-linked Parkinson's disease) but are not directly responsible for cytotoxicity. α-Synuclein oligomerization has also been implicated in inhibiting ER-Golgi transport, impaired synaptic vesicle release, mitochondrial dysfunction, and inhibition of chaperone-mediated autophagy (Figure 1) (Cookson 2009). Studies have shown that misfolded forms of α-synuclein are implicated in neuronal cytotoxicity (Auluck et al. 2010). In Gaucher-linked Parkinson's disease, α-synuclein preferentially accumulates in the dopaminergic neurons of the substantia nigra pars compacta (Figure 2) (Sidhu et al. 2004). Irreversible cytotoxicity in neurons eventually results in progressive neurodegeneration (Sardi 2011).

There are several keystone mutations in α-synuclein that have been shown to abrogate or enhance cytotoxicity.

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**Figure 1.** The left side of the figure shows key players in the process of Lewy body formation in neurons. The central panel illustrates the theorized pathway leading to α-synuclein oligomerization and Lewy body formation. α-synuclein exists in a helical form when bound to membranes and in an unfolded state when unbound. An equilibrium is proposed to exist between the two forms of α-synuclein. The red arrow demonstrates the effect of glucocerebrosidase dysfunction on formation of α-synuclein oligomers. Research groups have demonstrated that under Gaucher's disease conditions, α-synuclein monomers oligomerize and form fibrils. The fibrils eventually form Lewy bodies in neurons. The right side of the figure shows the ramifications of α-synuclein oligomerization. Adapted from Cookson 2009.

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**Figure 2.** This figure illustrates the dopaminergic neurons of the substantia nigra pars compacta. Irreversible cytotoxicity in neurons eventually results in progressive neurodegeneration (Sardi 2011).
Biochemical analysis of the A53T mutation show that the hydrophobic core (Δ71-82) is expanded from eleven amino acid residues to thirty amino acid residues (Auluck et al. 2010). Expanding the hydrophobic core destroys the normal thermodynamic stability of the α helical domain in amino acids 51-66; β-sheets are subsequently adopted as the most stable conformation (Auluck et al. 2010). An expanded hydrophobic core mediates oligomer formation due to α-synuclein's propensity to form β-sheets (Auluck et al. 2010). The A30P mutation in α-synuclein results in decreased β-amyloid plaque formation (Yonetani et al. 2009). The prevailing hypothesis concerning the ability of A30P to reduce plaque formation lies within an equilibrium shift from free α-synuclein monomers to oligomers (Yonetani et al. 2009). E46K mutations in α-synuclein are thought to increase the ability of charged residues in α-synuclein to interact with the negatively-charged phosphate heads of the phospholipid bilayer (Rospigliosi 2009). Increased membrane interaction is thought to facilitate the formation of dityrosine cross-linked dimers (Rospigliosi 2009).

**Figure 2:** The formation of α-synuclein oligomers forms a self-maintained neurotoxic cycle through downregulation of tyrosine hydroxylase (TH) activity, vesicular monoamine transporter 2 (VMAT2) activity, and dopamine transporter (DAT) activity. Subsequently, free dopamine (DA) is oxidized, forming reactive oxygen species (ROS). The genesis of ROS propagates the formation of α-synuclein fibrils. **Retrieved from Sidhu et al. 2004**

**Glucosylerceramide**

Glucosylerceramide is an amphipathic glycolipid composed of glucose and ceramide (Jennemann et al. 2005). Glucosylerceramide, as well as other glycosphingolipids, are an integral component of neuronal membranes in the central nervous system and are essential for proper brain development (Jennemann et al. 2005). Normal degradation of glucosylerceramide is mediated by the enzyme glucocerebrosidase. Glucocerebrosidase hydrolyzes the β-glucosidic bond between the fatty and sugar moieties of glucosylerceramide. Dysfunctional glucocerebrosidase results in a buildup of glucosylerceramide. Glucosylerceramide preferentially accumulates in macrophages and is internalized after receptor-mediated phagocytosis (Kok et al. 1989). A histological description of a "crinkled paper" morphology is commonly used to describe Gaucher cells (Zhang et al. 2011). The presence of glucosylerceramide has been demonstrated to stabilize the formation of α-synuclein oligomers (Choi et al. 2011). Glucosylerceramide accumulation in neurons and macrophages in Gaucher’s patients leads to neuronal death, particularly in dopaminergic neurons (Hisaki et al. 2004).

