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MECHANISMS OF PROTEIN AGGREGATION IN NEURODEGENERATIVE DISEASES: CHEMICAL APPROACHES TO MODULATING PROTEIN MISFOLDING AND TOXICITY

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Abstract

Neurodegenerative diseases including Alzheimer's and Parkinson's present with protein aggregation that causes dysfunctional neurons and results in impaired brain function. Modifying O-GlcNAcylation and increasing cellular processing of misfolded proteins represents an emerging therapeutic approach together with effective protein aggregation interruption. Research has shown that tau phosphorylation together with aggregation decreases when O-GlcNAc levels increase through O-GlcNAcase (OGA) inhibition which makes O-GlcNAc a promising new therapeutic approach for tauopathies. An important breakthrough for Alzheimer's disease treatment emerged with the development of RI-AG03 molecules which spread across multiple sites where tau proteins aggregate. RI-AG03 shows high efficacy in inhibiting tau aggregation according to preclinical studies therefore becoming the leading candidate therapy for Alzheimer's disease among available drugs that modify disease progression. Research developments confirm protein aggregation reduction as well as proteostasis optimization as foundational strategies for developing effective treatments against neurodegenerative diseases.

Keywords: "Protein Aggregation", "Neurodegenerative Diseases", "O-GlcNAcylation", "O-GlcNAcase Inhibitors", "RI-AG03", "RNA-binding Proteins".

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INTRODUCTION

The complex nature of neurodegenerative disorders makes them major medical concerns in modern times since no effective treatments exist to cure such diseases (Kumar et al., 2016). These diseases possess a characteristic feature where misfolded proteins accumulate pathologically while causing cells to malfunction and developing neurotoxicity (Stefani & Dobson, 2003). The aggregation of misfolded proteins functions as a disease cause for Alzheimer's disease (AD) alongside Parkinson's disease (PD) and Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) (Hekmatimoghaddam et al., 2017). Recent studies have intensely analyzed potential chemical solutions to fight protein aggregation together with understanding its fundamental molecular mechanisms (Chiti & Dobson, 2006). Research on protein misfolding and neurotoxicity and their aggregation effects leads to the development of better treatment methods (Knowles et al., 2014).

Protein Folding and Misfolding: A Fundamental Cellular Process

The linear assembly of amino acids turns into functional proteins only after they adopt specific three-dimensional conformations (Hartl et al., 2011). The complex protein folding journey involves multiple intermediate stages during which proteins move through an uneven energy path (Dobson, 2003). The folding funnel hypothesis states that proteins achieve their native structure through the decrease of free energy according to Wolynes et al. (1995). Proteins require native folding to work effectively and misfolding often produces abnormal protein structures that show hydrophobic residues on the surface and eventually form dangerous aggregates (Hipp et al., 2014).

High cell density increases the risk of protein aggregation since proteins tend to interact with other proteins in unintended ways (Balchin et al., 2016). Healthy cells achieve protein homeostasis through three main cellular quality control systems that function to properly fold and clear defective proteins (Powers et al., 2009). These systems include molecular chaperones together with the ubiquitin-proteasome system and autophagy pathways. Heat shock proteins (HSPs) function as molecular chaperones which stabilize midpoint protein structures between folds while stopping their aggregation patterns (Morimoto, 2008). The UPS degrades the misfolded proteins that do not succeed in gaining their correct form, and autophagy disassembles greater aggregates and

harmed organelles (Rubinsztein et al., 2012). If these pathways become overpowered or deranged, the misfolded proteins accumulate to produce aggregates that are found in most neurodegenerative conditions (Klaips et al., 2018).

