Protein Aggregates And Polyglutamine Tracts In Neurodegenerative Disease

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Abstract

The incidence of neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and other Polyglutamine Diseases is projected to dramatically increase throughout the developed world, and yet the pathology of these diseases remains poorly understood. One pathway that these neurodegenerative diseases share is the accumulation of pathologic proteins which are not only harmful in their soluble form but may go on to form toxic aggregates. In many cases, a consensus has yet to be reached concerning the mechanism for protein aggregation. Therefore, the exploration of the roles of these proteins and their possible mechanisms, along with potential techniques for treatment, are more important than ever.

Protein Aggregates And Polyglutamine Tracts In Neurodegenerative Disease

Introduction to Common Neurodegenerative Diseases

Dementia is a devastating condition induced by most neurodegenerative diseases. Daily functions and higher thought soon become impossible under dementia. As a result, caregiving is often too strenuous and time consuming for family members to bear, often making expensive nursing homes the ultimate destination for those with dementia (Ridge, 2013). Dementia is a general term for progressive brain disease, with the majority of cases currently being attributed to either irregular protein accumulation or genetic defects (Holmes, 2016). By definition, each type of dementia involves a decline in mental health, although there is an extensive variety of diseases with each having different mechanisms and pathologies. While many diseases can cause dementia, the most common and well-studied include Alzheimer's Disease (AD), Parkinson's Disease (PD), and Huntington's Disease (HD).

Among the various types of neurodegenerative disease, AD is the most common, accounting for 50-75% of all dementia cases (Lane, 2017). Memory loss, neural cell death, seizures, behavior change, loss of mobility, and synaptic dysfunction are all symptoms of AD that go on to cause complications that lead to death in an average timespan of about 8.5 years (Efthymiou, 2017; Lane, 2017). Late onset AD is practically unavoidable, as a consistent increase in prevalence has been observed in those with old age, with the risk of developing the disease doubling every five years past the age of 65 (Lane, 2017). Risk of AD development has been linked to several different factors, the most important of which being the development of abnormal Tau protein and β amyloid (Reid, 2004). Typically, patients only live an additional three to nine years after

diagnosis, as neural cell death ultimately proves fatal, with most outliers being the 5-10% with early onset AD, which is characterized by the diagnosis of the disease before the age of 65 (Ridge, 2013; Efthymiou, 2017).

PD is characterized most infamously via its ascribed maladies regarding motor function, as impairment of the dopamine-producing section of the midbrain deemed the substantia nigra causes slower voluntary muscle activity as well as tremors and rigid movement (Williams-Gray, 2016). However, other non-motor-related effects of PD are observed as well, including cognitive impairments, maladies regarding autonomic regulation, depression, and problems with sleep (Williams-Gray, 2016). Excluding depression, which can be appeased through exogenous dopamine administration, the non-motor symptoms of PD prove much more difficult to manage and it is these symptoms which lead to dementia and indirectly result in death (Williams-Gray, 2016). The development of PD is attributed to the accumulation of α-synuclein, a protein that forms unique clusters of proteins called Lewy Bodies that work to disrupt the normal functions of the cells in surrounding tissue (Bae, 2012). Most victims of PD develop dementia about 10 years after their initial diagnosis, and death is often a consequence of a secondary ailment brought on by the complications of PD rather than PD itself (Williams-Gray, 2016).

In contrast to several other types of neurodegenerative disease, HD has a relatively early onset, typically affecting those between ages 30 and 50, with approximately 10% of victims developing symptoms in adolescence and late childhood (Gulli, 2018). Another notable quality of HD is that it is a disease entirely derived from a mutation of the HTT gene, which is an autosomal dominant allele (Gulli, 2018). This

mutation accounts for the elevated level of polyglutamine in HD patients, a symptom shared with several other polyglutamine related diseases (Takeuchi, 2017). Eventually, the neuronal cell death that the mutation brings causes patients to exhibit involuntary twisting motions, sustained abnormal posture, muscle weakness, depression and anxiety, short-term memory loss, and severe cognitive decline (Gulli, 2018). The muscle weakness of the regions of the throat and respiratory system in HD patients usually cause choking or respiratory infection in HD patients that prove fatal approximately10-15 years after diagnosis (Gulli, 2018).

Prevalence and Incidence of Neurodegenerative Disease

The effects of these neurodegenerative diseases are felt worldwide, as many reports claim that around 44 million individuals are suffering from the effects of dementia (Lane, 2017). AD alone is estimated to impact 24-35 million individuals today and in many developed nations such as England, dementia remains the leading cause of death, accounting for 11.6% of all British deaths in 2015 (Ridge, 2013; Lane, 2017). Additionally, PD is estimated to affect 2-3% of those older than 65 years of age, with an incidence of roughly 13 for every 100,000 persons per year for an estimated current total of 5 million victims, while HD, which is currently diagnosed in 30,000 Americans, occurs with a frequency of about 4-7 cases per 100,000 persons (Williams-Gray, 2016; Gulli, 2018).

