(IJRMST) 2016, Vol. No. 1, Issue 1, Jan-Jun e-ISSN: 2455-5134, p-ISSN: 2455-9059

ROLE OF MISSFOLDING PROTEIN LIKE TAU TANGLES AND BETA AMYLOID AS MAJOR CULPRIT IN ALZIHMER DISEASE-THE FUTURE POTENTIAL THERAPEUTIC TARGET THERAPY

***Dr. Sandeep Kumar Mittan, **Dr K.S Anand**

**Dept. of Genetic Medicine Sir Ganga Ram Hospital, New Delhi*
***Prof. HOD, Dept. of Neurology PGIMER RML HOSPITAL, NEW DELHI*

INTRODUCTION

The accumulation of misfolded proteins is a hallmark feature in numerous human disorders including blood diseases like sickle cell anemia, neurodegenerative diseases such as Alzheimer's diseases (AD) and Parkinson's disease (PD), and metabolic diseases such as type II diabetes.(FEBS Lett. 2001 Jun 8; 498(2-3):204-7.). Misfolded protein aggregates may deposit intra cellularly or extra cellularly in tissues. The conformational changes accompanying Misfolding can result in disruption of the regular function of the protein or may result in a gain of function that is often associated with toxicity. Amyloid peptides represent a subset of misfolded proteins that share unique characteristics. (Cell Mol Life Sci. 2007 Aug; 64(16):2066-78.).

BACKGROUND AND SIGNIFICANCE

Accumulation of Neurotoxic proteins, including beta-amyloid plaques and Tau tangles are major culprits in Alzihmer Disease. In neurode generation, failure of cellular quality control mechanisms leads to inadequate protein degradation via the proteasome or autophagy, resulting in intracellular accumulation of toxic and pathological proteins. These Secreted proteins may not penetrate an adjacent cell via the synapse but they may be re-routed into the cell and recycled via the endosomal system to fuse with autophagic vacuoles like the autophagosome or the lysosome. These discoveries of toxic protein propagation from cell to cell leading to progression of neurodegeneration triggered a series of pre-clinical and clinical studies to limit protein propagation via antibodies (active and passive immune therapies) that can capture the protein and destroy it en route to healthy neurons. This approach is fraught with difficulties, including failure to stop neurocognitive decline and brain edema. This novel strategy essentially leads to unclogging the cell's disposal machine and degradation of toxic proteins, thus preserving neuronal survival via bulk digestion. As neurons are post-mitotic cells, pulsatile autophagy may promote protein degradation and provide an effective disease-modifying therapy for neurodegenerative diseases

Currently, the only pharmacological therapies available for AD are symptomatic drugs such as cholinesterase inhibitors or other drugs used to control the secondary behavioral symptoms in AD. Previously demonstrated therapeutic potential for anti-cancer tyrosine kinase inhibitors (TKIs) in neurodegeneration. TKIs are multi-target drugs and the role of specific tyrosine kinases (TKs) that are inhibited by TKIs is unknown. So the application propose to validate specific TKs as therapeutic targets for Tauopathies. Pazopanib (Votrient) is a multi-target TKI with highest affinities toward Vascular Endothelial Growth Factor Receptor 1-3 (VEGFR), Platelet Derived Growth Factor (PDGFR)α/β, and tyrosine-protein kinase.

ALZHEIMER DISEASE AND AMYLOID BETA AMYLOIDPROTEIN

AD is the most prevalent form of Neurodegeneration that is associated with severe cognitive impairment and memory loss primarily among elderly adults. Currently, over 5 million Americans are afflicted by AD, placing a severe burden on socioeconomic resources. (Alzheimer's Dement. 2011 Mar; 7(2):208-44.).Although the symptomatic and pathological signs of AD have been identified, a complete understanding of their origins remains elusive. This uncertainty has impeded progress toward developing effective preventive and disease modifying therapeutics; the presently available drugs alleviate the symptomatic burdens temporarily and are unable to deter or cure the disease.(Alzheimer's Dement. 2011 Mar; 7(2):208-44).Confirmation of the AD diagnosis is contingent upon the identification of misfolded protein aggregates, including the neurofibrillary tangles composed of hyperphosphorylated tau protein and senile plaques composed of Aβ peptides, as well as extensive oxidative damage and neuronal loss in the brain. (Angew Chem Int Ed Engl. 2009; 48(17):3030-59).