**Principal Approaches**

**Protein/Lipid Identification and Confirmation: Non-fluorescent Methods**

Most research groups utilized sodium dodecyl sulfate polyacrylamide gel electrophoresis to identify and quantitatively size target proteins—α-synuclein, glucocerebrosidase, ubiquitin, prosaposin, cathepsin D, lysosomal associated membrane protein 2 (LAMP2), hsc70, glucocerebrosidase, and glucosylphosphingosine (Alvarez-Erviti et al. 2010; Choi et al. 2011; Guschina et al. 2011; Manning-Boğ et al. 2009; Mazzulli et al. 2011; Neumann et al. 2009; Nishioka et al. 2011; Sardi 2011; Segarane 2009). α-Synuclein, glucocerebrosidase, ubiquitin, prosaposin, glucosylerceramide, glucosylphosphingosine, and cathepsin D are proteins involved in glycolipid catalysis, membrane structure, and protein degradation (Guschina et al. 2011; Manning-Boğ et al. 2009; Nishioka et al. 2011; Segarane 2009) LAMP2A and Hsc70 are autophagy-related proteins (Alvarez-Erviti et al. 2010). Several research groups used slightly different methods to achieve protein and glycolipid separation. These include native size exclusion chromatography, liquid chromatography, anion-exchange chromatography (due to the acidic nature of α-synuclein), NMR, 2D-TLC, electrospray ionization mass spectrometry, and gas chromatography (Guschina et al. 2011; Mazzulli et al. 2011; Sardi 2011; Xu et al. 2011; Yap et al. 2011). Although the methods among research groups differed, the results were consistent: defective glucocerebrosidase and aberrant lysosomal degradation pathways resulted in an accumulation of complex lipids and α-synuclein. Furthermore, the researchers noted the lack of self-limiting kinetics in the accumulation of the lipids and α-synuclein. Taken together, these results are consistent with the existence of a feed-forward mechanism of lipid and protein accumulation in patients with Gaucher-linked Parkinsonism.

While many research groups studied well-classified proteins and pathways, Tarantino et al. (2011) took a different approach. The group examined a protein, LRRK2, that was hypothesized to play a role in phosphorylation of α-synuclein at Ser129. Past research has shown that phosphorylated α-synuclein in Lewy bodies is a hallmark histological finding in patients with Parkinson’s disease (Tarantino et al. 2011). Reverse transcriptase PCR was used to identify four polymorphisms (rs871196, rs2420616, rs7069375, rs4752293) in LRRK2 (Tarantino et al. 2011). Mutated full-length Lrrk2 and Lrrk2 fragments maintaining kinase activity were shown to have a greater...
capacity to phosphorylate α-synuclein compared to wild-type Lrrk2 (Tarantino et al. 2011). The results suggested mutations in LRRK2 can lead to pathologic levels of phosphorylated α-synuclein. With this in mind, researchers used this finding as a building block to look for overlapping mechanisms in patients with Gaucher-linked Parkinsonism.

Table 1: Summary of key experiments demonstrating complex mechanisms associated with Gaucher-associated Parkinsonism.

<table>
<thead>
<tr>
<th>Molecule(s) of Interest</th>
<th>Observation</th>
<th>Physiological consequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-synuclein</td>
<td>Increased phosphorylation</td>
<td>Formation of insoluble oligomers leading to cell death</td>
<td>Tarantino et al. 2011</td>
</tr>
<tr>
<td></td>
<td>Mutation in Protein</td>
<td>Decreased clearance of malformed protein resulting in intracellular accumulation of protein, culminating in cell; ERAD malfunction</td>
<td>Mazzulli et al. 2011; Yakunin et al. 2010</td>
</tr>
<tr>
<td>Glucocerebrosidase</td>
<td>Dysfunctional Enzyme</td>
<td>Accumulation of complex lipids and perpetuation of formation of insoluble α-synuclein oligomers resulting in cell death</td>
<td>Mazzulli et al. 2011</td>
</tr>
<tr>
<td></td>
<td>Adeno-like virus-mediated expression in hippocampus</td>
<td>Reversal of memory deficits and motor dysfunction caused by Gb1 mutation</td>
<td>Sardi 2011</td>
</tr>
<tr>
<td>Prosaposin</td>
<td>Decreased expression</td>
<td>Accumulation of complex lipids due to lysosomal dysfunction, eventually leading to cell death</td>
<td>Mazzulli et al. 2001</td>
</tr>
<tr>
<td>Lysosomal hydrolases</td>
<td>Mutation in protein</td>
<td>Gaucher model demonstrating decreased catalabolism of complex lipids resulting in cell death</td>
<td>Parnetti et al. 2009</td>
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Induction of WT and Mutant α-Synuclein and Glucocerebrosidase: In Vitro Models