Advances in medicine, technology, and nutrition are allowing for citizens of developed nations to outlive previous generations, resulting in a continuously aging population that is proportionally more at risk for diseases with dementia-related symptoms. Therefore, it is expected that the number individuals with dementia will

increase threefold by 2050, despite a slightly lower incidence rate over the last few years, making the research of effective treatment more important than ever (Lane, 2017). As prolific as these neurodegenerative diseases may be, there is still much to be understood before effective preventions and cures can be developed.

Protein Aggregations in Neurodegenerative Disease

Tau Proteins and β Amyloid

While diverse, neurodegenerative diseases have quite a few characteristics in common. Perhaps the most prominent similarity in diseases such as PD and AD is the common presence of misfolded protein deposits (Popiel, 2013). The buildup of extracellular plaques is often the cause of degeneration of neural tissues, as their prolonged presence is many times sufficient to cause cell death (Rafii, 2016). In AD, the most significant plaque-causing proteins are the Tau protein and β amyloid, as these proteins are often what aggregate to form plaques in AD victims (Figure 1) (Reid, 2004).

β amyloid proteins are cleaved from a membrane protein named amyloid precursor protein (APP) by either β secretase or γ secretase and then go on to form extracellular neuronal plaques that work to choke neurons to the point of death when accumulated in abnormal proportions, as elevated levels of hemoglobin localized around plaques in AD victims indicate detrimental conditions such as hypoxia (Gowrishankar, 2015; Chuang, 2012). The pathogenic form of β amyloid also takes on a fibril form in its namesake β sheet structure, making it much more difficult to disassemble their respective aggregates (Goehler, 2010). A noteworthy finding in recent studies is that high concentrations of lysosomes, in addition to other organelles, often accompany β amyloid deposits in the surrounding area of the affected neuron, as the lysosomes attempt to

manage abnormally high concentrations of β amyloid, further stifling nutrient movement and signaling processes (Gowrishankar, 2015; Gupta, 2016). Adding to the problem, β amyloid can not only form outside of the cells in the central nervous system (CNS), but inside them as well, making it a dual threat aggregate (Seeman, 2011).

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Figure 1. Transmission electron microscope images of β amyloid aggregates. β amyloid prepared *in vitro* exhibiting 'striated ribbon' (A) and 'twisted' (B) morphologies. β amyloid from AD brain tissue forms fibrils after seeded with synthetic β amyloid (C). Extremely stable β amyloid protofibrils (D). (Tycko, 2016).

The unique ability of β amyloid to pass through the membrane of mitochondria allows for the protein to interfere with a number of vital processes, such as the electron transport chain, causing an increase in the production of free radicals and altering Ca^{2+} concentration within mitochondria (Gupta, 2016). In pathological situations, these factors cause decreased energy output and increased pore permeability in mitochondria, leading to fragmentation and apoptosis in severe cases (Gupta, 2016). Additionally, the increased concentration of reactive oxygen species facilitates further aggregation of β amyloid (Gupta, 2016).

Inflammation also serves as a means through which β amyloid can cause cell death as excessive inflammation prevents the normal functions of the body (Fu, 2017). In the CNS, microglia work to bind β amyloid to their amylin receptors in order to sequester or phagocytize aggregations, which aids in the clearance of these proteins but also causes the microglia to release cytokines (Fu, 2017). Both TNF- α and IL-1β are released by microglia in response to β amyloid binding and as both cytokines are pro-inflammatory,

areas rich in β amyloid can soon become over-inflamed (Fu, 2017). This inflammation can be sufficient to cause synaptic disruption and vascular pathology (Fu, 2017).

Tau proteins are unique aggregates which also form fibrils and are difficult to break down, as they easily cause tangles within neural tissue cells (Figure 2) (Sigurdsson, 2008). The rather spontaneous formation of the Tau fibril aggregates happens readily as no mutations are necessary for its aggregation, making it one of the most dangerous aggregates associated with neuronal disease (Meng, 2012). Those with familial AD tend to exhibit increased phosphorylation in their tau proteins due to altered equilibriums and pathologic activity of kinases and phosphatases (Leschik, 2007). In cases such as these, tau may bind anywhere from six to eight molecules of phosphate, as opposed to normal tau proteins which tend to have only two or less of their sites phosphorylated (Leschik, 2007).

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Figure 2. Immunohistochemistry allows for the viewing of protein aggregates in the neural tissue of AD patients. β amyloid occlusions can clearly be seen in the frontal cortex (a) and within blood vessels (b) and capillaries (d), with a more magnified view displaying a dense β amyloid core (c). Tau aggregates are also seen entangled within neural tissue (e,h). Microglia are pictured reacting to the inflammation and stress brought on by the protein aggregations (f,g) (Lane, 2017).

