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IRON AND A-SYNUCLEIN: HALLMARK OF A-SYNUCLEINOPATHIES

Sudeepa Srichandan[1]*, A.K. Panda[2], Pratima Ray[3]

[1]*,[2]National Institute of Immunology, [3]Jamia Hamdard University, New Delhi, India.

Introduction

Protein aggregation studies have presented amyloid aggregates as pathological agents responsible for several diseased conditions. β structures are difficult to degrade, that's may be the reason why amyloid deposits cannot be removed by the proteasome system of the cell (1). Deposition of misfolded oligomeric proteins having cross-β structure is a characteristic feature of a large number of disorders, collectively called "conformational diseases" (2,3,4). These diseases also known as Amyloidosis includes Alzheimer's disease (AD), Parkinson's disease (PD), Spongiform encephalopathies, Type-2 diabetes mellitus, Amyotrophic lateral sclerosis, Huntington's disease, systemic amyloidosis and all diseases caused by prion proteins (5,6). In recent years, number of studies has shown that the intermediates (oligomers, protofibrils) and not the final mature fibrils display toxicity (7). The amyloid fibril formation follows a nucleated growth mechanism. The nucleation phase is slow in which a nuclei is formed, followed by a fast elongation phase where the partially folded molecules of the protein or oligomers are formed, which binds to the nuclei forming amyloid fibrils (8,9). All amyloid-like proteins display a variety of common features like template-driven propagation, disruption of cellular defence mechanisms, mislocalisation of aggregated protein and glia-mediated neurotoxicity. (10) The structural analysis of these oligomeric species is difficult because of its unstable nature and they are short lived.

α-synucleinopathies

A group of neurodegenerative disorders called "α-synucleinopathies" are characterized by the presence of aggregated α-synuclein in the substantia nigra of the human brain. This includes Parkinson's disease (PD), Parkinson's disease dememtia (PDD), dementia with lewy bodies (DLB), multiple system atrophy (MSA) along with various neuroaxonal dystrophies. (11,12) The most common α-synucleinopathy phenotype is Parkinson's disease (PD). PD is caused by the death of dopaminergic neurons in the substsntia nigra, of the human brain. It involves presence of cytoplasmic inclusions in neurons. These proteinaceous intracellular inclusions called Lewy bodies, are hallmark of α-synucleinopathies. Lewy bodies contain a large number of molecules, the most predominant being α-synuclein (13,14). The neurochemical hallmark of PD patients is the accumulation of iron in the substantia nigra of the brain. (15) α-synuclein aggregates were first identified as the nonamyloid component of β-amyloid plaques in Alzheimer's disease (AD)

patients (16). Although AD has different pathology, majority of the patients have α-synuclein aggregates, which are restricted to the amygdala (17).

α-synuclein

α-synuclein is identified as the primary protein component of Lewy Bodies as found by different histochemical and biochemistry techniques. It is abundantly expressed in human brain as it is thought to be associated with the modulation of synaptic activity. It regulates the release of synaptic vesicles, neurotransmiter release and plasticity. (18) α-synuclein is a small protein of 140 amino acids, which consists of three different domains: an N-terminal domain, a central NAC domain and a C-terminal domain. The N-terminal domain (1–60) has a characteritic repetative conserved motif of six lysine-rich residues and has alpha-helical propensity. Most of the known clinical mutations are found in this region, increasing the propensity of protein aggregation. The central non-amyloid β-component (NAC) domain (61–95) is rich in hydrophobic amino acid residues, specifically contains a 12 aminoacid motif responsible for the aggregation and fibrillation of the protein. This region can undergo a conformational change from random coil to β-sheet structure and amyloid like fibrils. The C-terminal domain (96–140) is rich in acidic aminoacid residues responsible for the intricically disordered and flexible nature of the protein. (19,20,21)