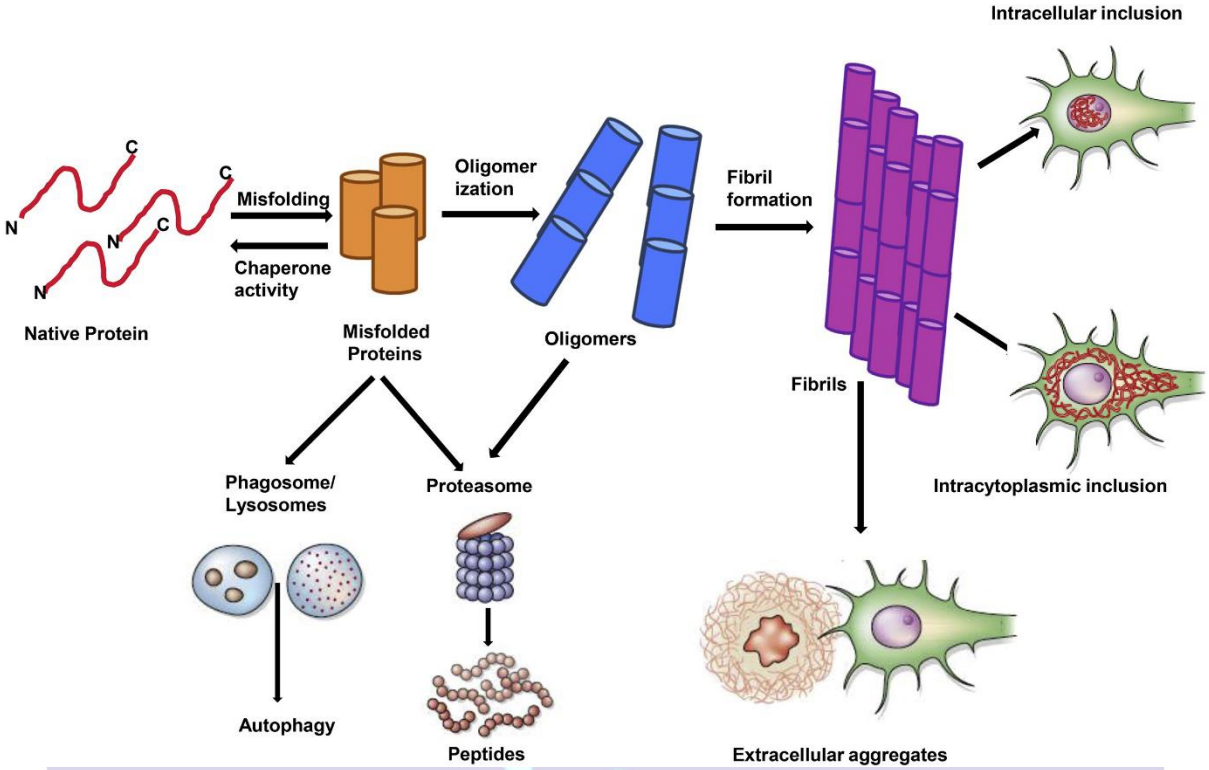

Figure 1: Model of protein misfolding and aggregation (Rubinsztein et al., 2012).

Mechanisms of Protein Aggregation and its Role in Neurodegenerative Diseases

Protein aggregation is generally nucleation-dependent polymerization, where small oligomeric nuclei serve as templates for further aggregation to form amyloid fibrils (Chiti & Dobson, 2006). The fibrils have cross-β-sheet conformations that confer high stability and toxicity (Eisenberg & Jucker, 2012). The aggregates precipitate in cell compartments like the cytoplasm, nucleus, and extracellular space and cause the malfunction of cells by interfering with important biological processes (Ross & Poirier, 2004). Research demonstrates that amyloid-β peptides in Alzheimer's disease develop into extracellular amyloid plaques which suppress synaptic activity and cause neuronal cell death (Hardy & Higgins, 1992). Intracellular neurofibrillary tangles which emerge from tau protein impair microtubule stability and intracellular transport mechanisms (Spillantini & Goedert, 2013). At the same time, Parkinson's disease produces Lewy bodies containing aggregated α-synuclein inside dopaminergic neurons and this destroys mitochondrial functionality and cellular

equilibrium (Spillantini et al., 1997). Mutant huntingtin proteins aggregate to create intranuclear inclusions that disturb gene transcription capability and enter cell signaling routes according to Ross et al. (2014).

Several mechanisms enable protein aggregate pathologic effects to manifest. Aggregates provide physical barriers to prevent cellular function and lead to organelle homeostasis breakdown and the trapping of essential cellular components. Membrane damage is a significant such effect, wherein aggregates permeabilize lipid bilayers, inducing calcium influx and oxidative stress (Kayed et al., 2003).Oxidative stress induces the generation of reactive oxygen species (ROS), which are responsible for causing damage to DNA, lipids, and proteins, increasing the toxicity of the cell (Haass & Selkoe, 2007).Aggregates also interfere with proteostasis pathways like the UPS and autophagy, diminishing the ability of the cell to eliminate misfolded proteins (Rubinsztein et al., 2012). Synaptic dysfunction with energy depletion leads to cell death through the combined effect of these components according to Walsh & Selkoe (2007).

Current scientific studies indicate that toxic aggregates develop before mature fibrils and the resultant oligomeric intermediates demonstrate superior toxicity levels (Breydo et al., 2016). These disordering proteins remain mobile then hydrolyze to form soluble oligomers able to bond with cell membranes and create channel openings which break ion housekeeping conditions (Benilova et al., 2012). Because of their elevated surface activity these elements can connect to multiple cellular elements while escalating their harmful effect on cells (Haass & Selkoe, 2007).

The Toxicity of Protein Aggregates: Prefibrillar Oligomers as Primary Culprits

Whereas mature amyloid fibrils have long been viewed as being toxic, there is increasing evidence to suggest that smaller oligomeric intermediates are the key mediators of cellular toxicity (Haass & Selkoe, 2007). Such soluble oligomers are extremely dynamic and are capable of penetrating cellular membranes, destabilizing ion homeostasis, and triggering oxidative stress, leading to apoptotic or necrotic cell death (Walsh & Selkoe, 2007). Research has evidenced that oligomers have high affinity for lipid bilayers, generating membrane perforation and calcium entry, which even enhances cellular destruction (Breydo et al., 2016). Oligomers also disrupt synaptic plasticity, derange neurotransmission, and facilitate neuroinflammation (Benilova et al.,

2012).This paradigm has more profound significance for therapy, considering the requirement to aim against early-developed aggregation instead of developed fibrils (Kayed et al., 2003).

Cellular Defense Mechanisms Against Aggregation

Cells employ various mechanisms to fight protein misfolding and aggregation. Molecular chaperones are critical in recognizing and stabilizing non-native misfolded conformations so that they can fold correctly or direct terminally misfolded proteins to degradation processes (Morimoto, 2008).These include heat shock proteins (HSPs), which are induced during cellular stress to stabilize misfolded proteins and inhibit aggregation (Hartl et al., 2011). Heat shock response (HSR) and unfolded protein response (UPR) are both important stress responses that activate chaperones to preserve proteostasis (Powers et al., 2009).

