A Persistent Stress Response to Impeded Axonal Transport Leads to Accumulation of Amyloid- β in the Endoplasmic Reticulum, and Is a Probable Cause of Sporadic Alzheimer's Disease

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Key Words

Alzheimer's disease • Amyloid- β precursor protein • Amyloid- β peptide • Axonal transport • Kinesin • Endoplasmic reticulum stress • Protein phosphorylation • Mechanism of neurodegenerative diseases

Abstract

Background and Objective: Could a normal – but persistent – stress response to impeded axonal transport lead to late-onset Alzheimer's disease (AD)? Our results offer an affirmative answer, suggesting a mechanism for the abnormal production of amyloid- β (A β), triggered by the slowed axonal transport at old age. We hypothesize that A β precursor protein (APP) is a sensor at the endoplasmic reticulum (ER) that detects, and signals to the nucleus, abnormalities in axonal transport. When persistently activated, due to chronically slowed-down transport, this signaling pathway leads to accumulation of A β within the ER. **Methods and Results:** We tested this hypothesis with the neuronal cell line CAD. We show that, normally, a fraction of APP is transported into neurites by recruiting kinesin-1 via the adaptor protein, Fe65. Under conditions that block kinesin-1-dependent transport, APP, Fe65 and kinesin-1 accumulate in the soma, and form a complex at the ER. This complex recruits active c-Jun N-terminal kinase (JNK), which phosphorylates APP at Thr⁶⁶⁸. This

phosphorylation increases the cleavage of APP by the amyloidogenic pathway, which generates A β within the ER lumen, and releases Fe65 into the cytoplasm. Part of the released Fe65 translocates into the nucleus, likely to initiate a gene transcription response to arrested transport. Prolonged arrest of kinesin-1-dependent transport could thus lead to accumulation and oligomerization of A β in the ER.

Conclusion: These results support a model where the APP:Fe65 complex is a sensor at the ER for detecting the increased level of kinesin-1 caused by halted transport, which signals to the nucleus, while concomitantly generating an oligomerization-prone pool of A β in the ER. Our hypothesis could thus explain a pathogenic mechanism in AD.

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Background and Objective

Herein, we propose a novel function of APP, which could explain why normal stress – well tolerated by the young and adult brain – could lead to intraneuronal accumulation of toxic amyloid- β (A β) oligomers and initiation of Alzheimer's disease (AD) in the aging brain. Old age is the major risk factor for sporadic AD [1]. The changes that accompany aging, which could trigger AD, are numerous. It was proposed that the age-related impairment

Fig. 1. The molecular mechanism of ER stress response triggered by arrested axonal transport. **a** The complex APP:Fe65:kinesin-1, normally targeted to neurites, accumulates at the ER when transport is arrested. **b** Recruitment of active JNK kinase complex by kinesin-1 leads to phosphorylation of APP. **c** Phosphory-

lated APP is cleaved by the amyloidogenic pathway to release A β into the ER lumen, and Fe65 into the cytoplasm. Fe65 then translocates into the nucleus, forming a transcriptionally active complex with Tip60 that regulates expression of stress-related genes.

Fig. 2. Knockdown of Fe65 with siRNA in CAD cells decreases A β levels in the soma by approximately 50%. GFP marks cells transfected with Fe65 siRNA. The histogram on the right shows the levels of A β along the indicated line. Bar = 10 μ m.

of axonal transport could lead to AD, but the mechanisms are poorly defined [2]. We hypothesize that a complex of APP and Fe65, a well-characterized APP-binding protein, is a sensor localized to the endoplasmic reticulum (ER) that detects and signals to the nucleus abnormalities in axonal transport. Prolonged activation of this signaling pathway due to chronically slowed-down axonal transport – likely to occur in AD – could lead to increased production and intracellular accumulation of A β . We propose a model (fig. 1) where a complex containing APP, Fe65 and kinesin-1 – normally transported into neurites – accumulates at ER when axonal transport is arrested and the level of kinesin-1 in the soma is increased. This complex recruits c-Jun N-terminal kinase (JNK), which phosphorylates APP, resulting in cleavage of the phosphorylated APP via the amyloidogenic pathway and accumulation of oligomerization-prone A β in the ER lumen. Concomitantly, Fe65 is released, and translocates into the nucleus to regulate expression of stress-related genes, including the AD-relevant kinase, GSK3 β .