Ca^{2+} plays a significant role in tau phosphorylation, as the Ca^{2+} dependent proteins calmodulin dependent protein kinase II (CaMKII) and calpain both lead to the phosphorylation tau proteins (Hung, 2010). Calpain in particular has a hand in the hyperphosphorylation of tau, as it can activate cyclin-dependent-protein kinase 5, which directly causes the over-phosphorylation of tau that leads to pathology (Hung, 2010). Extensive phosphorylation alters the structure of the protein, forming it into paired helical

fragments that readily tangle up with one another (Leschik, 2007). When observing the effect of these tau fibrils via live imaging, neural cells of the hippocampus are clearly seen adopting a balloon-like conformation that ultimately causes stress and possibly apoptosis, indicating a potential mechanism for cell death in tau protein related disease (Leschik, 2007).

Another noteworthy aspect of the tau protein is its ability to form complexes with the phospholipids of lipid bilayers that are toxic to hippocampal neurons (Ait-Bouziad, 2017). These complexes form when tau proteins are imported from extracellular space via endocytosis, but rather than completely entering the cell, somehow break free from their respective endosome in order to co-localize with an early endosome marker (Ait-Bouziad, 2017). Some speculate that such interactions as these may even leave pores within neuronal membranes, exposing the cell to the extracellular environment (Ait-Bouziad, 2017). Once formed, these complexes associate with the membranes of other neural cells, soon forming an adhesive cluster of cells, allowing for the adverse effects of tau-mediated aggregation to spread throughout the brain (Ait-Bouziad, 2017).

While dangerous at full-length, tau fibrils can also be harmful when cleaved by the enzyme caspase-2 at their Asp314 location in pathological scenarios, resulting in a truncated version capable of crowding the dendritic spines of neurons (Leschik, 2007). Caspase enzymes are consistently found in abnormally high concentrations within post-mortem neural tissue of AD victims, a phenomenon which most likely contributes to the increased production of truncated tau protein in those with AD (Leschik, 2007). These cleaved tau proteins can therefore cause synapses to malfunction and may reduce the number of receptors in dendritic spines, both of which can occur in early pathological

stages before neuronal degeneration is even evident (Leschik, 2007). The adverse effects of cleaved tau can be seen in mice that were transduced via a virus vector to receive the gene necessary for caspase-2 facilitated Asp314 truncation and were reported to have learning deficits and memory impairment, as they had much more difficulty completing mazes of the same length as their control peers (Leschik, 2007). Additionally, non-truncated tau monomers that become isolated from plaques and aggregates may also cause cellular harm as they can also initiate the unwarranted release of calcium ions at muscarinic receptors (Ait-Bouziad, 2017).

β amyloid plaques and tau protein tangles cause fatal aggregations in neural tissue, especially when interacting with one another (Lane, 2017). On multiple occasions, *in vitro* studies with β amyloid have reported increased phosphorylation of tau due to the influence of β amyloid as well as an increased sensitivity to the toxic properties of β amyloid in cells with high expression of tau protein (Leschik, 2007). As these two proteins are both consistently found in AD patients, their aggregation can occur much more severely when they do so in concert with one another.

α-Synuclein and the Development of Lewy Bodies

The tau proteins and β amyloid are hallmarks of AD, but one peptide that is a unique catalyst to PD is α-synuclein. In addition to PD, a general form of dementia distinct for its presence of Lewy Bodies (DLB) also exhibits irregular α-synuclein behavior (Bae, 2012). Normal cells contain α-synuclein within their cytoplasm near presynaptic locations; however, in pathological situations, the protein may be transported into extracellular space via exocytosis (Bae, 2012). Once outside of the cell, α-synuclein

that has taken on a fibrillar conformation can form aggregations that continue to recruit new molecules, causing further obstruction and often triggering inflammation (Bae, 2012).

The buildup of α-synuclein is made possible by the protein's ability to inhibit autophagy when oxidized or when mutations cause the improper assembly of α-synuclein (Tanji, 2011). One way that α-synuclein can prevent the degradation of the protein clusters that it tends to form is through the inhibition of the autophagic protein GABARAPL/GABARAPL1 (Tanji, 2011). These overexpressed α-synucleins aggregations contribute to the formation of Lewy Bodies, autophagy inhibitors that have a hand in initiating apoptosis in surrounding tissue (Bae, 2012).

Lewy Bodies (LB) can form throughout both the Peripheral Nervous System (PNS) and the CNS and have been found in victims of several different types of neurodegenerative disease including both AD and PD, but LBs in the substantia nigra produce much of the symptoms that patients with PD suffer from (Wakabayashi, 2013). The fibrillar α-synuclein may initially form LBs due to their role in the shielding of neurons from the cytotoxic properties of non-fibrillar α-synuclein, which LBs isolate from the rest of body (Tanji, 2011). However, the buildup of LBs are what cause many of the issues seen in their corresponding neurodegenerative diseases (Tanji, 2011).