Genetic factors for α-synuclein aggregation

α-synuclein is a small acidic protein of 14 kDa molecular weight consisting of only 140 amino acid residues that are highly conserved in vertebrates. It is encoded by the *SNCA* gene on the 4q21-q23 chromosome. Genetic studies indicate that single-nucleotide polymorphisms in the SNCA gene plays a direct role in disease pathogenesis. Multiplication of SNCA gene causes increased accumulation of α-synuclein, whereas missence mutations in SNCA gene locous enhances the propensity of α-synuclein aggregation. (22,23) Eight point mutations in SNCA gene: A18T, A29S, A30P, E46K, H50Q, G51D, A53E, and A53T are pathogenically associated with PD. These mutations mostly promote α-synuclein aggregation, but some mutations such as G51D, inhibits α-synuclein aggregation. (24,25) All these single amino acid substitutions lead to early onset of PD and has different effect on α-synuclein aggregation and oligomerisation. These mutations either increase the rate of aggregation or changes the oligomeric state of the protein providing evidence that prefibrillar oligomers are more toxic than the mature aggregated fibrils. (26) α-synuclein interacts with other neurodegenerative disease associated peoteins such as tau, amyloid- β and prion proteins describing its role in the pathogenesis of the neurodegenerative comorbidities (27). Evidence shows that abnormal aggregation of α-synuclein spreads from cell to cell in a prion like manner (28). So it can be hypothesized that along with α-synuclein aggregation other factors also contribute to the disease pathogenesis.

Microenvironment for α-synuclein aggregation

The atomic resolution structure of the α-synuclein fibrils showed that the NAC domain forms the β-sheet rich core where as the N and C-terminal forms a flexible random coil structure.

Intrinsically α-synuclein is an unfolded protein as it lacks a defined secondary structure but adopts a helically folded structure upon binding to biological membranes and other protein complexes. α-synuclein is known as a protein-chameleon due its significant conformational plasticity. It converts from its monomeric form to β-sheets by adding monomers onto itself eventually forming protofibrils and amyloid fibrils. It exists in different conformations and oligomeric states in a dynamic equilibrium regulated by small thermal fluctuations, that either accelerates or inhibits aggregation of the protein. (29,30) Solubility of α-synuclein on overexpression is supported by many intrinsic factors such as a positive average net charge, no cysteine residues, high hydrophilic index, no arginine which declines poly anion binding resulting in increased solubility, larger aliphatic index increasing thermostability of the expressed protein, a large number of turn forming amino acid residues, high frequency of dipeptides and tripeptides and a few adjacent hydrophobic amino acid residues. (31,32) α-synuclein adopts different aggregation states depending upon different micro environmental factors. Physical factors : Molecular crowding, temperature and Chemical factors : pH variations, metal ions, presence biomolecules such as proteins, nucleic acids, phospholipids affect the aggregation process. Physical factors affect the entrophy of the protein molecules where as chemical factors increase the neutralizing charge repulsion, favouring aggregation-prone structure of α-synuclein. Biomolecules act as a scaffold to promote protein aggregation. Post-translational modifications also contributes to the aggregation process by altering the surface charge or by changing the structure of the protein. (33,34) It has been hypothesized that the amyloid like insoluble fibrils or soluble protofibrillar intermediates might be the toxic species resulting in diseased condition (35). These pathologic aggregates induce fibrillation of endogenous α-synuclein which is self propagating (36).

Metal ions and α-synuclein aggregation

Essential trace elements like zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) acts as cofactors in many physiological cellular processes as well as organ and tissue development (37). Cellular homeostasis of iron (Fe), copper (Cu) and manganese (Mn) plays an important role in the normal functioning the brain. They are used as electron carriers by the enzymes or acts as catalytic centers for proteins involved in red-ox reactions. Many amyloidogenic proteins are known to readily oligomerize and aggregate in presence of metal ions *in vitro*. For example amyloid-β oligomerizes in presence of Cu and Zn (38), amylin oligomerizes in presence of Cu(II) (39), tau protein oligomerizes in presence of Al(III) and Fe(III) (40), and α-synuclein oligomerizes in presence of Al(III), Cu(II), Cd(II) and Fe(III) (41). Lewy bodies found in substantia nigra of Parkinson's patients brain have shown to have elevated levels of Al and Fe, specifically Fe(III) and Fe(III) binding protein ferritin (42). Cu and Fe controls necessary functions of the central nervous system including neurotransmitter synthesis, oxygen transportation and synaptic signaling. These redox active metals are used for most cellular functions yet remains a major source of reactive oxygen species (ROS). ROS production induces oxidation, misfolding and aggregation of essential proteins. Cells have mechnaism to control these damage due to ROS but their regulation is affected upon ageing, which is prevalent in Alzheimer's disease, Parkinson's

