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Microtubule-Tau Interaction as a Therapeutic Target for Alzheimer's Disease

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Introduction

Alzheimer's disease (AD) is an illness of progressive intellectual decline, presenting the most common age-related neurodegenerative disorder. AD is the most common cause of dementia and is characterized pathologically by the presence of extracellular plaques and intracellular neurofibrillary tangles (NFT) and by a selective loss of neurons and decreased synaptic density. In the middle 1980s, the major component of plaques was found to be β-amyloid (Aβ) (Glenner and Wong 1984a, b; Masters et al. 1985), generated by cleavage of amyloid precursor protein (APP) (Tanzi et al. 1987, 1992; Shoji et al. 1992). Later, tau protein was found to be the major component of NFT (Delacourte and Defossez 1986; Grundke-Iqbal et al. 1986a, b; Kosik et al. 1986; Goedert et al. 1988; Wischik et al. 1988; Lee et al. 1991; Bramblett et al. 1993). Substantial efforts have been made to find a cure or disease-modifying therapy for AD. However, whether a single target is sufficient to treat AD has come into question since the failure of all phase III clinical trials that used Aβ-centric approaches (for a review, Karran et al. 2011). In this review, it is aimed to present multiple factors that may be associated with AD concentrating on microtubules (MTs)/tau toward combinatorial therapeutics.

Zinc in Alzheimer's Disease

There is current evidence for a relative increase in intracellular zinc in vulnerable regions of the AD brain (Charton et al. 1985; Frederickson et al. 2005; Berti et al. 2015). Zinc is the second most abundant metal in the body after iron. The concentration of free zinc ions in the extracellular space of healthy brain tissue is in the range of 1 to 10 nM, and the cytosolic zinc concentration is in the picomolar range (Frederickson et al. 2005). But in the proximity of axon terminals, zinc rises to micromolar levels following release from synaptic vesicles that contain zinc in the millimolar concentration (Shen et al. 2007; Linkous et al. 2008). Synaptic zinc is involved in signal transmission/transduction across synapses and therefore modulates synaptic transmission and plasticity (Frederickson et al. 2005; Besser et al. 2009). Besides its physiological functions, zinc dyshomeostasis can contribute to neuronal and astrocytic cell death (Koh et al. 1996; Rossi et al. 2001; Bossy-Wetzel et al. 2004). It has been found that NFT and Aβ plaques contain abnormally high levels of zinc at millimolar concentrations (Bush et al. 1994a, b). In addition, it has been demonstrated that Aβ 1–40 (a major component of AD cerebral amyloid) specifically and saturably binds zinc (Bush et al. 1994a, b) that could accelerate the Aβ plaque formation (Bush et al. 1994a, b; Nair et al. 2010). Zinc can also interact with tau (Huang et al. 2014) and increase tau phosphorylation through activation of Erk1/2 (Yu and Fraser 2001; Harris et al. 2004; Boom et al. 2009; Kim et al. 2011) via increasing

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phosphorylation by GSK-3β, a major tau kinase (Lei et al. 2011), on tyrosine 216 (Björkdahl et al. 2005).

Microtubules and Tau

Microtubules (MTs) are the major component of the neuronal cytoskeleton, and MT dynamics plays a key regulatory role during axon regeneration. MT stability and organization are sufficient to induce axon formation (Witte et al. 2008). The MT shaft is composed of the heterodimer protein tubulin, the major protein in the brain, that exhibits developmentally determined microheterogeneity (Gozes et al. 1975; Gozes and Littauer 1978) and is decorated by the microtubule-associated proteins (MAPs). One of the major proteins of this family is the microtubule-associated protein tau. Tau is a MT-assembly factor that appears to be enriched in neuronal axons (Tucker et al. 1988; Trojanowski et al. 1989; Lee et al. 1991). Alternative splicing around exon 10 of the tau transcript yields tau protein variants including tau protein containing either 3 or 4 MT binding repeat domains (Tau 3R or 4R), associated with dynamic and stable MT, respectively (Goedert and Jakes 1990). The healthy human brain exhibits a 1/1 ratio of tau 3R/4R, and deviation from this ratio is a pathological feature of frontotemporal dementia (FTD) taupathies (Kalbfuss et al. 2001).

