

Literature Review

2.1 UNDERSTANDING THE MOLECULAR MECHANISMS OF NEURODEGENERATION

Degeneration of neurons in the body is a severe issue with multifaceted level of defects. A proper therapeutic strategy is still challenging because of the heterogeneity in the mechanism of disease progression. There are several mechanisms identified at the cellular and molecular levels in the literature as the probable cause of damage during neurodegeneration; few of them are explained here.

2.1.1 Oxidative Stress Damages: Almost Non-Recoverable

Most of the neuronal damages either originate or culminate into oxidative stress (Kagias et al., 2012). An imbalance between the functioning of cellular antioxidant systems and free radical generation is what can be understood as oxidative stress (Figure 2.1A). Pathogenic assault in the brain is actively combated by immune system, which is led by glial cells that involve cytokines based activation of leukocytes carrying out ROS/RNS mediated pathogen killing (Vaziri and Rodriguez-Iturbe, 2006). During an event of excessive pathogenic insult or mechanical damage over activation of immune system results in damaging of neurons by generating oxidative stress. Besides, mitochondria is known to generate energy for the cell, and it houses the electron transport chain (ETC) that ultimately results into energy formation and therefore, subjecting the cell to constant risk of oxidative stress due to the presence of redox carriers (Yan et al., 2013). Major neurodegenerative disorders fostered by the oxidative mechanisms include Amyotrophic lateral sclerosis (Barber and Shaw, 2010), Alzheimer's disease (Hwang, 2013), and Parkinson's disease (Chen et al., 2016).

2.1.2 Stressed Mitochondria Population

As discussed above, dysfunctioning in mitochondria is directly a reason for a free radical-mediated pathway for degeneracy (Figure 2.1B). Mitochondria houses the antioxidant system of neuron that continually runs to maintain the homeostatic balance ensuring proper functioning (Rabilloud et al., 2001). Mitochondria helps neurons by providing energy currency and uses antioxidative enzymes like glutathione-S-transferase (GST), catalase, superoxide dismutase (SOD), and glutathione peroxidase to maintain redox balance (James and Murphy, 2002). The presence of various electron carriers and subsequent flow of proton across the mitochondrial inner membrane possess a considerable risk for formation of free radical species along with oxidative phosphorylation generating superoxide ions, peroxides and highly reactive hydroxyl ion (Kausar et al., 2018). Mitochondria are also known as one of the principal regulators of the apoptotic mechanism, and their damage or dysfunction could directly lead to degeneracy in neurons (Guo et al., 2013).

2.1.3 Imperfect Neuronal Aging or Early Neurodegeneration?

Imperfect aging usually contracts accumulation of damages along with burdened and inefficient cellular homeostatic mechanisms ultimately leading to death (Collado et al., 2007). Neuronal glial and astrocyte cells that are undergoing senescence are seen to cause degeneration in neighboring neurons; however, their exact role in this etiology is yet to be understood (Figure 2.1C). Recent studies implicate certain factors are released by glial or astrocyte cells that lead to degeneration in neighboring neurons by the generation of protein accumulates (Bussian et al., 2018). There is a trend of late-onset of many nervous degenerative disorders, which may implicate increased tolerance to many of such stressful conditions. However, failure of neurons to deal with such toxic products generated by different stressful conditions can lead to their death. Additionally, the exact role by which the brain cells undergoing senescence leads to demise of neurons is not best known. Imperfect senescence is seen to be the source of the etiology leading to Alzheimer's and Parkinson's disease (Chinta et al., 2013; Boccardi et al., 2015).