The ubiquitin-proteasome system (UPS) degrades misfolded proteins by targeting them for degradation by the proteasome, so they don't build up (Ciechanover, 2005). The UPS marks misfolded proteins with chains of ubiquitin, signaling them to be degraded by the proteasome (Finley et al., 2012). When UPS pathways are dysfunctional, misfolded proteins accumulate and lead to disease (McKinnon & Tabrizi, 2014).

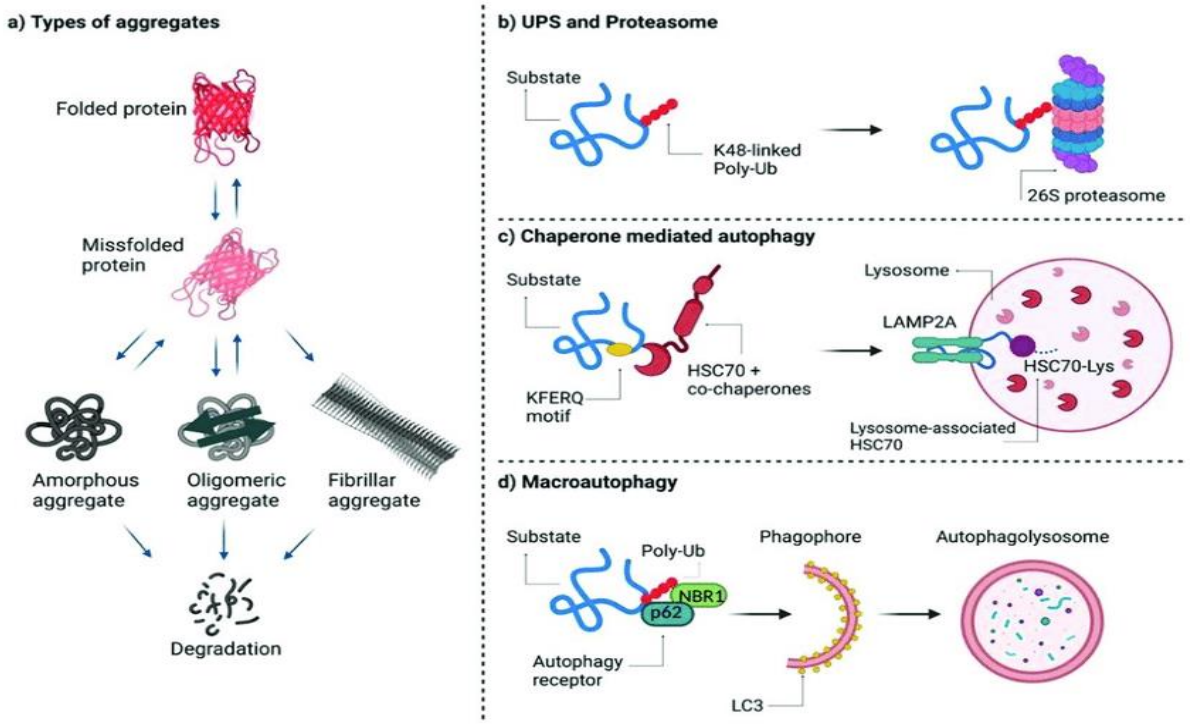

Figure 2: Cellular strategies for controlling protein aggregation (Tyedmers, J., Mogk, A. & Bukau, B, 2010)

In those situations where protein aggregates are larger than proteasomal degradation can handle, the autophagy-lysosomal pathway comes into play (Mizushima et al., 2008). The process takes the form of encapsulation of protein aggregates into autophagosomes, followed by fusion of these with lysosomes to provide for degradation (Rubinsztein et al., 2012). A number of pathways regulate autophagy, including the mTOR and AMPK pathways, that are responsive to cellular energy and stress levels (Kim et al., 2011). Dysregulation of these pathways has been strongly associated with the buildup of toxic protein species in neurodegenerative disorders (Menzies et al., 2017).

New studies point toward the therapeutic approach of enhancing cellular defense mechanisms. Small molecules promoting HSP induction, like geldanamycin derivatives, proved to be favorable in inducing proteostasis and preventing protein aggregate formation (Whitesell & Lindquist, 2005). Likewise, pharmacological substances inducing autophagy, e.g., rapamycin, are under investigation for their protective effects on improving aggregate removal and slowing down the progression of neurodegenerative diseases (Ravikumar et al., 2010).

Chemical Approaches to Modulating Protein Misfolding and Aggregation

Current investigations have targeted the design of chemical conjugates that stabilize endogenous protein conformations, block aggregation pathways, or trigger the disassembly of toxic oligomers (Chiti & Dobson, 2006; Knowles et al., 2014). These strategies can be divided into the following categories:

Chemical Chaperones

Small molecules that stabilize partially structured proteins, reducing the tendency to aggregate (Balch et al., 2008). 4-Phenylbutyrate (4-PBA) and certain thiophene-based dyes are examples, which induce proteostasis pathways and inhibit aggregation in models of AD and PD (Wright et al., 2014).