MT binding repeat domains are localized in the C-terminal region of tau protein, followed by a basic proline-rich region and an acidic N-terminal region (the last does not contribute to tau-MT binding). Tau proline-rich domain includes many phosphorylation sites (Biernat et al. 1992; Augustinack et al. 2002) and can associate with SH3 domains of other proteins (Reynolds et al. 2008), including the tyrosine kinase Fyn (Lee et al. 1998). When tau was first purified from porcine brain, it was found in association with tubulin (Weingarten et al. 1975). Later, tau was shown to be a regulator of tubulin assembly in vitro (Witman et al. 1976; Cleveland et al. 1977) and in vivo (Drubin and Kirschner 1986). Tau is predominantly expressed in neuronal cells and was shown to be co-localized with tubulin in the brain stem and basal ganglia (Migheli et al. 1988). MT binding ability appears to be dependent on the three or four repeat regions of tau (Lewis et al. 1988), although a single repeat is sufficient for MT binding (Lee et al. 1989). The ability of tau to bind MTs is also dependent on its phosphorylation status (Grundke-Iqbal et al. 1986b; Bramblett et al. 1993; Harada et al. 1994; Seubert et al. 1995). Tau hyperphosphorylation reduces its affinity to MTs, which leads to the destabilization of MTs; this process has been suggested to be a loss-of-function toxicity pathway in AD (Hanger et al. 2009; Spires-Jones et al. 2009).

Neurons possess long, highly branched axons and dendrites, which require a precise transportation system between pre-synaptic sites and cell bodies. This long-range MT-based system is required for neuronal survival; dysfunction of this system could lead to cell death and contribute to diseases, such as AD and PD (Morfini et al. 2007; Dixit et al. 2008). Tau has been shown to interact with kinesin and dynein, both of which are integral to this process. Overexpression of tau inhibits kinesin-dependent trafficking in neuroblastoma N2a cells (Ebneth et al. 1998) and primary neuronal cells (Stamer et al. 2002). Tau can bind to the light chain of kinesin-1 (Utton et al. 2005), and an 18-amino acid sequence of tau is required for the inhibitory activity upon kinesin (LaPointe et al. 2009). On the other hand, tau also binds to the dynein-activator complex, dynactin, which enhances its attachment to MTs (Magnani et al. 2007), and the 3-R isoform of tau is a more potent inhibitor of dynein binding to MTs (Vershinin et al. 2008). Therefore, tau differentially regulates MT-based axonal transportation. Lower levels of tau in the cell body enable kinesin to bind to MT (Dixit et al. 2008), which facilitates transportation of cargo along axons, which include tau itself (Utton et al. 2005). At the synapse, high concentrations of tau promote kinesin to release its cargo and facilitate dynein binding to MT (Dixit et al. 2008). Although this process has been well recognized, contradictory in vivo studies have shown that general rates of axonal transportation are not significantly affected by genetical overexpression or deletion of tau (Yuan et al. 2008; Vossel et al. 2010).

Since its discovery, tau has been recognized as a MT binding protein and thought to function in regulating the dynamics of MT assembly and associated axonal transport. However, the lack of overt phenotype in mice with gene deletion of tau up to 6 months of age (Roberson et al. 2007; Dawson et al. 2010; Ittner et al. 2010; Lei et al. 2012, 2014; Li et al. 2014, 2015; Ma et al. 2014) has led to the understanding that tau function is redundant. This supposition has been challenged by studies that revealed prominent motor and cognitive phenotypes in aged tau knockout mice (Lei et al. 2012, 2014; Ma et al. 2014). These observations were supported by the fact that in a *Caenorhabditis elegans* model, reduction in PTL-1, a tau homolog, significantly shortened the life span of the strain (Chew et al. 2013) and tau knockout *Drosophila* exhibited progressive neuronal degeneration (Bolkan and Kretzschmar 2014).