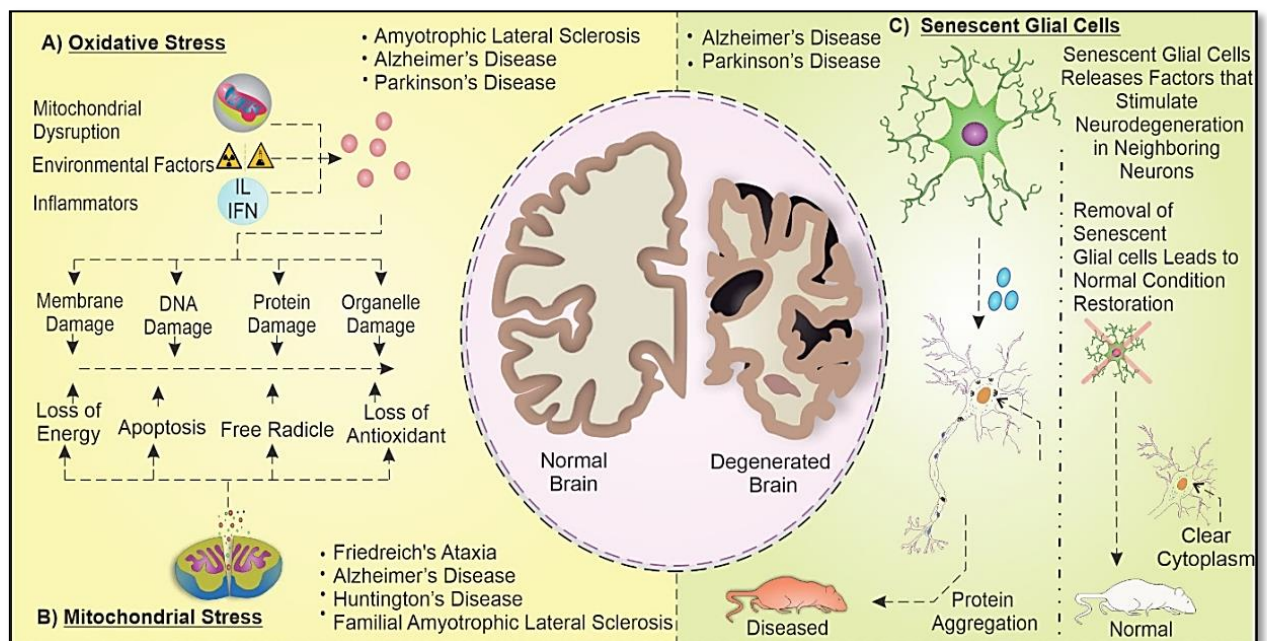


Figure 2.1 : Role of Free Radical and Aging Associated Damages in Degeneration of Neurons: The emergence of oxidative stress (A) and dysfunction in mitochondrial function (B) are collectively responsible for activation of several cellular toxicities. The accumulation of such toxic damages is responsible for activation of cell death pathway in neurons. Aging related senescent brain cells (C) are found to be responsible for propagating unknown factors that are seen to cause degeneration of neurons. (Source: Ankur R. Dubey and Amit Mishra et al, *Cellular Physiology and Biochemistry*, 2021)

2.1.4 Excitotoxicity

Several cellular mechanisms like unfolded protein response (UPR), autophagy, the UPS, DNA repair mechanisms, mitochondrial stress response, all of which are involved in maintaining general cellular homeostasis (Galluzzi et al., 2018). However, changes in their level of activation may be bestowed to diseased conditions. The over-activation of one such homeostatic secondary signal molecule, calcium ions are known to be one of the damaging factors of neurodegeneration (Bezprozvanny, 2010). The calcium signaling mechanism is known to be very diverse, performing different types of spatial and temporal functions in cells (Giorgi et al., 2018). Several excitatory synapses of the nervous system use glutamate as the

neurotransmitter and modulate two different kinds of receptors located at the postsynaptic neuron namely, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) (Li and Sheng, 2003). Binding of ligand (Glutamate) to AMPA receptor, a tetrameric protein containing binding site for glutamate in all its subunits, provides specificity to permeabilization towards calcium ions over sodium/potassium ions by exchanging one of the subunit GluR1 to GluR2 (Kim et al., 2001). Over-activation of NMDA receptor can be responsible for causing excitotoxicity, wherein excessive inflow of calcium ions in neurons disturbs the calcium balance and causes neuronal damage. Imbalanced calcium ions damages mitochondrial functioning and initiates intrinsic pathway of apoptosis. Besides this, excessive calcium ions may also en-route the cell to apoptotic pathways by directly interacting with the caspases, and cellular damages are amplified by damages to endoplasmic reticulum (Figure 2.2A) releasing the cellular calcium reserve back into the cytoplasm (Dong et al., 2009).

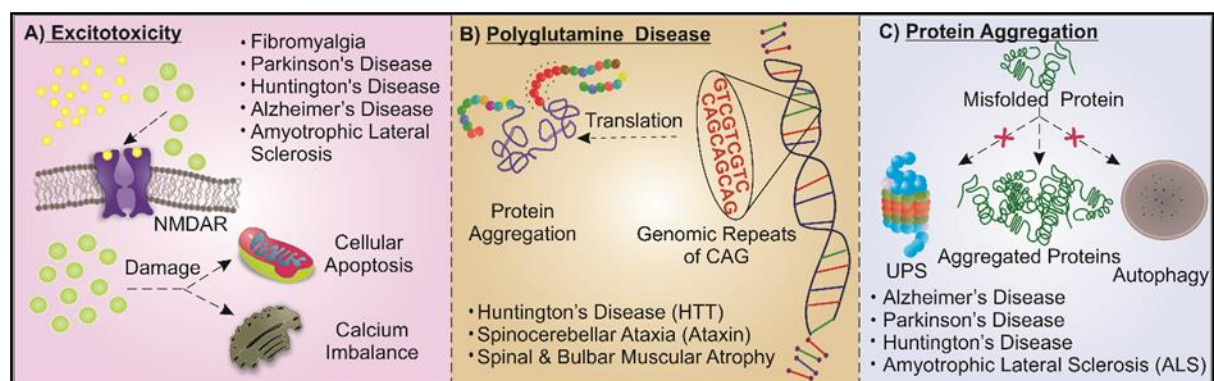


Figure 2.2 : Molecular Damages Responsible for Generation of Neurodegenerative Conditions: Several causative factors are linked to occurrence of neurodegenerative conditions. The molecular damage accumulation due to calcium imbalance (A) or genomic anomalies like increase in trinucleotide repeat (B) instigate the synthesis of misfolded aggregates. The aggregation of such aberrant proteins is responsible for blocking and impairing protein degradation mechanisms (C). (Source: Ankur R. Dubey and Amit Mishra et al, *Cellular Physiology and Biochemistry*, 2021)

2.1.5 Aberrant Polyglutamine Proteins Stress

Proteins are essentially a polymer of amino acids that share vibrant thermodynamic and electrostatic properties bestowed by their side chain. Genome is known to contain repeated units of "CAG" tri-nucleotide, which codes for amino acid Glutamine (Q). In polyglutamine disease there are long stretch of glutamine amino acid in the structure of protein (Figure 2.2B) and this forms an underlying cause for the beginning of pathogenesis of polyglutamine diseases (Gusella and MacDonald, 2000). Huntington's disease is known to be caused due to polyglutamine tract, which is an inherited autosomal dominant disorder (Dayalu and Albin, 2015). Polyglutamine tract is naturally present in the gene with shorter length (35-50 repeats) however, their length increases largely (40-100), rendering the polypeptide susceptible to aggregation (Cohen-Carmon and Meshorer, 2012). If we look onto the biophysical dynamics of such proteins there is potential change in the secondary structure of proteins with polyglutamine tract like for example, the extended track leads to a change of α -helix to β -folds which gets cross-linked to form aggregates by the process of polar zippers (Illarioshkin et al., 2018).