AMYLOID PRECURSOR PROTEIN (APP) AND AB GENERATION

The Aβ peptide is the predominant component of the senile plaques that accumulate in various brain regions and pathologically represent the AD condition. (Angew Chem Int Ed Engl. 2009; 48(17):3030-59.). Amyloid precursor protein involvement in AD neuropathogenesis has been extensively studied following the establishment of the Amyloid cascade hypothesis, which implicates Aβ as a causative agent in AD.(Science. 1992 Apr 10; 256(5054):184-5.). The Aβ peptide is composed of 38–43 amino acid residues and is generated through sequential proteolytic cleavage of the Amyloid precursor protein (APP) . Enzyme proteases responsible for APP cleavage and Aβ generation distinguish the resulting forms of Aβ as either non-amyloidogenic or amyloidogenic. Processing of APP by α-secretase initiates the non-amyloidogenic cleavage pathway, with initial scission between K687 and L688 (APP770 numbering) affording a large soluble fragment (sAPPα) and leaving a portion of the parent APP molecule membrane-bound.

AGGREGATED AB PROTEIN & THERE ROLE IN NEUROTOXICITY

Aβ is a relatively small peptide β monomeric protein features hydrophilic *N*-terminus and hydrophobic *C*-terminus, which play a role in driving its aggregation in an effort to the aqueous environment.(Chem Soc Rev. 2009 Sep; 38(9):2698-715.). Residues contained in the region from L17–A21 are identified as key contributors to self-recognition, due to their primarily hydrophobic nature, which might be influential in producing the higher-order structures. Aβ undergoes conformational changes during aggregation where the native random coil structure can be modified to either α-helical or β-strand-like forms. These plaques of Aβ naturally present in the body but, at higher concentrations both Aβ and especially the longer version become neurotoxices are mostly composed of amyloid, fibrol fiber. AB plaques may disrupt brain cells by clogging points to cell-cell communication. The progressive damage caused by Aβ sets up a cycle of cell death that eventually leads to almost complete deterioration of mental function.

EXPERIMENT (1)

a- To determine Tau levels, WB of total Tau using (1:1000) Tau-5 monoclonal antibody (Chemicon), and p-Tau will be probed (1:1000) with epitopes against polyclonal serine-396 (Chemicon), polyclonal AT8 (1:1000) Serine-199/202 (Biosource, Carlsbad, CA, USA), polyclonal AT180 (1:1000) threonine-231 (Biosource) and monoclonal (1:1000) human specific (HT7) antibody (Thermo Scientific). WBs will be quantified by densitometry using Quantity One 4.6.3 software (Bio Rad).

b- To measure Tau concentration, ELISA for total Tau and p-Tau at serine396 (Millipore) and/or threonine 231 (Millipore) will also be used in total brain lysates

C-To measure plasma and brain inflammatory markers,

Isolate blood and brain tissues to determine systemic and CNS immune profiles and identify possible adaptive and innate immune responses as we previously described (2,3).

SIGNIFICANT IMPACT OF RESEARCH IN FUTURE:

- This way will devolve a novel state-of-the-art approach to open a way of cure for neurodegenerative diseases, including Parkinson's, Alzheimer's ,Dementia's and other neurodegenerative diseases by perform biochemical and functional assays, including Western blot, ELISA, histology, proximity ligation and other behavioural assays.
- This way will open long range objective is to learn how Valuable therapy development, using a variety of disease models, such as cultured neuronal cells, primary neurons and animal models. In addition, will have the opportunity to conduct cutting-edge research in therapy development, as well as in the cellular basis of disease development. The outcome

of this research on genetic, molecular and next-generation sequencing aspects of various types of cancers and to identified a host of genomic alterations, and a number of pathways involved in some cancers, such as neurological, cervical, and hematologic malignancies.