Several cell lines and types were used in order to express and examine abnormal α-synuclein glucocerebrosidase interaction—MES23.5, PC12, SH-SY5Y, HEK293, and induced dopaminergic neurons (Alvarez-Erviti et al. 2010; Cullen et al. 2011; Manning-Boğ et al. 2009; Mazzulli et al. 2011; Yakunin et al. 2010). The MES23.5, PC12, and SH-SY5Y cell lines were used because the cells were of a neural cancer origin and are able to produce large amounts of protein as a result (Biedler et al. 1978; Liu 1999). HEK cells were used due to the ease to transfect the cells using an adenovirus vector (Yakunin et al. 2010).

Yap et al. (2011) took a slightly different approach to produce α-synuclein by using engineered Escherichia coli BL21(DE3)pLysS. Greater control of α-synuclein production led to the decision of Yap et al. (2011) to use the genetically-engineered E. coli system; BL21(DE3)pLysS contains an IPTG-inducible gene for T7 RNA polymerase.

To express mutant α-synuclein and dysfunctional glucocerebrosidase using an in vitro model, some research groups introduced the mutations through PCR-directed mutagenesis (Alvarez-Erviti et al. 2010; Cullen et al. 2011; Mazzulli et al. 2011; Saha et al. 2004; Xu et al. 2011; Yap et al. 2011). Some of the common mutations introduced into α-synuclein were G7C, A30P, A53T, S87D, S87A, Y125F, Y125D, S129D, S129A, and Y136C (Alvarez-Erviti et al. 2010; Cullen et al. 2011; Saha et al. 2004; Yap et al. 2011). The mutations introduced in α-synuclein were chosen based on past reviews of medical records and computerized molecular models. The most common mutations introduced into glucocerebrosidase were N370S, V394L, D409V, and L444P (Cullen et al. 2011; Mazzulli et al. 2011; Sardi 2011).
An additional model used to simulate dysfunctional glucocerebrosidase involved the use of a potent, irreversible antagonist of glucocerebrosidase, conduritol B epoxide (Cullen et al. 2011; Manning-Boğ et al. 2009; Xu et al. 2011). Conduritol B epoxide binds with high affinity to the acidic residues of glucocerebrosidase (Rempel and Withers 2008). Irreversibly inhibiting glucocerebrosidase provides an accurate and efficient in vitro model of Gaucher’s disease.

**Induction of WT and Mutant α-synuclein and Glucocerebrosidase: In Vivo Models**

*In vivo* methods to examine the role of normal and abnormal α-synuclein and glucocerebrosidase typically rely on genetically-modified animals, particularly mice. A common approach to mimicking dysfunctional glucocerebrosidase is to use knock-in or knock-out methods. Heterozygous mice (Gbai<sup>−/−</sup>) and homozygous recessive mice were used in behavioral experiments such as object recognition, fear conditioning, and field exploration (Sardi 2011). Sardi (2011) demonstrated Gbai<sup>D409V/D409V</sup> mice exhibited α-synuclein and ubiquitin aggregates in hippocampal neurons. Consistent with hippocampal degradation, Gaucher mice exhibited memory deficit, consistent with what would be expected in a human with Gaucher-linked Parkinsonism; however, Sardi (2011) probed the effects of adeno-associated virus-mediated expression of exogenous glucocerebrosidase. Gaucher mice treated with exogenous glucocerebrosidase in the hippocampus showed a regain of motor function and memory. Consistent with the data and what would be expected in humans, mutations in GBA1 resulted in a synucleinopathy resembling Parkinson’s disease. The effects of the mutation can be ameliorated by resuscitating normal glucocerebrosidase function.