2.1.6 Elimination of Non-Native Protein: Essential for Cellular Protection

The presence of mutations or other cellular damages, as described above, may contribute to the generation of over-accumulated aberrant proteins in the cell, one of the significant properties of neurodegenerative diseases. There are highly conserved and ubiquitously present mechanisms inside the cell that govern and implement the process of clearing out aberrant protein species from the cell (Figure 2.2C), UPS and Autophagy (Tyedmers et al., 2010). Pathological conditions that arise due to aggregation of protein molecules are recognized as proteopathies (Boland et al., 2018). The process of protein misfolding is not very well understood, and therefore, protein aggregation and their role in neurodegeneration is still a mystery. However, various implication are present and despite intensive work, pin-pointing to exact cause is still not possible (Soto, 2003). Toxic proteins may build-up and associate with itself or with other cellular elements, giving rise to inclusions of proteins that may in-turn become a factor for causing the above described cellular and molecular anomalies. Moreover, over-accumulation of such proteins is found to impair the cellular capacity to clear them from cytoplasm leading to apoptosis (Bence et al., 2001; Riera-Tur et al., 2022). It is therefore, imperative to focus on strategies that help in dissolving such toxic protein species during neurodegenerative conditions.

2.2 CELLULAR PROTEIN QUALITY CONTROL EFFECTORS

Protein quality control ensures the cellular proteome is devoid of any worn-out or misfolded protein elements. The regular functioning of this quality control mechanism ensures the overall health of the cell as it is involved in regulating several essential pathways and processes. The core elements forming the protein quality control mechanism involves the chaperone function and clearance of aberrant polypeptide molecules by the UPS or the autophagy. This section represents a concise understanding of the mechanistic details of different cellular protein quality control effectors.

2.2.1 Molecular Chaperones

The synthesis of protein and its subsequent folding into native conformation is an outcome of vigilant action of heat shock proteins (HSPs)/chaperones. Chaperones and chaperonins principally identify and help modulate the interaction between the hydrophobic residues in polypeptide chains (Kim et al., 2013). Molecular chaperones perform a significant function in regulating the proteins in native conformation under general and stressed cellular conditions. The misfolding of protein exposes water insoluble amino acids on the surface of protein. Chaperones with the help of hydrophobic amino acid stretches in their active site interact with these exposed hydrophobic patches on client protein and help them get buried into the core. Most often chaperones use ATP to perform the binding and release cycle to their client proteins (Figure 2.3). Some chaperones are also known to target the client protein towards degradation. In cell HSP70, HSP90, and HSP60 form the major class of chaperones that are invested in folding and refolding of polypeptides. Additionally, several small HSPs and high molecular weight HSPs are also involved in holding and folding of polypeptides.

2.2.2 The Ubiquitin Tag Based Protein Modification

Inside the cell, proteins are responsible for highly diverse structural and functional roles in an integrated, specific, and regulated fashion. Ubiquitin modification forms one of the essential tools in conciliating the functional regulation of proteins in the cell. Ubiquitin is a small protein containing seven different lysine residues (K-6,27,11,33,29,63,48) and, each of these lysine residues and the first methionine can act as a polymerization site to form a diverse

system of regulation (Pickart and Fushman, 2004; Braxton et al., 2019). Mono-ubiquitylation at single or multiple locations is also an essential form of post-translational modification, primarily modulating the subcellular localisation of proteins (Hicke and Dunn, 2003).

2.2.3 The Ubiquitin Proteasome System for polypeptide degradation

Similar to the synthesis of the polypeptide, the disintegration of proteins is also a highly regulated process (Figure 2.3). The polyubiquitin chains are considered as death tags for worn-out proteins, identified by the proteasomal machinery or autophagic elements at the lysosomal membrane (Lecker et al., 2006). Ubiquitin tagging is performed with the help of following conserved groups of enzymes; E1, E2, and E3. E1 protein or enzyme is accountable for interacting with the ubiquitin protein and, with the help of ATP, performs ubiquitin adenylation and later thioesterification to form covalently attached E1~ubiquitin intermediate (Haas and Rose, 1982). Later, ubiquitin is moved to the E2 protein, also understood as the conjugating enzyme. With the help of a transthiolation reaction, a thioester intermediate is formed between E2 proteins and ubiquitin (Stewart et al., 2016; Keszsei and Sicheri, 2017). E2~ubiquitin adduct may associate with the E3 ubiquitin ligating protein, which mediates the transference of ubiquitin to desired target (Scheffner et al., 1995). E3 ubiquitin enzymes on the basis of their ubiquitin ligase domain can be characterized as HECT (Homologous to the E6-AP Carboxyl Terminus) E3 ligases (~30 in humans), RBR (RING-between RING-RING) E3 ligases (~12 in humans), and the principal class RING (Really Interesting New Gene) E3 ligases (~600 in humans) (Morreale and Walden, 2016).