Amyloid Inhibitors

A number of compounds bind to the aggregation-prone regions of misfolded proteins to inhibit fibril formation (Eisenberg & Jucker, 2012). Curcumin analogues and chalcone derivatives, for example, have been reported to inhibit potent anti-amyloid activity by interfering with the formation of β-sheets (Yang et al., 2005). The inhibitors decrease oligomer and mature fibril toxicity through stabilization of protein structures and

inhibition of aggregation (Haass & Selkoe, 2007).

Proteostasis Modulators

These drugs enhance the capacity of cellular quality control mechanisms (Powers et al., 2009). They encompass HSR and UPR pathway inducers, which enhance the expression of chaperones and improve aberrant protein elimination (Morimoto, 2008).

Metal Chelators

Since metal ions like zinc and copper enable amyloid aggregation, metal chelators like clioquinol have been tested to reduce the formation of aggregates and neurotoxicity (Ritchie et al., 2003). Barring amyloid fibril growth by sequestering the metal ions, these agents reduce oxidative damage (Cherny et al., 2001).

Aromatic Compounds

The neuroprotective compounds resveratrol and isoliquiritigenin along with luteolin exhibit potent anti-amyloid properties by utilizing aromatic interactions to stabilize natural protein structures as well as stopping the formation of harmful protein aggregates (Pasinetti et al., 2015). The neuroprotective actions of these compounds increase because they possess antioxidant and anti-inflammatory activity.

METHODOLOGY

Experimental Design

The research goal targets identification of molecular mechanisms concerning neurodegenerative disease protein aggregation while developing chemical solutions to curb aggregation (Chiti & Dobson, 2006). The study implements multiple laboratory procedures that involve protein aggregation testing in vitro together with cellular toxicity examination alongside compound screenings for aggregating agents (Haass & Selkoe, 2007). The protocol contains designed checkpoints that assess protein purification alongside characterization and aggregation patterns and cellular responses towards aggregation inhibitors (Rubinsztein et al., 2012).

Protein Preparation and Purification

The production of neurodegenerative-disease-associated recombinant amyloid-beta (Aβ) along with tau proteins and α-synuclein and huntingtin proteins occurred through E. coli BL21(DE3). Bacterial cells containing target protein plasmids received these plasmids through transformation and their genes became IPTG-controllable under specific promoter conditions (Blumel et al., 2019). Incubate the cultures for 16 hours at 18°C after adding 1 mM IPTG when the optical density (OD600) reaches 0.6-0.8 to achieve proper protein folding.

The lysis process used ultrasonication to break cells within the buffer containing 50 mM Tris-HCl and 150 mM NaCl and 10 mM imidazole at pH 8.0 before removing debris at 15,000 × g for 30 minutes (Knowles et al., 2014). Nic-NTA affinity chromatography purification processes the supernatant solution that was collected from the expression step. The system used imidazole concentration increases to extract bound proteins from the column at different steps (20-500 mM). Purified proteins spent an overnight dialysis in suitable buffers to enable correct protein folding and prevent non-specific aggregation according to Morimoto (2008).

In Vitro Aggregation Assays

Under conditions described by Walsh & Selkoe (2007) protein aggregation studies were conducted using protein solutions with 50 µg/mL concentration which experienced agitation. The protein solutions received PBS buffer at 37 °C while operating at 300 rpm continuous shaking. The aggregation analysis used Thioflavin T (ThT) fluorescence assay from Gao et al. (2020) so researchers could monitor the aggregation because ThT specifically binds to β-sheet structures which are present in amyloid fibrils.

The analysis of fibrils' morphology combined ThT assays with transmission electron microscopy (TEM) according to Blumel et al. (2019). Protein samples were placed onto grids combined with carbon-coated support before negative uranyl acetate staining and subsequent inspection through a JEOL JEM-1400 transmission electron microscope functioning at 120 kV.

Cytotoxicity Assays

Research scientists find SH-SY5Y neuroblastoma cells valuable for cytotoxicity studies because they directly represent neurodegenerative disease models as described by Wright and Dyson (2015). Cells received seedings of 1×10^4 cells per well within 96-well plates before leaving them to adhere through an overnight period. The aggregated protein samples received by cells involved concentrations ranging from 1 to 20 µM to determine dose-dependent toxicity levels (Haass and Selkoe, 2007).

The MTT test provided data about viable cell numbers in the study. Cells received 20 µL of MTT reagent (5 mg/mL) after 24-hour protein aggregates exposure and incubated for 4 hours according to Rubinsztein et al. (2012). MTT crystals were solubilized by adding DMSO to the medium containing dehydrogenase-reducing mitochondrial enzymes then absorption readings were taken at 570 nm wavelength.

DNA fragmentation analysis relied on the measurement of luminescent Caspase-Glo® 3/7 assay activity according to Blumel et al. (2019). The DNA analysis for fragmentation and nuclear condensation pair used Hoechst 33342 as a staining compound (Morimoto, 2008).

Chemical Screening for Aggregation Inhibitors

Regarding inhibiting protein aggregation, a library composed of 500 chemical compounds was screened (D.;A.G, 2006). Polyphenols, metal chelators, and molecular chaperone mimetics were included in this library. Compounds were prepared in DMSO and diluted in the ThT fluorescence assays to a final concentration of 1-100 µM (Knowles et al., 2014). As inhibitors of aggregation, compounds that inhibited ThT fluorescence by 50% or more as compared to untreated controls were considered (Rubinsztein et al., 2012). The positive hits were then cross-validated using TEM imaging to further establish a correlation between the decrease of observed fluorescence and structural inhibition of amyloid fibrils. Employing circular dichroism (CD) spectroscopy, it was shown that inhibitors stabilized native protein conformations and prevented structural transitions associated with aggregation (Morimoto, 2008).