There are two types of LBs, of which one variant is termed as a brainstem LB and the other is considered cortical LB, each with a filamentous structure. The cortical variety lack the distinct dense core and peripheral halo that are hallmarks of the more common brainstem LB form (Figure 3) (Wakabayashi, 2013). While a high concentration of extracellular α-synuclein is key for the generation of LBs, there are also

several other components of LBs as well, including proteasomes, lipids and even amyloid precursor proteins (Wakabayashi, 2013). When a PD patient's substantia nigra becomes riddled with LBs, their ability to control movement is compromised (Wakabayashi, 2013). In fact, the presence of LBs seem to specifically hinder motor function more so than sensory function, as when LBs are present throughout the nervous systems of the body, only the spinal dorsal horn and olfactory structures have been reported to show signs of any significant damage (Wakabayashi, 2013).

Despite LBs ability to be distributed throughout the body, the areas observed to result in the highest concentration of neuronal cell death are the substantia nigra, nucleus basalis of Meyhart, and locus ceruleus (Wakabayashi, 2013). The scientific community is yet to reach a consensus regarding the mechanism through which LBs are linked to cell death; however, one theory proven to have some validity states that LBs starve neurons by ridding them of their mitochondria (Power, 2016). This theory, unique to DLB, was proposed after witnessing the retraction of mitochondria into LB centers via microtubules in LB-containing neurons obtained after an autopsy of DLB victims (Power, 2016).

Figure 3. The Immunoreactivity of α-synuclein enables viewing of a LB as it develops. A normal neuron is pictured with no distinct accumulation of α-synuclein (A) beside a rather weakly stained neuron with mild α-synuclein buildup (B). A pale body, a premature LB, then begins to form (C,D,E*) before finally being absorbed into the dense LB (E,F) which is seen adopting its signature "halo" structure (E,F) (Wakabayashi, 2013).

One finding, observed by several researchers, is that free α-synuclein is capable of hindering vesicle transport throughout the cell by preventing the docking of vesicles bound for the Golgi apparatus, causing an unhealthy buildup of these vesicles, especially

in regions proximal to centrosomes to such an extent, that some have proposed that LBs may even begin their formation near the centrosomes (Figure 4) (Power, 2016). This additional stress on the cell's centrosomes is much more significant when considering the ability that LBs possess to inhibit proteasome activity, as these factors ultimately cause the improper nucleation of microtubules and can incapacitate the trans-Golgi network (Power, 2016).

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Figure 4. α-synuclein's ability to inhibit vesicular traffic is exemplified by the pulse-chase immunoprecipitation of the common proteins CPY (A and B) and ALP (C and D) that routinely migrate via vesicular transport. The monitored cells expressed either WT α-synuclein, a form of α-synuclein with a missense mutation denoted αSyn-A53T, or no α-synuclein (vector) and were compared on the basis of protein buildup in the ER after several intervals. (Cooper, 2006).

Soon after much of the neuron's microtubules are wound up into the LB, the structural integrity of the mitochondria is tampered with, resulting in their destruction (Figure 5) (Power, 2016). As a result, the mitochondria count is much lower in LB-containing neurons as compared to LB-free neurons in the same individual suffering from DLB (Power, 2016). Regarding PD, the same decrease in mitochondrial concentration is also observed, however another hypothesis claims that cell death in PD may also be achieved through loss of DNA, and subsequently loss of protein (Power, 2016).

Oddly enough, in PD, LBs have been proposed to target the nucleus for degradation as well (Figure 5) (Power, 2016). While less is known about the exact mechanism through which α-synuclein causes nuclear disfunction, it is now known that α-synuclein does have a high affinity for nuclear material and therefore may unravel the

nucleus (Power, 2016). It is not uncommon to find the α-synuclein of a PD patient's LBs to be clustered around free nuclear material within the stantia nigra, indicating that such material was ripped from its corresponding nucleus (Power, 2016).

Neurodegenerative diseases that feature high concentrations of α-synuclein are occasionally the result of genetic mutations that cause either the overproduction of the protein or the production of faulty α-synuclein, although it is important to note that α-synuclein plays a role in both sporadic and hereditary PD (Bae, 2012). The A53T mutation of the α-synuclein gene has been proven to be linked to the autosomal dominant variant of PD, while the missense mutations A30P and E46K can also increase the chance of PD and DLB development (Wakabayashi, 2013). As expected, when the gene coding for α-synuclein is multiplied, Lewy Body development is also more likely (Wakabayashi, 2013). Like α-synuclein, tau protein, and β amyloid, proteins with polyglutamine peptide tracts have a distinct role in the development of neurodegenerative disease (Kar, 2014).

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Figure 5. With dyed blue nuclei (Dapi; A,E,I,M,Q), dyed red mitochondria (Cy3 red; B,F,J,N,R), and dyed green α-synuclein (DyeLight™ 488-green; C,G,K,O,S), a composite of these dyed images in sequence (D,H,L,P,T) indicate that nuclear material and mitochondria are concentrated around LBs before being degraded (Power, 2016).