This cycle of ubiquitin transthiolation reaction repeats creating a tag of four or more ubiquitin at a particular substrate. Often these tags are extended by the E4 ubiquitin ligase that works with E1, E2, and E3 enzymes to extend the chain (Koegl et al., 1999). The proteasome then identifies the ubiquitin-tagged protein clients, wherein its regulatory 19S subunit identifies the K48-tagged polyubiquitin chain and binds to it. The complete processing by the proteasomal machinery of the tagged substrate involves its identification, unfolding, transport through the 20S core particle (CP), removal of ubiquitin tags, and finally proteolysis at the active sites of CP with the help of nucleotide hydrolysis (Finley, 2009; Mishra et al., 2018). Often entire mechanism is regulated with the help of deubiquitinases that essentially performs the opposite functions to that of E3 ligases. Deubiquitinases breaks the ubiquitin chain in a distinct three-step process that involves recognising substrate, forming ubiquitin-acyl-enzyme intermediate, and finally undergoing hydrolysis to form free ubiquitin molecules (Love et al., 2007).

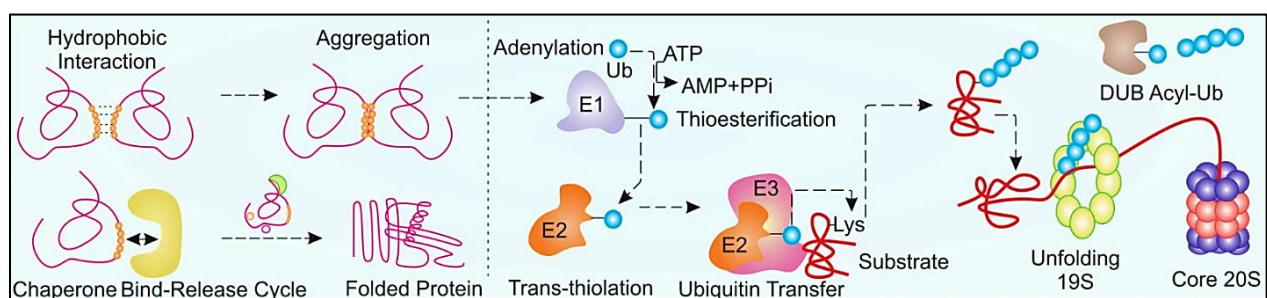


Figure 2.3 : Proteostasis by Chaperones and Proteasome Dependent Protein Clearance: Synthesis of aberrantly folded proteins are closely monitored by molecular chaperones by avoiding the non-native interactions. Functions of chaperone help to maintain aberrant polypeptides in soluble form in the cytoplasm. The generation of toxic intermediates of misfolded proteins is progressively tagged with ubiquitin for their degradation with the help of proteasome machinery. (Source: Ankur R. Dubey and Amit Mishra et al, *Molecular neurobiology*, 2022)

2.2.4 Lysosome-based Disintegration of Proteins and its types

Autophagy is considered as a homeostatic catalytic process of the eukaryotic cell, which is instigated in response to starvation conditions. Autophagy is identified in three majorly distinguishable forms as shown in Figure 2.4, namely macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Its mechanistic process was understood from extensive work carried in yeast by several groups, and the Nobel prize in the field of Physiology or Medicine was given to Yoshinori Ohsumi for his contribution in 2016 (Levine and Klionsky, 2017). Double membrane bound phagophore and autophagosome formation is the hallmark feature for macroautophagy. Damaged organelles, infective agents, and malicious proteins are the major target cleared from the cytoplasm with the help of macroautophagy selectively as well non-selectively (Feng et al., 2014).

The activation of macroautophagy and formation of autophagosome, enclosing target molecules/organelles, comprises of two distinct complexes; ULK1 and PI3KC3-C1/C2. A myriad of signaling events can activate ULK1 complex that essentially comprise of FIP200 (scaffold protein), ATG13, ATG101 and ULK1. Studies have revealed that ULK1 undergoes autophosphorylation in order to get activated which is promoted partly due to the formation of complex itself, increasing local level of ULK1 leading to its autophosphorylation (Wong et al., 2013). Post activation the recruitment of ULK1 complex at the site of phagophore activation (lipid rich section of ER membrane) is another regulatory event which in mammals is considered to be mediated by the association of LC3 and ATG9 with ULK1 (Hurley and Young, 2017).