Tau has traditionally been considered an axonal protein; however, dendritic localization and functions of tau are evidenced by altered long-term depression (LTD) (Kimura et al. 2014) and long-term potentiation (LTP) (Ahmed et al. 2014) in tau knockout brain slices. Regan et al. showed that tau knockout mice had uninterrupted spatial learning (Regan et al. 2015) using the Barnes maze test, which confirms previous studies investigating tau knockout mice at the same age (4–5 months) in other learning paradigms (Morris Water Maze, Y-Maze, T-Maze) (Roberson et al. 2007; Ittner et al. 2010; Lei et al. 2012, 2014; Ahmed et al. 2014; Ma et al. 2014). While tau knockout mice had unimpaired learning in

this initial setup, these knockout mice had defective reversal of this learning when the location of the escape hole was changed. In light of these findings, and compounded by the fact that tau mutations lead to various forms of non-AD dementia (Hutton et al. 1998; Spillantini et al. 1998; Goedert and Spillantini 2011), targeting tau function may be able to restore the microtubule network and prove beneficial for AD and related neurodegenerations.

Activity-Dependent Neuroprotective Protein, NAP, and Tau

Using small molecules to stabilize microtubules is hypothesized to offset loss of tau function in AD. Several stabilizers were tested in animal models and showed promising results, including preventing Aβ toxicity (Zempel et al. 2010), improving microtubule density (Divinski et al. 2006; Brunden et al. 2010), reducing tau phosphorylation (Vulih-Shultzman et al. 2007), and ameliorating behavioral disability (Zhang et al. 2005; Brunden et al. 2010). One of those drug candidates, NAP, showed a beneficial effect in a phase II clinical trial in amnestic mild cognitive impaired patients preceding AD (Gozes et al. 2009; Morimoto et al. 2013; Magen and Gozes 2014).

NAP (NAPV**SIPQ**), an eight-amino-acid peptide, identified as the smallest active element of activity-dependent neuroprotective protein (ADNP) (Bassan et al. 1999; Zamostiano et al. 2001). ADNP is vital for brain formation (Pinhasov et al. 2003; Mandel et al. 2007) and neurite outgrowth in vitro (Mandel et al. 2008). Furthermore, ADNP also provides glial protection (Pascual and Guerri 2007; Vulih-Shultzman et al. 2007). ADNP is a member of the SWI/SNF chromatin remodeling complex (Mandel and Gozes 2007), which is associated with transcription and splicing (Batsche et al. 2006). ADNP expression was previously shown to be correlated with tau 3R expression (Schirer et al. 2014). We also showed a direct interaction of ADNP with protein associated splicing factor (PSF) (Schirer et al. 2014), which was found to associate with the SWI/SNF-like complex (Ito et al. 2008) and also with tau splicing. PSF suppresses tau exon 10 inclusion by interacting with a stem-loop structure downstream of exon 10 (Ray et al. 2011). Moreover, Adnp$^{+/-}$ mice exhibit tauopathy (significant increase in phosphorylated tau and tangle-like structures), reduced neuronal survival, and age-driven neurodegeneration and behavioral deficits (Vulih-Shultzman et al. 2007).

NAP protects against ADNP deficiencies (Vulih-Shultzman et al. 2007) and exhibits potent neuroprotective activities against a number of toxic insults, including several relevant to neurodegenerative diseases such as the Aβ peptide (Bassan et al. 1999; Gozes et al. 2008), excitotoxicity (Bassan et al. 1999), oxidative stress (Steingart et al. 2000), and oxygen glucose deprivation-associated apoptosis (Zemlyak et al.

2009), which is paralleled by protection against tau hyperphosphorylation (Idan-Feldman et al. 2012). NAP was further identified as a neurotrophic factor, stimulating neurite outgrowth and dendrite formation (Smith-Swintosky et al. 2005; Oz et al. 2012, 2014). These results were corroborated by other investigators worldwide (Pascual and Guerri 2007; Chen and Charness 2008; Jehle et al. 2008).