Expanding on the experiments performed by Sardi (2011), an additional research method utilized mice with point-mutated GBA1 in addition to hypomorphic prosaposin (Mazzulli et al. 2011; Xu et al. 2011). Prosaposin is a precursor to saposin A, saposin B, saposin C, and saposin D (Morimoto et al. 1990). Saposins are required cofactors in the metabolism of certain glycosphingolipids in the lysosome by action of glucocerebrosidase (Mazzulli et al. 2011; Xu et al. 2011). Paralleling the findings of Sardi (2011), Mazzulli et al. (2011) showed hypomorphic prosaposin mice exhibit characteristics, both at the macroscopic and microscopic level, consistent with the existence of a synucleinopathy such as Parkinson’s disease.

Contrary to most research groups, Yakunin et al. (2010) examined the effects of α-synuclein aggregation independent of glucocerebrosidase dysfunction. The group used genetically-engineered Pex<sup>2</sup>−/−, Pex<sup>5</sup>−/−, and Pex<sup>13</sup>−/− mice. Due to the homology between the peroxisomal proteome and the endoplasmic-reticulum-associated protein degradation (ERAD) pathway, a Pex knock-out system models defective ubiquination and proteasomal catabolism (Schlüter 2006). A hypothesis proposed by research groups is that defects in the ERAD pathway perpetuate the neurodegenerative effects of α-synuclein fibril formation (Sardi 2011). Consistent with the hypothesis, defects in the ERAD pathway, as modeled by Yakunin et al. (2010), showed abnormal accumulation of lipids and α-synuclein resulting in non-specific neurodegeneration.

**Gene Analysis and Quantitative Transcript Analysis**

Numerous research groups relied on the ability to analyze genes in order to determine specific mutations in many types of samples and to confirm the success or failure of genetic modification in bacterial, cellular, and animal models (Cullen et al. 2011; Goker-Alpan 2010; Manning-Boğ et al. 2009; Neumann et al. 2009; Nishioka et al. 2011; Segarane 2009; Tarantino et al. 2011; Yakunin et al. 2010). Transcriptional analysis was performed to measure the amount of glucocerebrosidase mRNA and whether the mRNA was affected by α-synuclein aggregation or whether it was the source of α-synuclein oligomerization. If α-synuclein inhibited translation of glucocerebrosidase transcript, one would observe normal to increased amounts of glucocerebrosidase transcript and α-synuclein. In the case of decreased concentrations of glucocerebrosidase transcript, one would also observe α-synuclein accumulation. The preferred methods to measure glucocerebrosidase transcript were qPCR and long range PCR (Cullen et al. 2011; Goker-Alpan et al. 2010; Manning-Boğ et al. 2009; Neumann et al. 2009; Nishioka et al. 2011; Segarane 2009; Tarantino et al. 2011; Yakunin et al. 2011).

Alvarez-Erviti et al. (2010) analyzed and validated siRNA inhibition of LAMP2A by using PCR amplification. LAMP2A encodes a lysosomal receptor that binds certain proteins associated with chaperone-mediated autophagy (Alvarez-Erviti et al. 2010). The authors investigated if there was an alteration in autophagy due to the degradation of neurons observed in patients with Gaucher-related Parkinson's disease (Alvarez-Erviti et al. 2010). α-Synuclein oligomerization leads to reactive oxygen species production, which subsequently leads to impaired mitochondrial function. If mitochondrial function was impaired, one would expect to observe an increase in autophagy as an energy compensation mechanism. As expected in vitro, siRNA-mediated inhibition of LAMP2A resulted in increased autophagy levels in a dopaminergic cell line. In conjunction with the in vitro findings, examination of brain samples from seven Parkinsonism patients showed reduced chaperone-mediated autophagy and an increased half-life of α-synuclein in the substantia nigra pars compacta and amygdala, as measured by LAMP2A and hsc70 concentrations, when compared with age-matched brain samples (Alvarez-Erviti et al. 2010).