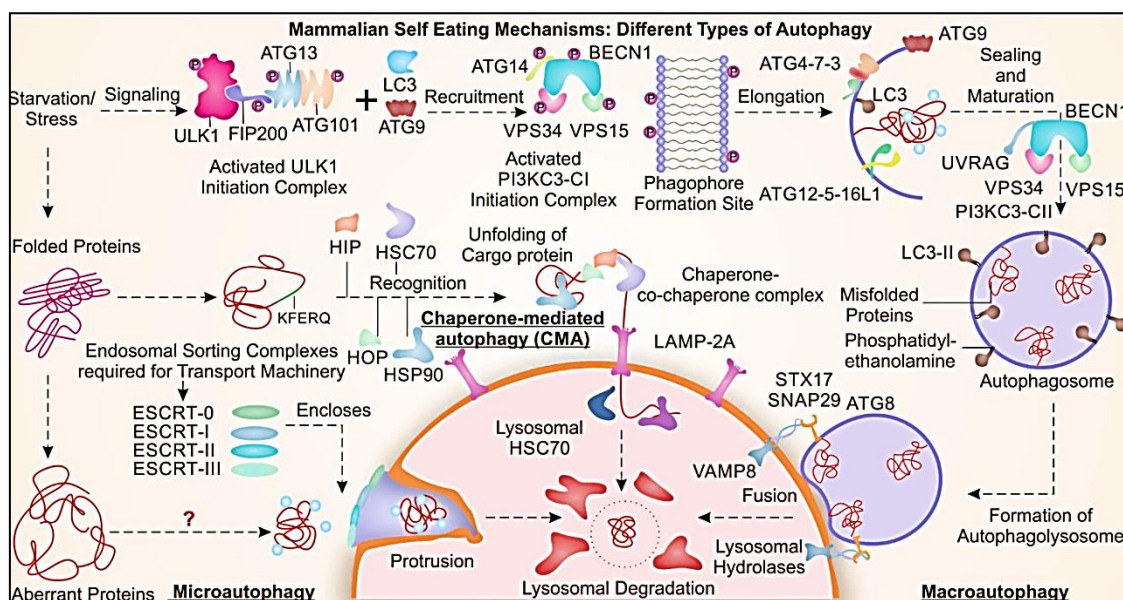


Figure 2.4 : Mechanistic Details of Autophagy and its Types: Autophagy is one of the central homeostatic process of the cell involved in the disposal of toxic or damaged elements. Three principle forms of autophagy are identified, with differences in their precise mechanism for target delivery into the lysosome lumen namely; the macroautophagy, the microautophagy and chaperone-mediated autophagy. (Source: Ankur R. Dubey and Amit Mishra et al, *Molecular neurobiology*, 2022)

Secondly, this activated ULK1 complex further goes and phosphorylates its substrates one of which involves PI3KC3-C1. The PI3KC3-C1 complex basically involves VPS34, VPS15, BECN1, and ATG14 whereas, PI3KC3-C2 consist of VPS34, VPS15, BECN1, and UVRAG (Obara et al., 2006; Itakura et al., 2008; Matsunaga et al., 2009). The complex I is accountable for

elongating the phagophore whereas the complex II is involved in the autophagosome maturation. Once, the cargo is loaded in the autophagosome it encloses and undergoes maturation process. The autophagosome is identified to contain LC3 and PI3P as critical feature, along with several ATG proteins. During maturation these ATG proteins, linked to membrane with the phosphatidylethanolamine, are released from the autophagosome by the action of several phosphatases (Reggiori and Ungermann, 2017). After maturation, autophagosomal outer membrane combines with the lysosome and forms an autolysosome wherein the inner membrane is removed, exposing and disintegrating cargo (Hurley and Young, 2017).

Similarly, during starvation conditions along with macroautophagy, microautophagy is also instigated in order to meet the metabolic requirements. The process of microautophagy involves protrusion of lysosomal membrane to eat up cellular elements. Lysosomal protrusions that carryout the microautophagic process are distinct and consist of definite bend at the top of protrusion (Knorr et al., 2017). These protrusions are believed to be formed with the help of lipid dynamics as the protrusion show a heterogeneous dispersion of protein to lipid content along with loss of protrusion when subjected to lower temperatures (Li et al., 2012). Once the cytoplasmic components are internalised by the protrusions the next step is to seal the protrusion forming an internalized lysosomal vesicle inside the lysosome lumen. In order to seal the protrusion ESCRT complex and VSP4 (AAA-ATPase) may seal any endosomal elements via identifying ubiquitinated endosomal membrane proteins (Adell et al., 2017; Vietri et al., 2020). Additionally, members of ATG family such as ATG8 and ATG11 (for generation of structure resembling to phagophore) also are involved in closing the membrane protrusion as well selectively docking cytosolic elements to lysosome membrane (Li et al., 2012; Schuck, 2020).

The process of autophagy can be selective as well as non-selective, which was first perceived from the fact that not all cellular proteins undergo lysosome-mediated clearance (Kaushik and Cuervo, 2018). In search of specific form of autophagy, a striking feature was noticed during the study of degradation of Ribonuclease A. This protein was found to have a unique sequence that was eventually described as KFERQ sequence. Proteins consisting of KFERQ sequence are thought to get degraded by CMA (Dice, 1982;1990;Kaushik and Cuervo, 2018). The process of CMA is regulated by critical functions of chaperones like HSC70 and co-chaperone proteins (Chiang et al., 1989). HSC70 recognizes the KFERQ motif of aberrant proteins along with the involvement of co-chaperones like HSP40, HOP, BAG-1, HIP, and HSP90 delivering the cargo towards the LAMP-2A (Agarraberes and Dice, 2001).

The protein cargo then undergoes unfolding using this chaperone-co-chaperone complex and moves into the lumen where lysosome lumen based chaperones HSP90_L and HSC70_L mediate the complete translocation of protein cargo into the lysosome lumen (Bandyopadhyay et al., 2008). The process of CMA is characterized by the involvement of the two critical elements HSC70 and LAMP2A (Cuervo and Dice, 1996; Cuervo et al., 1997). While other forms of autophagy have come up lately that utilise chaperones such as chaperone-assisted selective autophagy (CASA). During CASA the protein cargo again interacts with HSC70 however, this time their recognition is modulated by BAG family proteins and CHIP (Arndt et al., 2010). The process of CASA is presumed to be a subtype of macroautophagy; hence, often their target proteins are ubiquitinated, which are recognized by p62 (Arndt et al., 2010; Fischer et al., 2020).

2.3 PLANT DERIVED COMPOUNDS FOR TARGETING PROTEINOPATHIES

In order to circumvent such proteotoxic problems, several plant derived natural compounds have demonstrated promising outcomes. Flavonoids are color imparting compounds present in several plants and possess a basic 15 carbon structure. Depending on their chemistry flavonoids can be further categorised, of which flavones, flavonols, and anthocyanin share several antioxidant properties (Panche et al., 2016). Besides polyphenols, terpenoids have also found several applications in protein aggregation disorders some of which are discussed in upcoming sections.