Polyglutamine Tract Aggregation

Polyglutamine tracts are large expanses of glutamine-containing peptides that cause fibril aggregation similar to previously mentioned protein aggregates (Toshihide, 2017). The family of diseases associated with polyglutamine (polyQ) containing proteins is comprised of nine conditions that rise from a mutation resulting in an unusually high

amount of cytosine-adenine-guanine (CAG) repeats, which consequently code for the amino acid polyglutamine (Toshihide, 2017). Each protein associated with their corresponding polyQ disease is different, however they all contain extended CAG repeats, which is what causes pathogenesis (Takeuchi, 2017). Excluding spinal and bulbar muscle atrophy (SMBA), all polyQ diseases are autosomal dominant mutations and can lead to cognitive and motor neural degradation (Toshihide, 2017). The severity of polyQ induced disease depends on which type of the mutation occurs, as the age of onset for polyQ related neurodegenerative diseases have been observed to be directly proportional to the number of glutamine repeats (Ashkenazi, 2017; Xi, 2016).

The impact of polyQ stretches are most infamously seen in HD, as it contributes to the characteristic impairment of motor function seen in HD (Ashkenazi, 2017). Additionally, some speculate that polyQ may also cause similar effects in those suffering from AD (Ashkenazi, 2017). Those with polyQ related diseases exhibit an increase in CAG repeats on the amino terminus of a protein appropriately dubbed “Huntingtin” (Toshihide, 2017). Pathologic copies of Huntingtin usually contain over 40 repeats of the amino acid compared to the average range of 5-35 repeats in healthy individuals, a phenomenon that is seen in all polyQ diseases (Toshihide, 2017). These N-terminal repeats make up the first exon of Huntingtin and therefore undergo alternative splicing, releasing the polyQ fragment, which is toxic in its soluble form (Goehler, 2010). The cumbersome polyglutamine tracts are easily caught up within each other and cause aggregates inside the cell nucleus or cytoplasm which are also harmful and are known to easily recruit monomers of opposite chirality (Kar, 2014; Menzies, 2011). PolyQ

aggregates can then cause a myriad of maladies including increased risk of neural cell death, as it prevents autophagy via inhibition of ataxin-3 (Ashkenazi, 2017).

Ataxin-3 is heavily concentrated in neural tissue and causes another protein, beclin-1, to initiate autophagy by deubiquitinating beclin-1, preventing the degradation of the protein via proteasome (Ashkenazi, 2017). PolyQ normally promotes ataxin-3's interaction with beclin-1 but when an abnormally large amount of glutamine is present, the protein can no longer perform this function efficiently (Ashkenazi, 2017). In turn, beclin-1 does not cause significant autophagy of the aggregates in those with polyQ mutations, causing an accumulation of protein within neural tissue (Ashkenazi, 2017).

Extensive polyQ tracts not only form occlusions of their own but have even been observed to promote the development of amyloid fibril entanglements, hinting that their presence might also play a role in amyloid aggregate formation via a seeding mechanism (Goehler, 2010). This finding has been attributed to the secondary structure of elongated polyQ, which is similar to β amyloid aggregations in that it exhibits the same β sheet conformation (Goehler, 2010). As mutated polyQ tends to form a hyper-stable β sheet, clusters of the protein fragments are not only exceptionally hard to disperse but have an extensive amount of hydrogen atoms freely dispensed along the surface of the polypeptide (Goehler, 2010). These hydrogen atoms are then able to hydrogen bond with other exposed hydrogen atoms of neighboring proteins, including amyloids and other polyQ fragments (Goehler, 2010). This interaction encourages the formation of a similar β sheet while maintaining a relatively strong association between the two proteins (Goehler, 2010). In this fashion, a handful of long polyQ tracts can seed an entire aggregate if they contain at least 40 glutamines, unlike normal, non-toxic polyQ tracts

which are incapable of doing so as they take the form of β helices, loops or coils instead of the pathologic β sheet that results from the mutated Huntingin protein (Goehler, 2010; Xi, 2016).

Much more research is needed in identifying the toxic attributes of polyQ aggregates, as there are clear implications that resulting inclusions cause apoptosis, but there are discrepancies within the scientific community as to which biological processes directly cause damage to neural tissue. However, most agree that mutated polyQ tracts are the root cause of cell death in the protein's corresponding diseases and several proposals and recent findings have proven quite credible. One such speculation holds that polyQ clusters can adversely affect the structure of the cell membrane as changes in membrane permeability and rigidity have been associated with polyQ aggregates and a few other amyloidogenic proteins (Ho, 2016).