- The discoveries of toxic protein propagation from cell to cell, leading to progression of neurodegeneration triggered a series of pre-clinical and clinical studies to limit protein propagation via antibodies (active and passive immune therapies). This approach is a novel therapeutic approach that focuses on degradation of neurotoxic proteins at the manufacturing site in order to prevent their secretion and propagation. This novel strategy essentially leads to unclogging the cell's disposal machine and degradation of toxic proteins, thus preserving neuronal survival via bulk digestion.
- The significant of this research plan will determine whether Pazopanib reverses cognition via Morris water maze and open field test, brain Tau and p-Tau levels and inflammation using WB, IHC and ELISA use of Pazopanib safety via measurement of liver function enzymes, including serum alanine transaminase (ALT), glutamine oxaloacetic transaminase (AST), and blood urea nitrogen (BUN) and kidney function via creatinine.

REFERENCES

1. Selkoe, D.J. Folding proteins in fatal ways. Nature, 426, 900-904 (2003).

2. Selkoe, D.J. Cell biology of protein misfolding: The examples of Alzheimer's and Parkinson's diseases. Nature Cell Biology, 6, 1054-1061 (2004).h will provide viable new treatments for AD with an adequate therapeutic window.

3. Tutar L, Tutar Y. Heat shock proteins: An overview. Current Pharmaceutical Biotech-nology 2010; 11(2):216-222.

4. Wang W. Protein aggregation and its inhibition biopharmaceutics. International Journal of Pharmaceutics 2005; 289: 1-30.

5. Stefani M. Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world. Biochimiaet Biophysica Acta 2004; 1739: 5-25.

6. Chaudhuri TK, Paul S. Protein-misfolding diseases and chaperone-based therapeutic approaches. FEBS Journal 2006; 1331-1349.

7. Winkhofer KF,Tatzelt J,Haass C.The two faces of protein misfolding: gain –and loss-of – function in neurodegenerative disease. EMBO J.2008,27:336-349.

8. Hebron, M., Lonskaya, I., Olopade, P., Selby, S., Pagan, F., and Moussa, C. E. (2014) Tyrosine kinase inhibition regulates early systemic immune changes and modulates the neuroimmune response in α-synucleinopathy. *J. Clin. Cell. Immunol.* **5**

9.Hebron, M. L., Lonskaya, I., Olopade, P., Selby, S. T., Pagan, F., and Moussa, C. E. (2014) Tyrosine Kinase Inhibition Regulates Early Systemic Immune Changes and Modulates the Neuroimmune Response in alpha-Synucleinopathy. *J Clin Cell Immunol* **5**, 259.

10. Ciechanover, A., and Kwon, Y. T. (2015) Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. *Experimental & molecular medicine* **47**, e147

11. Polymenidou, M., and Cleveland, D. W. (2012) Prion-like spread of protein aggregates in neurodegeneration. *The Journal of experimental medicine* **209**, 889-893

12. Mellman, I. (1996) Endocytosis and molecular sorting. *Annual review of cell and developmental biology* **12**, 575-625

13.Jerram, A. H., Smith, P. F., and Darlington, C. L. (1996) A dose-response analysis of the behavioral effects of (+)MK-801 in guinea pig: comparison with CPP. *Pharmacology, biochemistry, and behavior* **53**, 799-807

14.Luzio, J. P., Rous, B. A., Bright, N. A., Pryor, P. R., Mullock, B. M., and Piper, R. C. (2000) Lysosome-endosome fusion and lysosome biogenesis. *Journal of cell science* **113 (Pt 9)**, 1515-1524