2.3.1 Flavonoids

Flavonoids form the biggest class of polyphenols and are well known for their antioxidant functions in nutraceutical industries. Structurally, flavonoids consist of basic structure of 15 carbons composed of two phenyl and one heterocyclic ring. The keto substitute of flavonoids is flavonols which are mostly formed in the aerial tissues. Flavones on the other hand consist of 2-phenyl-1-benzopyran-4-one substitution in their backbone and, are found in rich amounts in yellow or orange fruits and vegetables. Anthocyanins are water soluble pigments responsible for different colors based on pH consisting of charged oxygen atom in heterocyclic ring structure of flavonoids (Khoo et al., 2017). These flavonoids along with other classes are understood to possess antioxidant potentials creating their therapeutic value. Antioxidants are one such class of phytochemicals known to possess neuroprotective roles. Plants experience oxidative assaults due to the photo-oxidative cycles, and they combat it by the use of anti-oxidative molecules that are generated from the alteration of their normal metabolic pathways (Krishnamurthy and Rathinasabapathi, 2013).

Quercetin, Luteolin, and Eugenol are some of the bioactive molecules that are known to find application as quenchers of the free radicals inside the neuronal systems (Figure 2.5A). Quercetin is a flavonoid present in fruits rich in citric acid and is known for its many benefits such as anti-inflammatory, antioxidant, and anti-proliferative. However, Quercetin effects are restricted to be beneficial in a concentration-based manner (Jakubowicz-Gil et al., 2008). It has been seen that even nano-formulation of quercetin can relieve the oxidation induced damage to neurons by quenching the reactive species as well the caspase functions that otherwise would lead to degeneration (Ghosh et al., 2013). Another molecule belonging to the same group, flavonoids that are found in abundant amounts in broccoli is Luteolin. Luteolin, a flavone is crystalline and yellow used as dye for ages. It can be obtained from many of the vegetables and fruits and have different therapeutic roles mentioned in Chinese traditional medicine. Its therapeutic roles in nervous system comprise of anti-oxidant, immunomodulatory functions that provide neuroprotective effects against degeneration (Zhao et al., 2012; Shamsizadeh et al., 2017). Besides the antioxidant function, luteolin is also known to ameliorate amyloid β based toxicity in neurons (Cheng et al., 2010).

Apigenin is again a flavone that can be obtained richly from parsley, tea, cereals and is known to ameliorate the β amyloid toxicity in Alzheimer's (Zhao et al., 2013b). Myricetin and Genistein that are flavonoids and isoflavones present in tea, berries, fruits, lupine, fava beans, and soya beans respectively also have known to act as anti-amyloid compounds (Zelus et al., 2012; Ma et al., 2015; Velander et al., 2017). As illustrated in Figure 2.5B the action of these polyphenols is seen to act either by up-regulating the survival pathways of neurons, or as they essentially are flavonoids they act as anti-oxidants. Besides this they could directly interact with the amyloid β structure and prevent their association from different cellular functions rendering them inactive (Green et al., 2005; Porat et al., 2006). For example apigenin can reduce the synthesis of β amyloid toxic accumulates by downregulating the functional enzymes that are

responsible for their formation and processing; besides its role in signalling apigenin is found to be as anti-oxidative in function and reduce neuronal toxicity caused by the β amyloid induced oxidative stress (Zhao et al., 2013a). Myricetin is known to reduce amyloidogenesis by directly interacting and inhibiting the beta-secretase, enzyme responsible for amyloid β faulty processing, or by activating and up-regulating the alpha-secretase, enzyme responsible for amyloid β standard processing (Shimmyo et al., 2008). Apart from this it is involved in depolymerisation and oxidation of the monomeric units of amyloid and interferes with the build-up of the amyloid structures and prevents the hydrophobic peptides from interacting with each other in order to get aggregated (Fiori et al., 2012). β amyloid can activate apoptosis leading various cellular damages and genistein has been reported to reduce such cellular anomalies (Zeng et al., 2004). Genistein is involved as one of the significant neuroprotective molecules by up-regulating the cell survival pathways of neuron like JNK pathway and PK-C pathway (Luo et al., 2012; You et al., 2017).

The pathway of neuronal damage exhibited due over activation of NMDA receptors is majorly contributed by the action of calcium ions in the neuron that enters through the NMDA receptors (Beal, 1998). In addition to this the toxic insults could arise as a result of reactive species generation or by direct generation of reactive nitrogen species upon reaction with superoxide to form peroxynitrite. Such effect of nitric oxide gets amplified with the diffusion of the gaseous molecules to the neighboring neuronal sites leading to further damage. Callistephin and Kuromanin, which are extracted from fruits like pomegranate, strawberries and other berries are belonging to anthocyanin groups, known to quench the adverse effects of Excitotoxicity. Callistephin is a 3-O-glucoside of pelargonidin, a pigment responsible for orange color in plants and kuromanin (Chrysanthemin) is a 3-O-glucoside of cyanidin which is a color pigment responsible for red color. The action of both the compounds is seen to inactivate the nitric oxide synthase enzyme to reduce the synthesis of nitric oxide (Figure 2.5C) and may quench the damage resulting from the glutamate-induced excitotoxicity (Winter et al., 2017).