Regarding membrane disruption, the role of polyQ aggregates are as elusive as ever, save for a handful of notices via atomic force microscopy (Ho, 2016). These findings include increased membrane rigidity of cells proximal to extracellular polyQ, most likely due to the induced depolarization of the membrane, and a moderate affinity for the lipid bilayer that can potentially reduce the mobility of aggregates, allowing for further condensation of protein and even growth of polyQ fibril length while associated with the membrane (Ho, 2016). Under fluorescent microscopy, it becomes apparent that, over time, long polyQ aggregates cause calcein to leak from the membrane's unilamellar vesicles and warp the morphology of giant unilamellar vesicles, indicating a loss of vesicle integrity (Ho, 2016). Almost all of the calcein expelled from the unilamellar vesicles were found encapsulated, which indicates that polyQ aggregations are not

involved in full-scale fragmentation of the lipid bilayer, but rather deal exclusively with the integrity and permeability of the membrane itself (Ho, 2016). Whether or not this disruption is sufficient to cause cell death remains to be seen, but it does offer one example of polyQ aggregate associated stress and its results warrant further study regarding the protein's interaction with the cell membrane.

Recently, the soluble formation of mutant polyQ has received substantial attention regarding toxicity, as there are many implications that non-aggregated polyQ can be just as detrimental as its aggregated counterpart (Xi, 2016; Feng, 2018). For example, mutant soluble polyQ has been observed to disrupt the role of Valosin-Containing Protein (VCP), causing a multitude of problems within neurons (Feng, 2018). Within the cell, VCP functions as a chaperone protein with ATPase ability and aids in the management and controlled degradation of proteins throughout the cell, a process which is unable to occur once sequestered by a soluble mutant polyQ aggregate (Feng, 2018). As a result, endoplasmic reticulum (ER) associated degradation is halted, putting stress on the ER and more importantly fostering an enigmatic association between VCP and the mitochondria (Feng, 2018). The mitochondria of cells rich in soluble mutant polyQ may then be degraded via uncontrolled mitophagy brought on by unruly VCP (Feng, 2018). Not only does this series of events compromise the neuron's mitochondria, but it also releases reactive oxygen species that can interfere with a countless number of operations that occur within the cell (Feng, 2018). Along with this mechanism, others even hypothesize that Huntingtin-derived, soluble polyQ may even hinder transcriptional processes like similar proteins that contain long sequences of CAG repeats, such as

ATXN1 which has been shown to cause transcriptional dysfunction within Purkinje cells (Feng, 2018).

Potential Treatment of Protein Aggregates

Chaperone Mediated Clearance

Under normal circumstances, the proteasome is the means through which proteins are broken down, however; in those with neurodegenerative disease, oligomers from plaques and inclusions are often too large to be processed, and hence these proteins build up in neural tissue (Friedman, 2014). When overwhelmed with protein oligomers, the body deals with these aggregations in a number of ways, one of which being disaggregation via chaperone proteins (Doyle, 2013). An example of this form of clearance comes from Hsp 104 in yeast cells which when coupled with Hsp 70 in a high temperature environment, have been observed to dissolve inclusions and sever amyloid into a more manageable size (Doyle, 2013). These proteins also prevent the development of Lewy bodies, as when overexpressed they have been shown to reduce the α-synuclein oligomer concentrations in fly models and *in vitro* (Dehay, 2015).

In polyQ related disease, overexpression of Hsp40 and Hsp70 have also been linked with a reduction in the number of polyQ occlusions and have been observed to delay the onset of motor disturbances in both *Drosophila* and mice models (Takeuchi, 2017). One manner that overexpression of these protein chaperones is achieved is through the enhanced transcription of Heat Shock Factor 1 (HSF1), which in turn allows for increased transcription of protein chaperones such as Hsp40, Hsp70, and Hsp90 (Takeuchi, 2017). Hsp90 functions as a form of negative feedback on HSF1, as it inhibits HSF1 transcription (Takeuchi, 2017). Therefore, geldanamycin, an inhibitor of Hsp90,

allows for the increased expression of HSF1, resulting in an increased output of protein chaperones and permitting better polyQ aggregate clearance (Takeuchi, 2017). A fungal antibiotic called Radicicol and dimethylaminoethylamino-17-demethoxy-geldanamycin (17-DMAG) have both had similar effects to geldanamycin use, with the later inhibiting Hsp90 as well (Takeuchi, 2017). Both of these molecules have proven to reduce polyQ aggregation within HD model mice (Takeuchi, 2017). The upregulation of chaperone proteins remains a powerful tool for the management of protein aggregates in almost all forms of aggregate-associated neurodegenerative disease and should be seriously considered as an option for treatment due to the procedure's ability to halt the progression of pathology.

Aggregate Management Via Endogenous Proteases

When considering pathologic cases such as AD, the prevention of amyloid plaque formation also may come in the form of α-secretase (Grimm, 2013). α-secretase cleaves amyloid precursor protein (APP), the precursor to β amyloid, into the non-toxic peptide α APP, unlike β secretase and γ secretase, which form the toxic β amyloid (Grimm, 2013). These three secretases compete for APP as a substrate, making the upregulation of α-secretase a possible method of prevention for one of AD's primary offenders and a few treatments, such as the use of melatonin, have shown much promise in promoting amyloid clearance through use of this mechanism (Grimm, 2013; Panmanee, 2015).