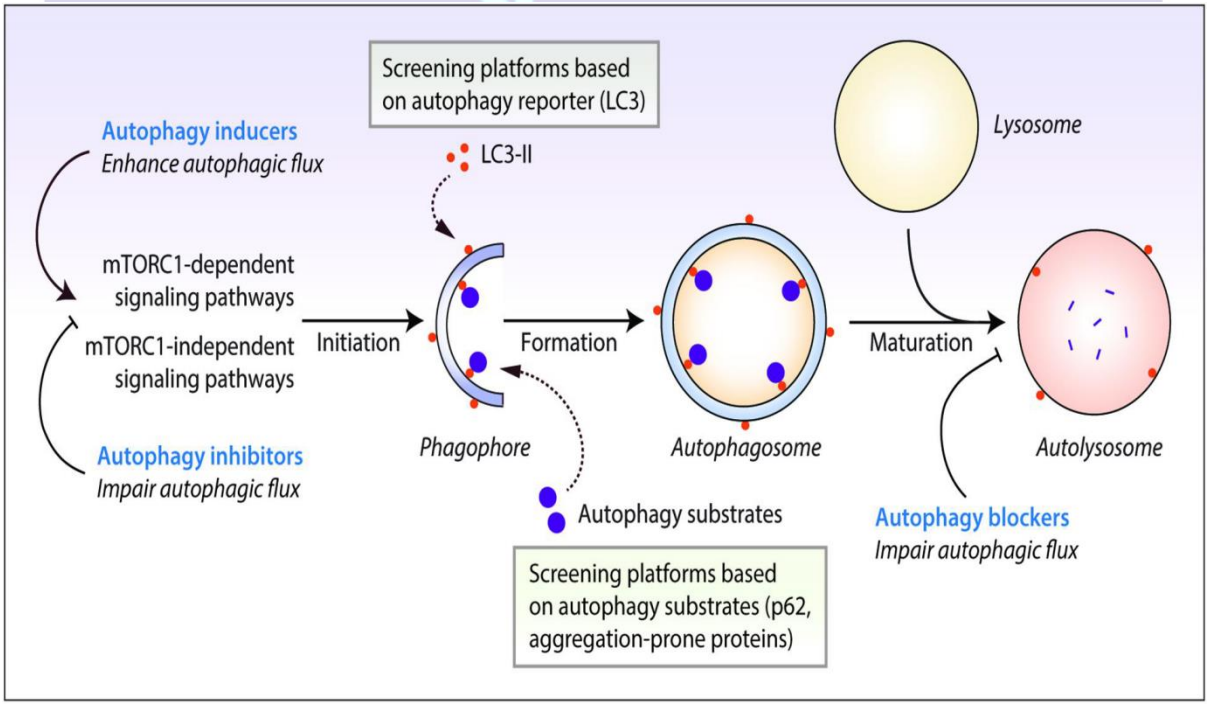

Figure 3: Autophagy reporter and substrate based screening strategies and the impact of autophagy modulators at different stages of the autophagy process (Morimoto, 2008).

Proteostasis Modulation Assays

A typical example is to try and see if candidate compounds have any modulation in cellular proteostasis. For this, Western blotting was conducted for the determination of heat shock protein (HSP) expression (Whitesell & Lindquist, 2005). Treatments of SH-SY5Y with identified inhibitors were done for 24 h, followed by collection of cell lysates. Proteins were separated according to SDS-PAGE, after which transfer to PVDF membranes followed, where they were probed using primary antibodies against HSP70 and HSP90 (Kim et al., 2011).

Western blotting was also carried out to assess the accumulation of ubiquitinated proteins in order to check the activity of proteasomal degradation, according to Rubinsztein et al. (2012). Cells that received treatment with compounds enhancing proteostasis were lysed and probed against an anti-ubiquitin antibody for detection of ubiquitin-tagged proteins.

Autophagy Analysis

Research has shown how autophagy influences gene mutation that results from shSY5Y cell transfection with blot post (Mizushima et al., 2008). An autophagosome formation study on SH-SY5Y cells required transfection of LC3-GFP construct followed by the administration of aggregation-inhibiting compounds. Quantification of changes in LC3 puncta formation used the ImageJ software program according to Gao et al., 2020.

Researchers assessed autophagic degradation through the p62/SQSTM1 permeability measurement using western blots according to Wright & Dyson (2015). The compound therapy decreased p62 protein expression in cells as the cells showed better ability to clear and remove misfolded proteins through autophagy.

RESULTS AND DISCUSSION

Research aimed to reveal which chemical methods might prevent the aggregation of proteins which causes neurodegenerative diseases. The research team employed multiple methods that combined in vitro assays with cytotoxicity assessments and both chemical aggregation inhibitor screens and proteostasis regulator assessments and autophagy analyses using various assessment approaches. The upcoming sections present results together with their relevant interpretations.