Kono et al. (2010) examined GBA1 genetic variations by examining restriction fragment length polymorphisms (RFLPs). Although RFLP analysis is considered to an archaic practice by some due to the abundance of other, efficient, DNA-sequencing technology, observing RFLPs in homologous DNA molecules allows for a practical method to localize genes that are implicated in genetic disorders. This is important as it allows researchers to examine and characterize all of the mutations associated with similar pathologies.
Assaying Enzyme Activity

Two principal approaches were taken to assay the activity of WT and mutant glucocerebrosidase. One approach involved simply incubating purified glucocerebrosidase with a proper substrate; the primary substrate used in incubations was β-D-glucoside (Choi et al. 2011; Xu et al. 2011). An additional method used to measure the amount of glucocerebrosidase activity involved the use of glucocerebrosidase substrates coupled to a fluorescent molecule, 4-methylumbelliflone (4-MU) (Parnetti et al. 2009; Sardi 2011; Xu et al. 2011). The substrates that were coupled with 4-MU included β-D-glucocerebroside-N-acetate, β-D-glucocerebroside, and β-D-galactoside (Parnetti et al. 2009; Sardi 2011; Xu et al. 2011). When bound to the substrate, 4-MU is non-fluorescent; however, when cleaved by enzymes, 4-MU fluoresces. In essence, greater enzyme activity will result in a higher observed fluorescence and vice-versa for lower enzyme activity.

Parnetti et al. (2009) examined an additional enzyme class, lysosomal hydrolases. Lysosomal hydrolases (e.g., cathepsins and nucleases) are involved in the proteolysis of amyloid-precursor proteins. Defects in lysosomal hydrolases have been shown to cause formation of β-amyloid plaques in various neurodegenerative disorders (Mazzulli et al. 2011). Reactions consisted of 4-MU-coupled substrates (α-mannose, β-mannose, and β-glucose) and isolated lysosomal hydrolase enzymes from the cerebrospinal fluid of patients with pathologically-confirmed cases of dementia with Lewy bodies, frontotemporal degeneration, and Alzheimer's disease (Parnetti et al. 2009).

PET Scans

Few groups chose functional medical imaging to analyze Gaucher's disease effect on α-synuclein aggregation. Kono et al. (2010) decided to analyze subjects with clinically diagnosed Gaucher-associated Parkinson's disease. Three types of PET scans were used: fluoroxyglucose-PET (18F; FDG), (−)-2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane-PET (11C; CFT), and raclopride-PET (18C) (Kono et al. 2010). The purpose of the study was to demonstrate the presence of dopaminergic neuron deficit in patients with Parkinsonism secondary to Gaucher's disease.

The FDG molecule is rapidly taken up by neurons and is metabolized to 18F-FDG-6-phosphate. Due to the absence of a 2'-hydroxyl group, the phosphorylated metabolite is trapped inside the cell. Metabolically active neurons will display a higher concentration of 18F-FDG-6-phosphate intracellularly (Ewers et al. 2011). Concentration of 18F-FDG is a good indicator of healthy cells versus damaged or dying cells.

CFT is a derivative of cocaine and is a functionally useful molecule to assess the location and distribution of dopamine transporters in neurons (Tang et al. 2011). The use of CFT in patients with Parkinsonism would most likely demonstrate a lack of dopamine transporters in the brain, particularly in motor cortices and in the substantia nigra pars compacta (Kono et al. 2010).

Raclopride is a 13C radiolabeled D2 dopamine receptor antagonist (Antonini et al. 1997). Raclopride is useful for examining dopaminergic neuron function and useful in differentiating Parkinson's disease from multiple system atrophy (Antonini et al. 1997). The use of raclopride-PET was important in confirming Parkinson's disease as well as assaying the functionality of dopaminergic neurons in a Gaucher-Parkinson model.

The experiments performed by Kono et al. (2010) radiographically confirmed a loss-of-function in dopaminergic neurons, consistent with other experiments with Parkinsonism-affected Gaucher’s patients.