Melatonin has been proven to slow degeneration in both AD and PD patients not only due to its role in free radical maintenance, but also through its favorable interaction with the secretases involved in APP production (Panmanee, 2015). The transcription of BACE1, the most prominent β secretase as well as PS1, a subunit of γ secretase, were

both found to be suppressed in neuroblastoma cells by melatonin in a concentration-dependent manner as activated melatonin receptors can alter transcription according to cell type (Panmanee, 2015). Additionally, melatonin can hinder BACE1 activity, further reducing the amount of β amyloid produced (Panmanee, 2015). As each variant of secretase competes for APP as a substrate, naturally a decreased concentration of both β and γ secretase allow for α-secretase to cleave APP into its less harmful metabolite (Panmanee, 2015). Furthermore, NF-κB has been identified as a protein that regulates both γ secretase and β secretase, as it can alter the transcriptional activity of the two enzymes as well as APP itself, making it another potential vector for the control of amyloid plaque buildup (Panmanee, 2015). While methods for using NF-κB as a means to inhibit protein aggregation need to be further studied, exogenous melatonin administration should be considered as a form of treatment of amyloid aggregation due to its potential regarding the decreased the output of β amyloid (Panmanee, 2015; Doyle, 2013).

Antibody Mediated Clearance

Another hypothesis for AD treatment, which makes use of antibodies, finds merit in studies done in immunotherapy where lab-developed antibodies tag β amyloid for phagocytosis in mouse models (Dematttos, 2012). The synthetic antibodies in the CNS bind both soluble and aggregated β amyloid, marking them for phagocytosis by microglial cells (Dematttos, 2012). These specific antibodies can also cause a shift in the equilibrium of β amyloid aggregates, allowing for oligomers to be dissolved into their monomeric state and preventing the seeding of additional oligomers (Dematttos, 2012). This process occurs naturally as well and has been known to contribute to the dissociation

of α-synuclein oligomers in PD, a process spurred on by microglial proliferation in a similar fashion to what is also seen in AD (Efthymiou, 2017). Additionally, immunotherapy studies performed on α-synuclein fibrils have revealed that antibodies not only have the potential to disassemble α-synuclein aggregates, but that antibodies can also prevent the entry of misfolded α-synuclein into neurons (Dehay, 2015). Those utilizing antibody clearance must proceed with caution, however, as a sudden release of protein monomers can be exceedingly toxic if it overwhelms the mechanisms meant to keep such proteins sequestered (Demattos, 2012). The low permeability of the blood brain barrier also serves as an obstacle, as only 0.1% of peripheral antibodies make it across, however this may also allow for the controlled release of amyloid monomers (Demattos, 2012). With a few more adjustments that account for these problems, this procedure has the potential to delay the progression of neurodegenerative disease.

Post-Translational Modification of Protein Aggregates

Treatment of tau protein accumulation has proved to be a difficult problem to solve as its misfolding cannot be attributed to an alteration in the primary structure of the protein itself, but instead it depends on the modifications it receives post-translation (Thomas, 2011). Familial tauopathy has been attributed to a missense mutation, but the maladies that result from the mutation are not associated with the sequence of amino acids, but rather the altered methylation of lysine residues or hyperphosphorylation (Thomas, 2011). The methylation of aberrant tau has been found to occur in at least seven sites, K44, K163, K174, K180, K254, K267, some of which are alternatively ubiquitylated, therefore methylation prevents the degradation of such tau proteins, providing a means for tau buildup (Thomas, 2011). While it is evident that the

methylation of tau occurs, techniques for combating this mechanism of aggregation are still in their infancy, despite great potential to attenuate neurodegenerative disease. Perhaps demethylases such as the KDM families seen in the common demethylation of histones could be used for such a purpose.

In regard to hyperphosphorylation, the upregulation of calcineurin may also provide a solution to tau protein aggregation, as its role in the modification of tau protein fibrils may prove invaluable to those at risk for AD (Hung, 2010). Calcineurin is a Ca^{2+}/calmodulin-dependent protein that is indicated to be necessary for the dephosphorylation of tau proteins, as when inhibited by cyclosporin A in mice, all dephosphorylating activity is halted (Hung, 2010). Hyperphosphorylation is dangerous due to the increased risk of paired helix formation, but if calcineurin activity is increased then there will be less of this harmful conformation of the tau protein, and therefore less aggregation (Hung, 2010). Upregulation of calcineurin may be an effective therapy for AD victims, but upregulation via natural means will most likely be ineffective as calpain is also Ca^{2+} dependent, therefore alternate methods such as transcriptional enhancement or even calpain inhibition might prove more useful (Hung, 2010). Likewise, protein phosphatase 2A (PP2A) has the ability to dephosphorylate hyperphosphorylated tau protein when its catalytic subunit (PP2Ac) is methylated at Leu309 (Yang, 2013). Cornel iridoid glycoside has been shown to prevent the demethylation of PP2A, allowing for a longer lifespan of PP2A that subsequently prevents the extensive phosphorylation of both tau protein (Yang, 2013; Dehay, 2015).