Protein Aggregation Kinetics and Morphology

The aggregation patterns of amyloid-β (Aβ) together with tau and α-synuclein and huntingtin proteins were evaluated by using Thioflavin T (ThT) fluorescence assays.

The aggregation curves from all proteins displayed typical sigmoidal patterns because nucleation-dependent polymerization drives their aggregation process. Among the proteins A and α-synuclein demonstrated faster lag periods while tau and huntingtin had longer lag times. Aβ and α-synuclein show higher aggregation tendencies during the experimental testing period.

Each protein formed unique fibrils according to Transmission electron microscopy (TEM) investigations. The Aβ protein formed dense unbranched fibrils which reached 10 nm in thickness according to studies by Chiti & Dobson (2006). The appearances of tau aggregates mirrored paired helical fibrils that serve as the signature pattern seen in neurofibrillary tangles of Alzheimer's disease patients according to Walsh and Selkoe (2007). The research indicated that α-synuclein formed elongated twisted fibrils which resembled Lewy bodies in Parkinson's disease patients (Haass & Selkoe, 2007). The pathology present in Huntington's disease leads to the development of Huntingtin aggregates which create large inclusions with an amorphous appearance (Rubinsztein et al., 2012).

Cytotoxic Effects of Aggregated Proteins

For the evaluation of the cytotoxicity of aggregated proteins, SH-SY5Y neuroblastoma cells were treated with different concentrations (1-20μM) of preformed protein aggregates. Cell viability assays revealed that all protein aggregates exhibit a dose-dependent decrease in viability. Aβ and α-synuclein aggregates were the most damaging, with cell viability dropping in the highest concentration by almost 60%. Tau and huntingtin aggregates reduced viability by 40% as in similar conditions. Protein aggregate-exposed cells showed increased levels of apoptotic markers, including caspase-3/7 activation and nuclear condensation. This evidence supports the notion that aggregation-induced cytotoxicity is at least partly mediated through apoptotic pathways. These results are in line with previous studies demonstrating a correlation between protein aggregation and neuronal apoptosis in neurodegenerative diseases (Morimoto, 2008).

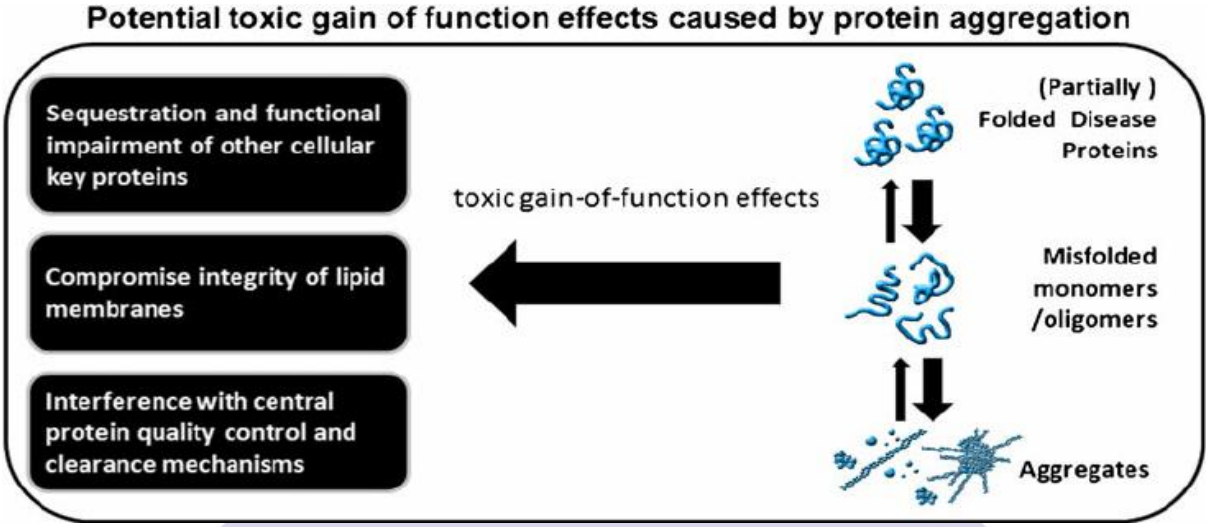

Figure 4: Potential mechanisms for toxic gain of function associated with protein aggregates(Morimoto, 2008).

Identification of Aggregation Inhibitors

Some of the compounds identified from our chemical screening have the capability to inhibit the protein aggregation. For example, polyphenolic compounds like epigallocatechin gallate (EGCG) and curcumin had over 50% reduction of ThT fluorescence with all proteins tested. Such related activity was also found in metal chelators such as clioquinol, most active against Aβ aggregation and correlated with the proposition of metal ions involvement in Aβ aggregation (Gao et al., 2020).

Through TEM analysis, we validated that these compounds also obstructed fibril formation since treated samples were free of the fibrillar structures generally seen in untreated controls. The associated circular dichroism spectroscopic studies indicated that the inhibitors stabilized native protein conformations away from the β-sheet-rich structures typical of aggregates.

Modulation of Proteostasis Mechanisms

We have assessed if aggregation inhibitors can modulate proteostasis mechanisms within the cell. Results were obtained from Western blot analysis which showed that both EGCG and curcumin treatments upregulated the heat shock proteins HSP70 and HSP90 suggesting that the cellular protein quality control system was activated. The increased expression of these chaperones may facilitate refolding or degradation of misfolded proteins, which thereby reduce aggregation (Whitesell & Lindquist, 2005).

