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Mini Review

TRAPPC6AΔ is a Potential Marker for the Progression of Alzheimer's Disease from Middle Age

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Abstract

Alteration of trafficking protein particle (TRAPP) family proteins has been implicated in the pathogenesis for neurodegeneration. For example, a missense mutation in TRAPPC6A gene could cause protein accumulation in patients with a neurodevelopmental syndrome and dysmorphic features. TRAPPC6AΔ (or TPC6AΔ) is an N-terminal internal-deletion isoform of intracellular TRAPPC6A protein. TPC6AΔ aggregates, with Ser35 phosphorylation, can be found in the hippocampus and cortex of middle-aged postmortem normal humans, whereas amyloid beta (Aβ) is barely detectable. Downregulation of WWOX (WW domain-containing oxidoreductase) in the brain progressively induces TPC6AΔ aggregate formation and the aggregates serve as seeding posts for the subsequent aggregation of TIAF1 (TGF-β-induced antiapoptotic factor) and SH3GLB2 (SH3 domain-containing GRB2 like, endophilin B2), followed by caspase activation and ultimately generation of amyloid β plaques and tau tangles. Overall, pathogenic TPC6AΔ is an extracellular matrix protein that forms aggregates in the hippocampus and cortex of middle-aged healthy individual. If both wild type TPC6A and TPC6AΔ are present in the blood circulation, the ratios of TPC6AΔ/TPC6A protein can be regarded as a potential index for AD progression from middle age.

***Keywords**: TRAPPC6A; TRAPPC6AΔ; TIAF1; SH3GLB2; WWOX; Neurodegeneration; Alzheimer's Disease*

Initiation of brain protein aggregation in middle age

Age-dependent protein misfolding, instability and aggregation in the brain has been documented [1-4]. Despite protein stability is reduced with age, the underlying mechanism is largely unknown. Our knowledge regarding the initiator(s) and the downstream protein/protein interaction pathways for causing protein instability and aggregation is indeed lacking. Of particular concern is that the molecular nature of the aggregated proteins in the middle-aged humans is largely unknown. Conceivably, these aggregated proteins can be degraded with time as one gets older. Or, these proteins continue to exist or persist in the brain from middle age toward old AD patients (> 70 years old). Interestingly, aspirin is able to block protein aggregation that causes neurodegeneration [5].

AD patients have accumulation of protein aggregates such as fibrillar β-amyloid (Aβ) plaques in the extracellular matrix and intracellular deposition of neurofibrillary tangles (NFT) in the hippocampus and cortex [6-8]. However, the protein levels of Aβ and tau aggregates are very low in the middle-aged healthy individuals [6-9]. Aβ and tau aggregates are known to potently invoke neuronal death and block neurogenesis and learning and memory capabilities in AD patients. It is reasonable to assume that protein aggregates in the middle-aged brain are stable and may play a crucial role in inducing additional brain protein aggregation progressively with age, and thereby leads to accumulation of Aβ plaques and tau tangles in old AD patients. While research focus on the brain pathologies of healthy individuals at

their middle age is largely lacking, the presence of pathogenic proteins in the middle-aged brain cannot be ignored. In the following sections, I will present supporting evidence that initiation of protein aggregation in the brain starts to occur in the middle-aged humans and the aggregation cascade lasts over 20 to 30 years to generate pathogenic levels of Aβ and tau aggregates as shown in the AD patients [6-8].

TGF-β signaling is downregulated in AD

Among secreted cytokines, transforming growth factor beta (TGF-β) is known to play a crucial role in the pathogenesis of AD and other types of dementias [8-10]. TGF-β is a potent inhibitor of cell growth such as epithelial and endothelial cells, an inducer of cellular senescence and stem cell aging, and a strong enhancer of cellular protein aggregation [8,9]. However, downregulation of TGF-β signaling occurs with age. This is probably due to rapid receptor protein degradation, inhibitor proteins in the signaling cascade, chronic inflammation and microglial cell activation. The dysregulated TGF-β signaling is manifested in old patients with AD or vascular dementia [8-12]. Restoration of TGF-β signaling in AD or vascular degeneration is likely to rebuild neuronal plasticity [11,12].