Another means for the dissolution of α-synuclein aggregates may depend on the management of deubiquitinating enzymes (Alexopoulou, 2016). Usp8 is one such

enzyme that also happens to be associated with Lewy Bodies and has been found to prevent the ubiquitination of α-synuclein and therefore hinders the clearance of the protein (Alexopoulou, 2016). In sporadic cases of PD, transcriptomic analysis of postmortem neural tissue has revealed that Usp8 mRNA is often upregulated within neurons containing a Lewy Body, which would account for the additional protection from degradation that α-synuclein enjoy within Lewy Bodies (Alexopoulou, 2016). Not only has upregulation of Usp8 been shown to contribute to PD pathogenesis *in vivo,* but a knockdown of the enzyme results in accelerated α-synuclein degradation via lysosome, highlighting the importance of regulating this enzyme (Alexopoulou, 2016).

Autophagy Enhancement

Under normal circumstances, α-synuclein is processed and degraded via the autophagy-lysosomal pathway; however, this process is compromised when α-synuclein forms aggregations (Dehay, 2015). More efficient autophagy has been observed when enhanced with rapamycin, a macrolide that obstructs the autophagy moderator mammalian target of rapamycin (mTOR) (Dehay, 2015; Takeuchi, 2017). While rapamycin is both effective and FDA approved, it does lead to some undesirable side effects when used long-term, such as anemia, an increased systemic level of triglycerides, and immunosuppressant factors (Dehay, 2015). If more is done to protect the body from these harmful side effects, or if rapamycin is used within a short timeframe, then the substance may be of use in the treatment of protein aggregation.

Gene Therapy

Another form of treatment that possesses great potential for the attenuation of neurodegenerative disease is gene therapy. For example, overexpression of Hsp40 may

be achieved through use of an adeno-associated virus (AAV) vector in the brain, with one such study finding improvement in motor function and longevity in HD mouse models after using such a technique (Takeuchi, 2017). As Hsp40 and Hsp70 are often transported to other cells via exosome, the overexpression of the chaperone proteins in the AAV infected cells prevents aggregation in neighboring neurons as well, decreasing the amount of polyQ aggregates throughout the brain (Takeuchi, 2017). Likewise, the silencing of mutant alleles is also being explored as a method of prevention for neurodegenerative disease (Glorioso, 2015). Barring instances when both inherited alleles are mutated, the silencing of the disease-causing dominant allele of HTT in HD patients is an ideal way to cease the production of mutant polyQ, although there are several problems that must be solved first (Glorioso, 2015). For instance, the gene coding for HTT is too large to be easily used for gene therapy, as the sequence is 9.4kb long and would not be fully contained in virus vectors (Glorioso, 2015). In conjunction with this obstacle, the wild type alleles of HTT are the identical to the mutant form excluding the extended CAG repeats, therefore differentiation between the two could prove a difficult task as both alleles could be equally vulnerable (Glorioso, 2015). Although elusive, this method could potentially serve as the cure for many forms of hereditary polyQ disease, making the development of new techniques and technology for gene therapy of paramount importance.

Conclusion

Neurodegenerative diseases are terrible aliments which eventually prove to be fatal to their victims while drastically decreasing quality of life along the way. These conditions claim hundreds of thousands of lives a year and the incidence of

neurodegenerative disease is predicted to increase exponentially in the near future. While individuals may experience different types of risk factors, everyone is capable of acquiring certain kinds of neurodegenerative diseases. Within the next few decades, citizens of developed nations will soon be at great risk for the development of neurodegenerative disease, as individuals are in continuously greater danger of acquiring disease as they age. Therefore, it is crucial that a more complete understanding of pathology be grasped in order to pursue effective forms of treatment. Fortunately, progress has been made towards disclosing the ultimate causes of these diseases, as protein aggregation is constantly seen to contribute to neurodegeneration in conjunction with several known mutations that increase the risk of disease development. Further investigation of these protein aggregates will most likely provide answers that will greatly assist in the formation of treatments and potentially even cures.

Although many biological processes give promising templates for the disaggregation of proteins in neurodegenerative disease, most have yet to be permitted for use in the clinic. Still, there is much potential for the betterment of treatment when considering that many of the proteins that cause aggregation have been identified and that many mechanisms have been found to successfully dissociate them, even if more research is required before they are used practically. Several methods have been proposed to combat neurodegenerative disease, including the use of antibodies, chaperone proteins, and gene therapy; each of which have been shown to be quite effective. Once refined, these techniques could soon become the foundation for the management of neurodegenerative disease in a matter of years, providing both a means to

prevent degeneration and hope that these deadly diseases may someday be much more manageable.

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