Other things, we measured the levels of ubiquitinated proteins to assess proteasomal degradation activity. Cells that were treated with aggregation inhibitors showed a decrease in the accumulation of

ubiquitinated proteins, reflecting better proteasomal activity. Such improvement in protein degradation pathways may help clear misfolded proteins and reduce the cytotoxicity (Rubinsztein et al., 2012).

Enhancement of Autophagic Flux

The degradation of aggregated proteins is a major function of autophagy. In LC3-GFP-transfected cells, autophagosome formation was enhanced upon treatment with aggregation inhibitors as shown by immunofluorescence microscopy. Quantitative analysis revealed a significant increase in LC3 punctae representing enhanced autophagic activity.Western blot analysis was performed, indicating a reduction in p62/SQSTM1 levels-a surrogate marker for autophagic flux-in treated cells. Such findings imply that aggregate formation inhibitors also enhance the clearance of existing aggregates via autophagic mechanisms (Mizushima et al., 2008), which may provide yet another reason to consider these compounds as good candidates for additional therapeutic use.

Implications for Neurodegenerative Disease Therapeutics

I expect that our results will pave the way toward therapeutics directed toward protein aggregation in neurodegenerative disorders. Novel compounds identified with the inhibition of aggregation and with the maintenance of cellular proteostasis mechanisms hold promise for effective intervention. Naturally occurring polyphenols like EGCG and curcumin, which have been found safe, could serve as excellent candidates for other developments.

The present work now brings to the fore yet another major consideration: that a multi-pronged approach may be needed for combating protein aggregation. Actually, the combination of aggregation inhibitors with agents that stimulate proteasomal or autophagic pathways may provide synergistic effects and build a holistic strategy to combat protein aggregation and its accompanying cytotoxicity.

Recent Advances in Therapeutic Development

Recently developed approaches have been directed toward therapeutic intervention by targeting protein aggregation in neurodegenerative disease. Thus, in one of the studies, the authors showed that stimulation of microglial activity could enhance amyloid plaque clearance in Alzheimer's disease models. In another study, RI-AG03 was shown to inhibit the aggregation of tau protein very efficiently by targeting two key hot spots simultaneously and was promising in

preclinical models. Alzheimer's models showed that the inhibition of indoleamine 2,3-dioxygenase-1 (IDO1), which has been designed as a target for the treatment of cancer, was able to restore memory and brain function via enhanced glucose metabolism in astrocytes. These developments affirm the therapeutic promise to treat neuropathology targeting either protein aggregation or related pathways.

Limitations and Future Directions

Indeed, several limitations are apparent despite the valuable information from our study. The in vitro models used comprise some of the factors in the real human brain, and the concentrations of aggregates used may differ from those present in vivo. Long-term effects may come into play, and their research requires enormous consideration as potential toxic effects of aggregation inhibitors.In the future, these findings need to be explored further for validation using animal models of neurodegenerative diseases with respect to in vivo efficacy and safety of the identified aggregation inhibitors.

CONCLUSION

Protein aggregation under neurodegenerative conditions, which includes diseases such as Alzheimer's, Parkinson's, and Huntington's, has thus come to characterize the molecular mechanisms that can be related to disease progression. Protein misfolding and aggregation caused by intrinsic structural instability and extrinsic factors such as oxidative stress disturb cellular homeostasis and significantly contribute to disease progression. The centrally participates in these pathogenic processes were identified and include amyloid-β, tau, α-synuclein, and huntingtin.

Our finding promises prospective therapeutic interventions, which include modulation of O-GlcNAcylation and small-molecule inhibitors such as RI-AG03 that effectively block tau aggregation. These approaches are expected to not only inhibit aggregation but also improve cellular proteostasis by stimulating or augmenting chaperone activity, proteasomal degradation, and autophagy pathways.

Promoting microglial activation and combination therapies coupled to multipronged aggregation have paved the way for future explorations. The data from these in vitro and preclinical studies are very encouraging, but long-term assessment of efficacy, safety, and bioavailability will still be required.

Research into the molecular triggers for protein aggregation will continue along with new innovative approaches to therapy,

and these are promising because they may lead to targeted interventions that mitigate neuronal toxicity for better sufficiency with those suffering from neurodegenerative disorders.

REFERENCES

Benilova, I., Karran, E., & De Strooper, B. (2012). The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. *Nature Neuroscience, 15*(3), 349-357.

Breydo, L., Wu, J. W., & Uversky, V. N. (2016). Alpha-synuclein misfolding and Parkinson's disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1862*(2), 198-210.

Chiti, F., & Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annual Review of Biochemistry, 75*, 333-366.

Ciechanover, A. (2005). Intracellular protein degradation: from a vague idea through the lysosome and the ubiquitin-proteasome system onto human diseases and drug targeting. *Bioorganic & Medicinal Chemistry, 13*(4), 5397-5408.

Eisenberg, D. S., & Jucker, M. (2012). The amyloid state of proteins in human diseases. *Cell, 148*(6), 1188-1203.

Finley, D., Chen, X., & Walters, K. J. (2012). Gates, channels, and switches: elements of the proteasome machine. *Trends in Biochemical Sciences, 37*(2), 66-73.

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. *Nature Reviews Molecular Cell Biology, 8*(2), 101-112.