TGF-β regulates protein aggregation in the brain

TGF-β-regulated protein aggregation has been shown in the hippocampi of middle-aged human brains [6-8,13-16]. We have identified three TGF-β1-induced proteins, namely TIAF1 (TGF-β-induced antiapoptotic factor), TRAPPC6A (Trafficking Protein Particle Complex Subunit 6A) and SH3GLB2 (SH3 Domain Containing GRB2 Like, Endophilin B2) [8,13-16]. The aggregated TIAF1 is found in the hippocampus and cortex of normal humans at middle ages [8] and in the peri-tumor region between brain cells and metastatic cancer cells from lung [13,14]. Aggregated TIAF1 is toxic to neurons [8]. The induced cell death is due, in part, to activation of SMAD promoter activation [8]. Seeding of cells onto a matrix derived from another cell line leads to upregulation and intracellular aggregation of TIAF1 [13,14].

We determined that TIAF1 aggregates cause degradation of membrane amyloid precursor protein (APP) and generation of Aβ and amyloid fibrils *in vitro* [6,8,13,14]. TIAF1 aggregates do not undergo degradation with age. Worse, the aggregates progressively cause formation of Aβ, fibrils and plaques as one gets older [6,8]. Polymerized TIAF1 binds Smad2/3/4 to prevent nuclear translocation, and thereby restricts SMAD-mediated gene transcription [6,8,13,14]. The observations may account for the downregulation of TGF-β/Smad2/3/4 signaling in old AD patients.

TRAPP family proteins are associated with neurodevelopmental disorders

TRAPPC6A (TPC6A) is a TGF-β-inducible intracellular vesicle trafficking protein [7,15]. Wild type TPC6A is one of the components in the transport protein particle (TRAPP) complex, which is needed in the vesicle trafficking and tethering with the ER-Golgi system in yeast [15]. TRAPP family proteins tend to have mutations. Consequently, these proteins are unstable and susceptible to aggregation. For example, a structural alteration in TRAPPC6A by a single mutation leads to protein build up in patients with a neurodevelopmental syndrome and dysmorphic features [16]. Also, TRAPP complexes is disrupted by the association of TRAPPC6A with a rare homozygous predicted deleterious missense variant, p.(Ala2Gly), in TRAPPC2L, which leads to neurodevelopmental disorder [17]. Human *TRAPPC6A* gene is involved in nonverbal reasoning in 2 Scottish cohorts and is suggested for a role in AD [18]. *TRAPPC6A* gene is significantly downregulated in the blood of AD patients of around 75 years old [19]. Whether this downregulation occurs in the brain is unknown.

TPC6A shuttles between nucleoli and mitochondria

TGF-β induces shuttling of wild-type TPC6A and TPC6AΔ from the nucleus, nucleolus and then to mitochondrion, and again travel back to the nucleus [6]. Isoform TPC6AΔ possesses a 14-amino-acid internal deletion at the *N*-terminus [7]. The nucleolus-mitochondrion shuttling is unusual. TPC6A has a Ser35 phosphorylation site. Upon phosphorylation at Ser35, both TPC6A and TPC6AΔ tend to readily undergo aggregation [7]. When TPC6A or TPC6AΔ relocates from the nucleoli to the mitochondria, TPC6AΔ binds TIAF1 and undergoes excessive aggregation in the mitochondria. This event leads to activation of caspases, cytochrome c release, and apoptosis [6,7]. Caspase activation induces membrane APP degradation and thereby causes formation of amyloid β.

TRAPPC6AΔ is an extracellular aggregated protein

A protein isoform of wild type TPC6A participates in neurodegeneration [7]. TPC6A is an intracellular protein, whereas TPC6AΔ is found in the extracellular matrix as aggregates [7]. TPC6AΔ protein aggregates are identified in the hippocampi of postmortem normal controls (40-70 years old) and AD patients (70 to 95 years old), suggesting that TPC6AΔ protein aggregates are difficult to remove from the brain and can last from middle to old ages [7]. A phosphorylation site at serine 35 is identified in TPC6AΔ, and the phosphorylation is needed for protein aggregation [7]. Alteration of Ser35 to Gly35 abolishes the aggregation of TPC6AΔ. Finally, TGF-β1 increases TPC6AΔ aggregation and this leads to caspase 3 activation and Aβ production [7].

Wild-type TPC6A and isoform TPC6AΔ are expressed in distinct brain areas. TPC6AΔ is a plaque-forming protein in the brain extracellular matrix, whereas wild-type TPC6A is a cytosolic protein [7]. TPC6A, for instance, is expressed in the pyramidal layer and TPC6AΔ in the adjacent molecular layer of the hippocampus. TPC6AΔ, but not the wild type, forms cortical plaques. TPC6A is abundant in the Purkinje cells of cerebellum, but TPC6AΔ is shown as polymerized protein in the white matter.