disease and Amyotrophic lateral scerosis (43). Reports have shown that genetic mutations and phosphorylation of certain amino acids in α-synuclein, increases its affinity for Cu and Fe. This induces oxidative damage of the protein, enhancing aggregation (44). α-synuclein in association with Cu changes its redox properties resulting of ROS production. α-synuclein contains an Fe binding site at the 5' region possibly increasing expression of α-synuclein with increase in Fe concentration (45,46). PD brains have shown to have loss of neuromelanin, an Fe chelating protein, which is thought to protect the substantia nigra from Fe mediated oxidative stress (47,48,49). So it can be hypothesized that elevated Cu and Fe levels initiates the dopaminergic neurodegenetation in PD.

Iron and α-synuclein aggregation

Iron accumulation and dysfunction of the IRP-IRE (iron response protein – iron response element) signaling pathway are linked to α-synuclein induced toxicity in neurons. The intrinsically unfolded protein α-synuclein has a high affinity to bind metal redox active metals. α-synuclein mRNA consists of iron response element (IRE) on its 5' untranslated region, which regulates the translation of the protein by the binding of iron response protein (IRP). In presence of iron, the IRPs are blocked by iron binding overexpressing α-synuclein transcripts and aggregation. α-synuclein is reported to act as a ferrireductase which influences iron homeostasis. (49,50) Mutations in the SNCA gene make α-synuclein aggregation prone or amylogenic, which increases its affinity for iron. This Fe- α-synuclein complex acts as a nucleation seed for the formation of oligomers and amyloid fibrils. This can be confirmed from reports showing oligomerization of α-synuclein upon addition of Fe during *in vitro* aggregation and during α-synuclein expression in *E.coli.* Like amyloid aggregates, inclusion body aggreagtes formed during expression in *E.coli*, can seed aggregation of similar proteins. These are highly dynamic in nature and are characterized by the continuous addition and removal of polypeptide chains. Protein molecules participating in inclusion body formation can reversibly disaggregate and fold into its native form. The presence of different structural forms of recombinant protein in the inclusion bodies depend upon the conditions used during the expression of the protein. Bacterial inclusion bodies have been reported to have native-like secondary structures and are considered biological active. Using bacterial inclusion bodies as a model, the interplay between iron and α-synuclein in protein expression and promoting protein aggregation supports the involvement of ferroptosis (iron mediated cell death) in the α-synuclein-mediated toxicity in PD. (51,52) Fe- α-synuclein fibrils are cytotoxic, propagates like prion protein and damage the cells by deleterious mechanisms such as increased ROS production, cell apoptosis, mitochondrial dysfunction etc. (53) The mutations are pathogenic as they help in transmission of the α-synuclein fibrils from host cells to receptor cells. (54)

Conclusion

Dyshomeostasis and accumulation of iron are related to several neurodegenerative diseases as they facilitate protein aggregation. Presence of iron facilitates α-synuclein expression and promotes aggregation which in return influences iron uptake and storage. α-synuclein is an essential factor

to spread the pathology in Parkinson's disease in a comprehensive manner, along with metabolic initiation factors, like iron, carbon monoxide, viral infections, oxidative stress, reduced antioxidative capacity, mitochondrial dysfunction, proteasomal and lysosomal dysfunction. Both iron and α-synuclein can be used as potential therapeutic targets in case of neurodegenerative diseases. Iron chelators and IRE inhibitors can also be used to prevent iron-dependent α-synuclein toxicity and neuron degeneration in PD.

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