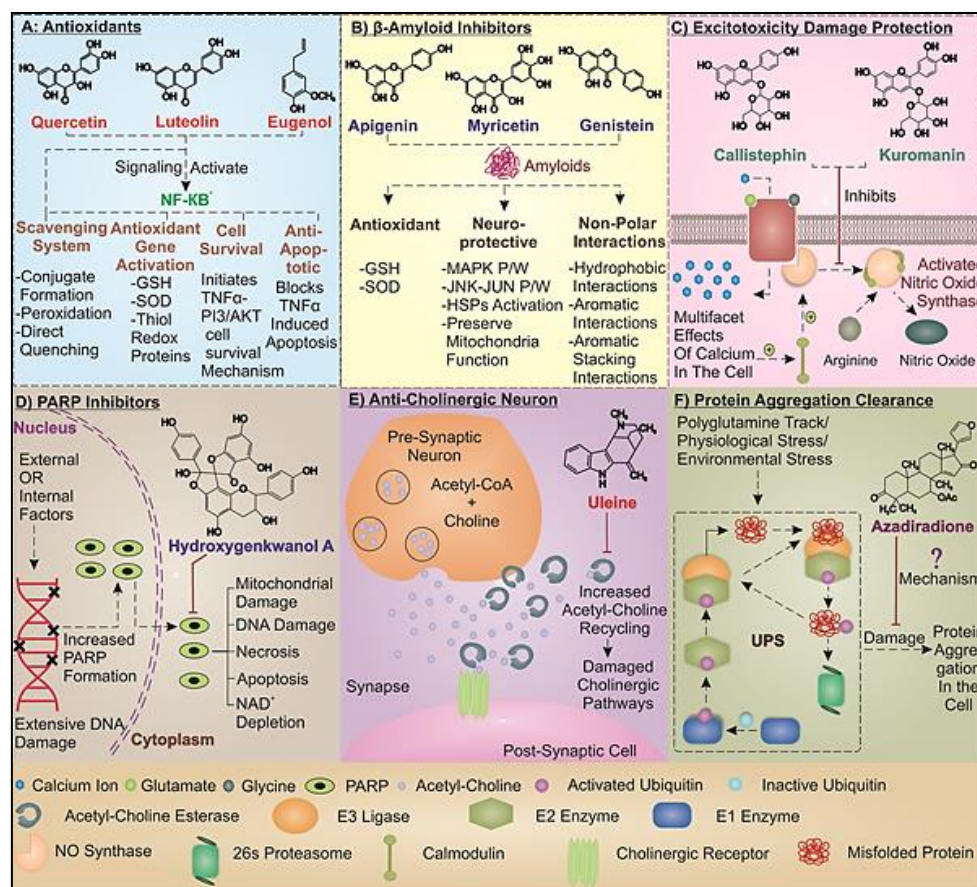


Figure 2.5 : Plant Dependent Compounds with Neuroprotective Properties: Critical homeostatic decline underlies the progressive build-up toxic damages in neurons resulting into neurodegenerative disorders. Several plant-derived phytochemicals are reported to inherit neuroprotective properties upregulating the cellular homeostasis potential that are found to be damaged during protein aggregation related neurodegenerative diseases. (Source: Ankur R. Dubey and Amit Mishra et al, *Cellular Physiology and Biochemistry*, 2021)

The genetic lesions do participate in the pathogenesis of degeneration in neurons. Figure 2.5D tries to give a schematic representation to describe the effects of excessive DNA damage resulting into initiation of repairation pathways for excessive DNA damage in neuron. DNA damage attracts the initiation of poly (ADP-ribose) polymerase 1 (PARP 1) which is the primary molecular catalase that is responsible for activation of various repair pathways (Soos et al., 2004; Morales et al., 2014). PARP-1 binds with the impaired DNA and undergoes conformation change and performs polymerization to form poly ADP-ribose polymer by using NAD⁺ as client molecule (Martire et al., 2015). Extensive oxidative insults results into the identification of open DNA ends by PARP-1 which then undergoes auto modification and leads to activation of a cascade of polymerization reaction forming poly ADP-ribose polymer attracting the DNA repair elements at the site of DNA damage (Ko and Ren, 2012). Moreover PARP-1 is also involved in triggering necrosis due to depletion of limiting co-factors (Ha and Snyder, 1999). Plant kingdom houses compounds that can neutralize the overproduced PARP-1 and one such example is Hydroxygenkwanol A which again is a flavonoid found in *Daphne* belonging to thymelaeaceae family. It is recommended for use by medicinal knowledge of Jordan for treating inflammatory disorders (Dal Piaz et al., 2015).

2.3.2 Phenylpropanoids

Phenylpropanoids are derivatives of the shikimate pathway and are found in almost all the plants. They are responsible for protection against infestants and ultra violet radiations. Eugenol that is found most abundantly in clove, cinnamon, bay leaf is a phenol molecule acting as anti-oxidant in nervous pathologies. It has found profound application in manifesting its neuroprotective (Said and Rabo, 2017) effects by inhibiting the NMDA receptors mediated Excitotoxicity and as well superoxide radical modulation (Wie et al., 1997). Scavenging system is majorly involved in directly interacting with the reactive species and performs their function by the formation of conjugates; inhibit peroxidation reactions or directly neutralizing the reactive species (Ahmadinejad et al., 2017). Intracellular signaling is mostly the role played by these bioactive, and they have been seen to converge their mode of action to single point in activating NF- κ B molecule which tends to activate different cellular system in the neuroprotection. NF- κ B can be considered as one of the essential modulator of cellular homeostatic pathways, whose modulation can either lead to cellular survival or cellular death fates (Wooten, 1999; Zhang et al., 2005). Activation of anti-oxidant genes and TNF α -PI3/AKT signaling improves survival functions whereas NF- κ B is also seen to provide neuroprotective effects by blocking the apoptosis (Mattson, 2005). The overall effects of these antioxidants bioactive comprise of (Figure 2.5A) terminal activation of antioxidant genes, modulation of different cell survival signaling pathways, and direct interaction with the reactive species, which help to maintain a healthy homeostatic balance in neurons.

