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Pathogenic Role for Microtubule Stabilization Pathways in Alzheimer's Disease

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Currently, around 50 million people worldwide suffer from Alzheimer's disease (AD)[1]. The chief clinical manifestation of AD is memory loss, while the two main neuropathological hallmarks are the accumulation of amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). The consequences of the accrual of Aβ plaques and NFTs are widespread loss of synapses and eventual cell death, particularly in the hippocampus, a brain region important in learning and memory[2,3]. There is no cure for AD, and existing drugs offer only temporary cognitive gains. A deeper understanding of the molecular mechanisms behind AD is necessary so that new pathways can be targeted for treatment.

Microtubules (MTs) are cytoskeletal protein filaments comprised of repeating α and β tubulin subunits. In neurons, microtubules are particularly important because they support complex, branching structures, like the dendritic tree and axonal arbors[4]. Besides providing structural stability, microtubules also act as an intracellular highway, creating a road for protein motors to deliver important cargoes to various regions of the cell. Loss of efficient transport is detrimental to the health and normal functioning of neurons[5]. Additionally, it has been recently shown that microtubules contribute to the maintenance of synapses, which are the connections between neurons in the brain[6].

NFTs arise from the intracellular aggregation of hyperphosphorylated tau proteins. Tau, a protein residing mostly in axons in association with microtubules, binds at the interface between two tubulin dimers[7]. The function of tau in neurons has been controversial: It has been shown to increase MT stability *in vitro*[8], but recent work indicates that tau's true function may be to maintain the labile domains in microtubules[9]. Hyperphosphorylation reduces tau's affinity for MTs and creates an increase in the population of soluble tau. This hyperphosphorylated tau amasses into NFTs. When comparing brain images of people who are cognitively normal to patients with mild AD, measures of hyperphosphorylated tau deposits better predict symptoms of dementia than plaques[10].

The pathogenic mechanisms that trigger the formation of NFTs are complex and are still not completely understood. This project explores how changes in neuronal microtubules can contribute to the tau hyperphosphorylation and the tau-dependent neuronal damage seen in AD.

Neurons have both stable and dynamic MTs. Dynamic MTs differ from stable MTs in their ability to undergo stochastic transitions from depolymerization to polymerization and vice versa. It is the dynamic population of MTs that can enter into dendritic spines to regulate spine maintenance[11-13]. Dynamic MTs are also critical for presynaptic neurotransmitter release by providing the tracks for transport of synaptic vesicles (SVs). Interestingly, oligomeric Aβ decreases MT dynamics through a Rho/mDia1 pathway that leads to tau hyperphosphorylation and tau-dependent synaptotoxicity[14]. This finding indicates that changes in microtubule dynamics could serve as a pathological pathway between Aβ and tau phosphorylation.

Dynamic MTs can be stabilized by a variety of proteins, such as MT associated proteins (MAPs)[15]. Once stabilized, MTs live long enough to become substrates of tubulin modifying enzymes and accumulate a variety of posttranslational modifications (PTMs), which can then further affect stability[16]. One common tubulin PTM is cleavage of the terminal tyrosine of the α subunit at its C-terminal by vasohibins 1 and 2 (VASH1/2)[17]. The reverse pathway, the re-addition of tyrosine, is performed by tubulin-tyrosine ligase (TTL)[18]. Interestingly, TTL are reduced in the hippocampi of AD patients[19].

A tubulin PTM that derives from detyrosinated tubulin is Δ2-tubulin (D2). More dynamic MTs tend to cycle between tyrosinated/detyrosinated states. However, when detyrosinated MTs are further cleaved of an additional amino acid on their α-tubulin subunit by carboxypeptidases CCP1/4/6, this modification permanently prevents re-tyrosination[20-22]. The function of this irreversible tubulin PTM is not well understood, although D2 is seen in 35% of neuronal structures[23] as well as in long lasting MTs conformations in cilia[23,24].

Recent work from our lab has shown that oligomeric $A\beta_{1-42}$ can induce an increase in tubulin detyrosination[14] in hippocampal neurons, which is the first step in the process of D2 tubulin accumulation, and a role for D2 has been underscored in acute axonal injury (Pero et al., 2020). To test this new MT-based

pathogenesis model in the etiology of AD, we are exploring how abnormal accumulation of D2 by premature tubulin longevity could represent a feature of both familial and sporadic AD and also a molecular driver of synaptic injury and tau hyperphosphorylation.

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