High zinc concentration (from 200 μM and more) caused significant increase in cell death (Oz et al. 2012). These results are compatible with previous publications reporting that an increase in the intracellular free zinc is neurotoxic and its accumulation may contribute to neuronal injury in several diseases, including neurodegenerative conditions such as AD. Moreover, aberrant zinc metal homeostasis has been reported in the brains of AD patients and this metal could contribute to the development of the lesions (Religa et al. 2006). However, NAP treatment, added together with zinc, significantly increased cell viability under zinc toxic condition (Divinski et al. 2004, 2006; Oz et al. 2012, 2014) and that proved again its neuroprotective ability (Bassan et al. 1999; Wilkemeyer et al. 2003; Busciglio et al. 2007; Gozes and Divinski 2007; Pascual and Guerri 2007; Gozes et al. 2008).

Zinc activity on MTs may contribute to the development of tau pathology (Pei et al. 2006; Boom et al. 2009). Zinc toxicity decreased tubulin and tau content in the polymerized fraction of MTs in the PC12 cell line, and NAP treatment protected against tubulin and tau loss from assembled MTs in the PC12 cells in the face of the toxic agent—zinc (Oz et al. 2012). Similarly, in a *Drosophila* model of tauopathy in which abnormal human tau mediates neuronal dysfunction, NAP enhanced tau-MT interaction (Quraishe et al. 2013).

Binding of tau to MTs is regulated through phosphorylation, and increased GSK-3β activity reduces the association of tau with MT (Lovestone et al. 1999; Leroy et al. 2000). We (Vulih-Shultzman et al. 2007) demonstrated a NAP-dependent reduction in activated GSK-3β that is associated with the pathological hyperphosphorylation of tau. In this respect, ADNP deficiency resulted in increased GSK-3β active form, tau hyperphosphorylation, and neurofibrillary tangle-like structure formation, which have been prevented by NAP treatment. Corroborating results showed that NAP requires Fyn kinase for activity (Chen and Charness 2008). NAP requires the neuronal marker, tubulin β3 for MT interactions in neurons (Divinski et al. 2006; Sudo and Baas 2011), and NAP treatment enhances tubulin β3 expression (Oz et al. 2012). ADNP deficiency was associated with deregulation of tubulin expression, in a developmental and sex-dependent manner, in vivo (Amram et al. 2016), and in depletion of the axonal marker MAP2, in vitro (Mandel et al. 2008), while NAP increased MAP2 expression (Smith-Swintosky et al. 2005). Together, these results suggest the NAP can serve as a multi-targeting compound to treat AD.

Mechanism and Future Horizons

Our most recent work (Gozes laboratory) identified the SIP (Ser-Ile-Pro) motif in NAP (NAPVSIPQ) as the NAP binding site for MT fortification, namely, binding to the MT end binding proteins EB1 and EB3 (Oz et al. 2014). NAP enhances ADNP interaction with EB3 and also with a key component of the autophagy process, the initiator of the autophagosome formation, MT-associated protein 1 light chain 3 (Merenlender-Wagner et al. 2015). Thus, NAP protects MTs and the autophagy process (Esteves et al. 2014) inhibiting apoptosis. Importantly, NAP protects MT-dependent axonal transport (Jouroukhin et al. 2013; Quraishe et al. 2013), with postmortem AD brains showing depletion in MTs (Cash et al. 2003). Capitulating on the NAP target, we have designed SKIP (Ser-Lys-Ile-Pro) and shown that it mimics NAP protection, as well as protects ADNP-deficient impairment in axonal transport (Amram et al. 2016). Together with new diagnostic tools, such as changes in blood ADNP in parallel to brain cognitive decline (Malishkevich et al. 2015), this and other pipeline products (Shiryaev et al. 2011; Gozes et al. 2014a, b) are ready for future development, at the basic mechanistic understanding and at the clinical frontiers.

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