Methods

CAD cells, a CNS-derived neuronal cell line [3], provide an excellent system for studying APP transport and metabolism, including intracellular accumulation of A β [4–10], a process typical

for neurons of the aging brain [11] that is poorly reproduced in primary cultures of embryonic neurons. Mouse hippocampal neurons were used for confirmation purposes. Immunocytochemical, biochemical, and molecular biology approaches were employed as described previously [4–10]. Moderate overexpression of the HA-tagged, cargo-binding, tetratricopeptide repeat (TPR) domain of the kinesin-1 light chain (KLC), HA-KLC-TPR, was used to experimentally mimic the halted axonal transport in CAD cells, when kinesin-1 accumulates in the soma.

Results

The model of ER response to stress caused by impeded axonal transport proposed here (fig. 1) is supported by our previous studies, as well as by the new results shown herein (fig. 2–4). Key findings, pertinent to each step of the model, are listed below.

(1) We showed that, under normal conditions, a fraction of APP is transported into neurites by kinesin-1, recruited by Fe65, an adaptor protein that simultaneously binds the cytoplasmic domain of APP, and kinesin-1 (via the TPR domain of KLC) [12] (fig. 1a).

(2) We showed that, blocking axonal transport by various means – e.g. osmotic stress – leads to accumulation of kinesin-1, Fe65, and APP in the soma [9], and formation of an APP:Fe65:kinesin-1 complex at the ER [13].

Fig. 3. Oligomeric species (anti-oligomer antibody, A11) accumulate in ER. CAD cells were transfected with HA-KLC-TPR. This construct expresses the 6 TPRs of KLC, which can simultaneously bind 2 proteins. Insets are magnifications of the cell in the lower, left corner of the images. The graph shows direct correlation between the levels of oligomers and expressed HA-KLC-TPR. Bars = 20 μ m.

Fig. 4. Overexpressed KLC accumulates in the cell soma of transfected CAD cells, causing an approximately 70% increased translocation of Fe65 in the nucleus. Plane of focus is through the nucleus. Bar = 10 μ m.

(3) Our previous results indicate that the APP:Fe65: kinesin-1 complex, accumulates at the ER, recruits active JNK, binds to the JNK-interacting protein-3 (JIP-3) scaffold, and phosphorylates APP (fig. 1b). Indeed, this APP phosphorylation is facilitated by, and requires, JIP-3 [6], Fe65, and KLC [13], and is augmented by stress [9].

(4) We hypothesize that the Fe65- and kinesin-1-dependent phosphorylation of APP facilitates its cleavage via the amyloidogenic pathway, and thus increases the generation and oligomerization of A β in the ER lumen (fig. 1c). Consistent with this scenario, we show that the amount of oligomerized A β that accumulates in the ER region is positively regulated by the levels of Fe65 (fig. 2) and kinesin-1 (fig. 3), manipulated experimentally with Fe65 siRNA, or expression of HA-KLC-TPR.

(5) We hypothesize that phosphorylation and cleavage of APP, caused by increased levels of kinesin-1 in the soma, leads to the release of Fe65, which translocates into the nucleus (fig. 1c). Our data show that the nuclear level of Fe65 is increased upon expression of HA-KLC-TPR (fig. 4).

The proposed model is consistent with findings from other laboratories, showing that: (1) Thr⁶⁶⁸-phosphorylated APP is a better substrate for the β -secretase, BACE1 than nonphosphorylated APP [14]; (2) phosphorylation of APP at multiple residues reduces its affinity for Fe65 [15, 16], and facilitates release of Fe65, and (3) Fe65 is part of a transcriptionally active complex, together with Tip60

[17, 18]. Interestingly, one of the genes upregulated by Fe65 could be GSK3 β ; this kinase is activated in AD, and phosphorylates the microtubule-associated protein tau, leading to its release from the microtubules and enhancement of axonal transport.

Conclusions

The ER stress response triggered by slowed-down axonal transport described here explains both the A β and the tau pathology characteristic for AD. According to our model, APP is a sensor that measures the pace of axonal transport. It detects variations in kinesin-1 levels, and triggers a stress response that signals to the nucleus abnormalities in axonal transport. A persistent stress response to impeded axonal transport, causing oligomerization and accumulation of A β in the ER, and abnormal phosphorylation of tau, could be a pathogenic mechanism in sporadic AD. This and other studies [19, 20] confer to the ER a prominent role in the disease mechanism in AD.

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