2.3.3 Terpenoids

Isoprenoids or terpenoids are large class of multicyclic compounds composed of 5 carbon isoprene units. Terpenoids are found in most of the medicinal plants and some microbes forming the largest class of natural bioactive compounds. Their further categorisation is related to the amount of isoprene units that constitute them (Thoppil and Bishayee, 2011). Terpenoids are also known to help ameliorate the degenerative effects in neurons. As we can see from the causative factors described above, it is clear that the level of diverseness is vast if we look at the *in-vivo* condition. Apart from these mechanisms there is another effect that is not described in the earlier sections and is found to be associated with certain types of degenerative disorders, cholinergic dysfunctions (Figure 2.5E). Cholinergic neurons are those that use the primary neurotransmitter that is acetylcholine, which is the first neurotransmitter to be identified with exciting history and nature of activity, as chemical messenger at their synapses (Semba, 2004). These cholinergic pathways are found to be the primary mode of transmission in the parasympathetic system and are involved in development and storage of memory (Bartus et al., 1982; Blake and Boccia, 2018) and therefore are associated with dementia mediating neurodegenerative disorders especially with Alzheimer's disease. There is mounting proof of the participation of Neural Growth Factor (NGF) mediated pathway in pathogenesis of the cholinergic dysfunctions (Cuello et al., 2019). So the approach is to target the process of formation of acetylcholine in the neuron itself which is carried out by the critical enzyme choline acetyltransferase that uses acetyl-CoA subunits to be added onto choline residues (Nunes-Tavares et al., 2012). It is then further released into the synaptic cleft upon neural stimulation with the help of calcium-mediated vesical fusion (Kelly, 1993).

The discharge of acetylcholine into synapse is followed by immediate binding to their receptors onto the postsynaptic neuron. It is then released from the receptor of acetylcholine and then acted upon by an interesting molecule that degrades the acetylcholine, Acetylcholinesterase (Volle and Koelle, 1961). Our discussion is, however, limited to plant-based approach in treating the condition wherein Uleine that is obtained from *Himatanthus lancifolius* (Agoniada) which essentially is an Indole Alkaloid acting as an inhibitor of the enzyme acetylcholinesterase (Seidl et al., 2010). Uleine is relatively new compound that has

been identified to find application in treating degenerative problems. Apart from anti-cholinesterase function it is also reported to be possessing anti-amyloidogenic effects by inhibiting β secretase that is responsible for generation of altered length fragments in Alzheimer's Disease (Seidl et al., 2017). It can, therefore, act as an active agent in targeting cholinergic dysfunctions associated with development of degenerative diseases like Alzheimer's disease. Uleine besides this is recognized for its anti-inflammatory functions (Nardin et al., 2008), gastroprotective (Baggio et al., 2005) and anti-malarial (Dolabela et al., 2015) activities as well.

Celastrol, a quinone methidetriterpene isolated from *Celastrus orbiculatus Thunb*, is one such bioactive that can modulate protein quality control system by modulating the HSF-1 activation (Westerheide et al., 2004; Calamini et al., 2011). Also, endoplasmic reticulum plays active role in maintaining the protein quality inside a cell. A report by Denzel et al. showed that via modulating the hexosamine pathway, branch of glycolysis, by introducing precursors of the pathway, helps with ER based protein quality control and hence, maintaining proteostasis (Denzel et al., 2014). Green compounds, those isolated from plant sources, also find application in modulating the activity of autophagic flux of cell and may find various anticancer applications, for example, Rg2 a steroid glycoside is known to increase the autophagic status of cell (Fan et al., 2017). As discussed, E3 ligases are largely versatile and inherits the potential to particularly identify different protein clients for mediating their degradation (Upadhyay et al., 2017). Apart from different chemical modulators, plant derived phytochemicals are identified (Figure 2.5F) that are found to potentially enhance the UPS functioning by upregulating the E3 enzymes. Azadiradione is a tetracyclic triterpenoid found in *Azadirachta indica*, recently reported to help in removal of toxic protein aggregates from cytoplasm (Singh et al., 2018). Neem or *Azadirachta indica* is a well-known medicinal tree known to possess several probable therapeutic value as describe by several old heritable medicinal knowledge systems such as Ayurveda, Homeopathy and Unani (Subapriya and Nagini, 2005). Azadiradione is seen to up-regulate the chaperone system via HSF-1 pathway as well increasing the activation of E3 ubiquitin Ligase (Ube3a) and ameliorate the pathogenesis of Huntington's disease in mouse models.

Such reports implicate that improving the cellular PQC process can help in targeting the toxic build-up of aberrant polypeptides during neurodegenerative disorders. The aggregation of mutant or non-native proteins is found to harm the cellular capacity to degrade the mutant proteins and rather become trapped in such inclusions (Riemenschneider et al., 2022). Further the phenomenon of imperfect aging is found to be associated with decline in overall cellular capacity to perform efficient protein quality control functions (Blumenstock et al., 2021). Mutations with essential PQC members such as E3 ubiquitin enzymes are considered to be linked with the disease conditions of neurodevelopmental and neurodegenerative disorders (Kishino et al., 1997; Wettermann et al., 2012; Engelholm et al., 2014). Such indications implicate a strong linkage between the faulty proteostasis with the development of neurodegeneration. From above discussion it is evident that compounds derived from plants have inherent antioxidant property and may have allied functions to improve cellular capacity to fight neuronal insults. It is important to target the aggregation of toxic proteins to regain the neuronal homeostasis during protein aggregation disorder for coming up with therapeutic strategies. Thus, in the work presented here, we have tried to search for compounds or molecules that can help in clearing the accumulation of aberrantly folded protein species by enhancing the cellular protein quality control capacity.

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