Discussion

Much time and effort has been dedicated to the comprehension of the understanding of Gaucher-linked Parkinsonism etiopathogenesis. Although a great deal is already known regarding the link between dysfunctional glucocerebrosidase and synuclein oligomerization, there are still many gaps to fill with regard to the effect of β-amyloid formation and non-functional glucocerebrosidase on the ubiquitination-proteasome mechanism to catabolize malformed proteins. Misfolded α-synuclein is involved in the onset and perpetuation of Gaucher-linked Parkinsonism (Choi et al. 2011). A typical cellular response to prevent incorporation of malformed proteins is to tag the protein with ubiquitin for subsequent degradation by proteasomes (Pickart 2001). If possible, manipulating normal physiological ubiquitination to tag aberrant α-synuclein and glucocerebrosidase could become a pharmacological targeting strategy against neurodegeneration.

Related to ubiquitination, the unfolded protein response (UPR) should also be the subject of further study in Gaucher-linked Parkinsonism. Concerning the brain, there is only one favorable alternative for the UPR, refolding of the defective protein. In order to refold the protein, certain chaperone proteins must be mobilized (Ron and Walter 2007). Manipulating the UPR to refold proteins should be investigated as a potential therapeutic target. One possible method to alter the UPR includes examining pharmacophores of key proteins involved in the UPR and designing small-molecule therapeutics to promote cell survival, sense malformed proteins, and trigger cell death when appropriate. The one fault in targeting the UPR is that it is an essential cell response perfected through evolution. Tilting the UPR one way or the other can have profound, unforeseen impacts on the organism. This is not to say the UPR should not be explored as a possible therapeutic target but rather it must be extensively studied in in vitro and in vivo models before beginning human studies.

The development of novel therapeutics to treat Gaucher-linked Parkinsonism should also be an area of exploration. Although Parkinson-like symptoms may be treated with L-DOPA, the eventual depletion of dopaminergic neurons due to α-synuclein oligomerization renders L-DOPA treatment...
ineffective. Enzyme replacement therapies and glucosylceramide inhibitors for Gaucher's disease have received much attention in the past few years. The use of recombinant glucocerebrosidase or glucosylceramide synthase inhibitors are expensive, chronic treatments. The estimated annual cost of glucocerebrosidase replacement therapy ranges from $200,000 to over $640,000 for a 60 kg individual (MacKenzie et al. 1998; Sidransky et al. 2009).

Recently, researchers have demonstrated cyclin-G associated kinase (GAK) interacts with α-synuclein to enhance dopaminergic neuronal death (Dumitriu et al. 2011). Targeting GAK could afford a valuable therapeutic target to mitigate the neurotoxic effects of α-synuclein aggregation. Studies should be conducted to examine dose-responsiveness and means to enhance drugs' half-lives. Due to the strong genetic link between Gaucher's disease and Parkinson's disease, individualized gene therapy should be considered as an alternative to enzyme replacement and substrate reduction therapies.

With regard to gene therapy, manipulating the post-translational modification and primary structure of α-synuclein also serves as a potential therapeutic focus. One target to consider is the Glu83 residue of α-synuclein. E83A mutations enable the ability of α-synuclein to form insoluble plaques (Waxman et al. 2010). Like manipulating the UPR, altering post-translational modification of proteins would have dire consequences if not examined extensively using in vitro and in vivo models. Examining critical residues in α-synuclein that contribute to fibrillization or defibrillization should also be explored as alternatives to enzyme replacement therapy.

Conclusion

The emergence of early onset Parkinson's disease in patients with Gaucher's disease has prompted researchers to search for an etiopathological link between the two seemingly different diseases. There is evidence that suggests that a positive feedback mechanism exists between mutant glucocerebrosidase and α-synuclein oligomerization. The effect of Gaucher's disease is not solely limited to systemic accumulation of glucosylceramide. In vitro and in vivo models demonstrate that glucosylceramide stabilizes α-synuclein oligomers, particularly in dopaminergic neurons of the substantia nigra pars compacta. The lack of response from the ubiquitin-proteasome mechanism exacerbates the pathology. The accumulation of α-synuclein in dopaminergic neurons eventually leads to the development of Gaucher-linked Parkinson's disease. Elucidating the etiopathogenesis of Gaucher-linked Parkinson's disease has been the goal of many research studies (Table 1). Areas of additional research should include the role of ubiquitination, the role of the UPR, and the development of novel therapeutics in Gaucher-linked Parkinson's disease. The existence of many potential mechanisms that sustain and promote α-synuclein neurotoxicity in the presence of mutant glucocerebrosidase demonstrates the complexity of linking two "unrelated" pathologies.

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