WWOX restricts the aggregation of TPC6A, TPC6AΔ, and TIAF1

Wild type TPC6A physically binds tumor suppressor WW domain-containing oxidoreductase (human WWOX or FOR, and mouse WOX1) [20]. TPC6A acts as a carrier for WWOX to undergo nuclear translocation [6]. *WWOX* gene has recently been determined as a risk factor for AD [21]. WWOX binds Tau and Tau-hyperphosphorylating enzymes (e.g. GSK-3β) and thereby prevents Tau aggregation [22,23]. *Wwox* gene ablation induces TPC6AΔ and tau aggregation in the brain of 3-week-old knockout mice [7]. Knockdown of WWOX by siRNA rapidly induces aggregation of TPC6AΔ and TIAF1 *in vitro*. Heterozygous *Wwox* mice exhibit an accelerated rate of neurodegeneration compared to that of triple transgenic mice for AD [24]. Significant downregulation of WWOX and pY33-WWOX occurs in the AD hippocampus, which correlates with increased activities of enzymes in hyper-phosphorylating tau [22]. Thus, WWOX is crucial in preventing the initiation and progression of AD.

WWOX molecular actions

Most strikingly, TPC6AΔ plaques can be found in the brain cortex of *Wwox* knockout mice of less than 3 weeks old [6,7]. WWOX is frequently downregulated in the hippocampi of AD patients [22]. Upon WWOX downregulation TPC6AΔ undergoes polymerization rapidly. Also, TGF-β1 causes dissociation of WWOX from TPC6AΔ, thus leading to the aggregation of TPC6AΔ and TIAF1, activation of caspases, β-secretase upregulation, Aβ production, and formation of tau tangles and amyloid fibrils [6,7]. Alternatively, downregulation of WWOX leads to p53 destabilization and subsequent aggregation of TPC6AΔ and TIAF1 [15]. Together, TPC6AΔ contributes a critical role in the aggregation of neuronal proteins and neurodegeneration.

Concluding Remarks

The molecular mechanism underlying protein misfolding and instability with age is largely unknown. When protein aggregation occurs in *C. elegans* in middle age, this event does not induce significant aggregation of amyloid β [2]. This finding is in agreement with our observations that there is little or no amyloid β associated with the TPC6AΔ/TIAF1 aggregates in hippocampus or cortex in middle-aged normal humans [6-8]. As a marker of AD [21], WWOX downregulation-mediated aggregation of TPC6AΔ and TIAF1 and aberrant downstream events provides a clear signaling cascade for the formation of tau tangles and amyloid β plaques in old-aged AD patients [6-8].

In light of our discovery that both TPC6AΔ and TIAF1 are present, in part, as aggregates in the brain of middle-aged individuals, we propose that both proteins can be regarded as markers of AD initiation and progression from middle to old ages [6-8]. The TPC6AΔ/TIAF1 aggregates or plaques in the hippocampi of normal individuals at middle ages possess very low levels of Aβ. The Aβ levels in the plaques are accumulated progressively with age and reach maximally in the hippocampi of older AD patients [6-8]. Mechanistically, aggregating TPC6AΔ activates caspases and contributes to Aβ generation. Based upon the Genecard database (https://www.genecards.org/cgi-bin/carddisp.pl?gene=TRAPPC6A&keywords=trappc6a), TPC6A is present in the serum. Thus, it is feasible to determine the ratio of wild type TPC6A versus TPC6AΔ isoform from serum and predict the tendency of AD progression in normal individuals.

Conflict of Interest

The author declares no competing interests.