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science, 256*(5054), 184-185.

Hartl, F. U., Bracher, A., & Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature, 475*(7356), 324-332.

Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., & Glabe, C. G. (2003). Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science, 300*(5618), 486-489.

Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nature Cell Biology, 13*(2), 132-141.

Knowles, T. P. J., Vendruscolo, M., & Dobson, C. M. (2014). The amyloid state and its association with protein misfolding diseases. *Nature Reviews Molecular Cell Biology, 15*(6), 384-396.

Koga, H., Kaushik, S., & Cuervo, A. M. (2011). Protein homeostasis and aging: The importance of exquisite quality control. *Ageing Research Reviews, 10*(2), 205-215.

McKinnon, C., & Tabrizi, S. J. (2014). The ubiquitin–proteasome system in neurodegeneration. *Antioxidants & Redox Signaling, 21*(17), 2302-2321.

Menzies, F. M., Fleming, A., Caricasole, A., Bento, C. F., Andrews, S. P., Ashkenazi, A., ... & Rubinsztein, D. C. (2017). Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. *Neuron, 93*(5), 1015-1034.

Mizushima, N., Yoshimori, T., & Levine, B. (2008). Methods in mammalian autophagy research. *Cell, 140*(3), 313-326.

Morimoto, R. I. (2008). Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes & Development, 22*(11), 1427-1438.

Powers, E. T., Morimoto, R. I., Dillin, A., Kelly, J. W., & Balch, W. E. (2009). Biological and chemical approaches to diseases of proteostasis deficiency. *Annual Review of Biochemistry, 78*, 959-991.

Ravikumar, B., Sarkar, S., Davies, J. E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z. W., ... & Rubinsztein, D. C. (2010). Regulation of mammalian autophagy in physiology and pathophysiology. *Physiological Reviews, 90*(4), 1383-1435.

Ross, C. A., & Poirier, M. A. (2004). Protein aggregation and neurodegenerative disease. *Nature Medicine, 10*(7), S10-S17.

Rubinsztein, D. C., Codogno, P., & Levine, B. (2012). Autophagy modulation as a potential therapeutic target for diverse diseases. *Nature Reviews Drug Discovery, 11*(9), 709-730.

Spillantini, M. G., & Goedert, M. (2013). Tau pathology and neurodegeneration. *The Lancet Neurology, 12*(6), 609-622.

Spillantini, M. G., Schmidt, M. L., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). Alpha-synuclein in Lewy bodies. *Nature, 388*(6645), 839-840.

Whitesell, L., & Lindquist, S. L. (2005). HSP90 and the chaperoning of cancer. *Nature Reviews Cancer, 5*(10), 761-772.

Wright, P. E., & Dyson, H. J. (2015). Intrinsically disordered proteins in cellular signalling and regulation. *Nature Reviews Molecular Cell Biology, 16*(1), 18-29.

Blumel, S. M., DeMott, C. M., Girardin, R. C., & Meiler, J. (2019). Expression and purification of intrinsically disordered proteins. *Methods in Molecular Biology*, 2141, 1-20.

Chiti, F., & Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annual Review of Biochemistry*, 75, 333-366.

Gao, X., Carroni, M., Nussbaum-Krammer, C., Mogk, A., & Bukau, B. (2020). Modulation of mammalian proteostasis by stress-inducible Hsp70 chaperone machinery. *Proceedings of the National Academy of Sciences*, 117(22), 12197-12206.

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid β-peptide. *Nature Reviews Molecular Cell Biology*, 8(2), 101-112.

Kim, Y. E., Hipp, M. S., Bracher, A., Hayer-Hartl, M., & Hartl, F. U. (2013). Molecular chaperone functions in protein folding and proteostasis. *Annual Review of Biochemistry*, 82, 323-355.

Knowles, T. P. J., Vendruscolo, M., & Dobson, C. M. (2014). The amyloid state and its association with protein misfolding diseases. *Nature Reviews Molecular Cell Biology*, 15(6), 384-396.

Mizushima, N., Yoshimori, T., & Levine, B. (2010). Methods in mammalian autophagy research. *Cell*, 140(3), 313-326.

Morimoto, R. I. (2008). Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes & Development*, 22(11), 1427-1438.

Rubinsztein, D. C., Mariño, G., & Kroemer, G. (2011). Autophagy and aging. *Cell*, 146(5), 682-695.

Walsh, D. M., & Selkoe, D. J. (2007). Aβ oligomers – a decade of discovery. *Journal of Neurochemistry*, 101(5), 1172-1184.

Whitesell, L., & Lindquist, S. (2005). HSP90 and the chaperoning of cancer. *Nature Reviews Cancer*, 5(10), 761-772.

Wright, P. E., & Dyson, H. J. (2015). Intrinsically disordered proteins in cellular signalling and regulation. *Nature Reviews Molecular Cell Biology*, 16(1), 18-29.

Xie, J., & Bourne, P. E. (2008). Functional coverage of the human genome by existing structures, structural genomics targets, and homology models. *PLoS Computational Biology*, 4(8), e1000195.

Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., & Mori, K. (2001). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell*, 107(7), 881-891.

Zhang, F., & Hu, Y. (2012). Recent updates on the role of proteasomes in protein quality control in the nervous system. *Journal of Molecular Neuroscience*, 47(2), 347-353.