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Bibliography

1. David DC. "Aging and the aggregating proteome". *Frontiers in Genetics* 3 (2012): 247.
2. Groh N., *et al*. "Age-Dependent Protein Aggregation Initiates Amyloid-beta Aggregation". *Frontiers in Aging Neuroscience* 9 (2017): 138.
3. Stefanova NA., *et al*. "Amyloid accumulation is a late event in sporadic Alzheimer's disease-like pathology in nontransgenic rats". *Oncotarget* 6.3 (2015): 1396-413.
4. Ayyadevara S., *et al*. "Proteins in aggregates functionally impact multiple neurodegenerative disease models by forming proteasome-blocking complexes". *Aging Cell* 14.1 (2015): 35-48.
5. Ayyadevara S., *et al*. "Aspirin-Mediated Acetylation Protects Against Multiple Neurodegenerative Pathologies by Impeding Protein Aggregation". *Redox Signaling* 2017 27.17 (2017): 1383-1396.
6. Chang JY and Chang NS. "WWOX dysfunction induces sequential aggregation of TRAPPC6AΔ, TIAF1, tau and amyloid β, and causes apoptosis". *Cell Death Discovery* 1 (2015): 1-11.
7. Chang JY., *et al*. "Trafficking protein particle complex 6A delta (TRAPPC6AΔ) is an extracellular plaque-forming protein in the brain". *Oncotarget* 6.6 (2015): 3578.
8. Lee MH., *et al*. "TGF-β induces TIAF1 self-aggregation via type II receptor-independent signaling that leads to generation of amyloid β plaques in Alzheimer's disease". *Cell Death Disease* 1.12 (2010): e110-e110.
9. Tominaga K., *et al*. "TGF-beta Signaling in Cellular Senescence and Aging-Related Pathology". *International Journal of Molecular Sciences* (2019): 5002.
10. Fessel J. "Caveolae, CD109, and endothelial cells as targets for treating Alzheimer's disease". *Alzheimer's and Dementia: Translational Research and Clinical Interventions* 6.1 (2020): e12066.
11. Kandasamy M., *et al*. "TGF-β signaling: a therapeutic target to reinstate regenerative plasticity in vascular dementia?" *Aging and Disease* 11.4 (2020): 828.
12. Estrada LD., *et al*. "Transforming growth factor beta type I role in neurodegeneration: implications for Alzheimer s disease". *Current Protein and Peptide Science* 19.12 (2018): 1180-1188.
13. Chang JY., *et al*. "TIAF1 self-aggregation in peritumor capsule formation, spontaneous activation of SMAD-responsive promoter in p53-deficient environment, and cell death". *Cell Death and Disease* 3.4 (2012): e302-e302.
14. Hong Q., *et al*. "Self-aggregating TIAF1 in lung cancer progression". *Translational Respiratory Medicine* 1.1 (2013): 1-8.
15. Sacher M., *et al*. "The TRAPP complex: insights into its architecture and function". *Traffic* 9.12 (2008): 2032-2042.
16. Mohamoud HS., *et al*. "A missense mutation in TRAPPC6A leads to build-up of the protein, in patients with a neurodevelopmental syndrome and dysmorphic features". *Scientific Reports* 8.1 (2018): 1-9.

17. AI-Deri N., *et al*. "A novel homozygous variant in TRAPPC2L results in a neurodevelopmental disorder and disrupts TRAPP complex function". *Journal of Medical Genetics* 0 (2020): 1-10.

18. Hamilton G., *et al*. "Alzheimer's disease genes are associated with measures of cognitive ageing in the lothian birth cohorts of 1921 and 1936". *International Journal of Alzheimer's Disease* 2011 (2011): 505984.

19. Park YH., *et al*. "Association of blood-based transcriptional risk scores with biomarkers for Alzheimer disease". *Neurology Genetics* 6.6 (2020): e517.

20. Liu CC, *et al*. "WWOX phosphorylation, signaling, and role in neurodegeneration". *Frontiers in Neuroscience* 12 (2018): 563.

21. Kunkle BW., *et al*. "Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing". *Nature Genetics* 51.3 (2019): 414-430.

22. Sze CI., *et al*. "Down-regulation of WW domain-containing oxidoreductase induces Tau phosphorylation in vitro: a potential role in Alzheimer's disease". *Journal of Biological Chemistry* 279.29 (2004): 30498-30506.

23. Wang HY., *et al*. "WW domain-containing oxidoreductase promotes neuronal differentiation via negative regulation of glycogen synthase kinase 3 β". *Cell Death and Differentiation* 19.6 (2012): 1049-1059.

24. Lee MH., *et al*. "Zfra restores memory deficits in Alzheimer's disease triple-transgenic mice by blocking aggregation of TRAPPC6AΔ, SH3GLB2, tau, and amyloid β, and inflammatory NF-κB activation". *Alzheimer's and Dementia: Translational Research and Clinical Interventions* 3.2 (2017): 189-204.

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