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Novel Pathways Regulating Function and Metabolism of ß-Amyloid Precursor Protein in Alzheimer's Disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide, defined by two classical hallmark pathologies: extracellular senile plaques and intraneuronal neurofibrillary tangles (NFTs) (*1, 2*). NFTs are composed of the hyperphosphorylated microtubule-associated protein tau that is abnormally phosphorylated primarily by glycogen synthase kinase-3 (GSK-3) and cyclin D kinase 5 (Cdk5) (*2*). Senile plaques are composed of heterogeneous small peptides collectively called β-amyloid (Aβ), derived from the β-amyloid precursor protein (APP) through sequential cleavage by β- and γ-secretases. APP is synthesized in the endoplasmic reticulum (ER) and transported through the Golgi/trans-Golgi network (TGN) to the plasma membrane, where it can be cleaved by α-secretase to produce sAPPα. Non-cleaved APP is re-internalized and is subjected to amyloidogenic processing for Aβ generation (*1*). Multiple lines of evidence suggest that overproduction/aggregation of Aβ in the brain is the primary cause of AD: Aβ is highly toxic to neurons and can trigger a cascade of pathogenic events leading to cell death. Therefore, detailed delineation of the function, processing, and regulated trafficking of APP is crucial for understanding the mechanism underlying AD pathogenesis and for developing AD therapeutic strategies.

APP PROCESSING TOWARDS Aß GENERATION

Full-length APP is a type I transmembrane protein transported through the constitutive secretory pathway. During its endocytic trafficking, APP can first be cleaved by β-secretase to release a soluble APP extracellular domain called sAPPβ. The remaining membrane-associated APP carboxyl-terminal fragment-β (β CTF) can then be cleaved by γ-secretase to generate Aβ and an APP intracellular domain (AICD).

The type I transmembrane aspartyl protease beta-site APP-cleaving enzyme (BACE1) is the primary β-secretase species. Optimal enzymatic BACE1 activity requires acidic environments such as those found in the TGN and endosomes where BACE1 is present in abundance (*3*). Mechanisms regulating BACE1 trafficking and activity have not been fully elucidated. Sorting nexin (SNX) family members contain a conserved lipid-binding PX domain and play important roles in membrane trafficking and protein sorting (*4*). We recently found that a member of the SNX family, SNX12, interacts with BACE1. Downregulation of SNX12 accelerates BACE1 endocytosis, thus increasing Aβ production, whereas overexpression of SNX12 has the opposite effect. In addition, SNX12 levels are decreased in AD brains, suggesting that changes in SNX12 levels may contribute to AD pathology (*5*). We also found that the human CUTA protein, another novel protein that interacts with BACE1, regulates its intracellular trafficking. Downregulation of CUTA can decelerate intracellular trafficking of BACE1 from the Golgi/TGN to the cell surface and increase BACE1-mediated APP processing/Aβ generation (*6*).

In addition to its function as a β-secretase for APP, we recently found that BACE1 may also contribute to memory and cognitive deficits associated with AD through an Aβ-independent mechanism: BACE1 interacts with adenylate cyclases via its transmembrane domain, resulting in a reduction in cellular cAMP levels and thus decreased protein kinase A (PKA) activity and CREB phosphorylation (*7*). Interestingly, during our search for new genes that regulate Aβ generation, we identified a new gene family, designated *Rps23rg*, whose encoded proteins also interact with adenylate cyclases via their transmembrane domains. However, RPS23RG proteins increase cellular cAMP levels to activate PKA, causing increased CREB phosphorylation and GSK-3 phosphorylation. Phosphorylation of GSK-3 inhibits its activity, resulting in reduced Aβ generation and tau phosphorylation (*8, 9*).

γ-cleavage is the last step in APP processing to generate Aβ peptides. In addition to cleaving APP, the high molecular mass, membrane-bound γ-secretase complex cleaves many other substrates such as Notch, Cadherin, and ErbB4. γ-secretase consists of four essential components: presenilin (PS, PS1, or PS2), nicastrin, anterior pharynx-defective-1 (APH-1), and presenilin enhancer-2 (PEN-2). We and others have shown that deficiency in any one of these may dramatically affect the stability and intracellular trafficking of other components and impair γ-secretase activity (*1*).

PS1 is the catalytic component of the γ-secretase complex. In addition to cleaving γ-secretase substrates, PS1 has been shown to have other functions, some of which are independent of γ-enzymatic activity. For example, we and others show that PS1 can reciprocally regulate the intracellular trafficking of APP (see next page) as well as several other membrane proteins (*1, 10*).

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FUNCTIONAL ROLES FOR APP AND ITS METABOLITES

Since its identification as the precursor of Aβ, APP has been studied extensively. However, the physiological function of APP remains largely undetermined. APP is proteolyzed into various fragments during intracellular trafficking and these APP metabolites mediate various and sometimes opposing functions. The net effect of APP on cellular activity may be determined by the relative amounts of APP itself and its various metabolites.

In cells and brains deficient in APP, we observed an elevation of Cdk5 activity where tau phosphorylation can be inhibited by re-expressing APP or sAPPα. In addition, APP-deficient neurons exhibit reduced metabolism and survival rates and are more susceptible to excitotoxic glutamate-induced apoptosis through a mechanism involving Cdk5 activation. Our results define a novel neuroprotective function for APP, specifically the extracellular APPα domain, in preventing tau hyperphosphorylation through the suppression of Cdk5 overactivation (*11*).

APP undergoes rapid anterograde transport in neurons. During its transport, APP interacts with kinesin-I and functions as a membrane-associated kinesin-I receptor to mediate axonal transport of β-secretase and PS1 (*12, 13*). We find that APP can regulate cell surface delivery of the PS1/γ-secretase; APP deficiency accelerates transport of PS1 from the TGN to the cell surface and increases cell surface levels of PS1, which can be reversed by restoring APP levels (*14*). APP dosage also markedly decreases retrograde transport of nerve growth factor (*15*). Moreover, APP interacts with the choline transporter and affects its endocytosis (*16*). Together, these findings suggest that APP plays a critical role in regulating protein trafficking.

Using AICD as bait, we identified a mitochondrial carrier protein as an APP-interacting protein and designated it as appoptosin. We found that elevated appoptosin-mediated heme biosynthesis induced the release of reactive oxygen species and activated intrinsic caspase-dependent apoptosis. Importantly, appopotosin levels were upregulated in neurons treated with Aβ and in AD brains, whereas downregulation of appoptosin prevents cell death and caspase activation caused by Aβ insult, thereby implicating a novel appoptosin-dependent mechanism underlying Aβ neurotoxicity. Moreover, we found that although APP interacts with appoptosin through the AICD domain, AICD was unable to rescue appoptosin-induced cell death. These results suggest that membrane-associated domains in the full-length APP and APP CTFs are required to inhibit appoptosin-induced apoptosis. Hence, membrane-anchored APP may interact with and retain a certain amount of appoptosin in the cytosol, thus keeping the level of appoptosin in mitochondria from being elevated for more heme production in the presence of cellular insults or under pathological conditions. Since membrane-dissociated AICD has little effect on appoptosin-induced caspase activation, this could imply that membrane-associated APP/appoptosin complexes can be released and transported to mitochondria upon γ-cleavage to increase heme synthesis and apoptosis. These results demonstrate a function of APP in mediating trafficking of nascent appoptosin from the cytosol to mitochondria (*17*).

Cytosolic AICD within the cell may translocate into the nucleus to regulate the transcription of several genes such as APP, GSK-3β, BACE1, and low density lipoprotein receptor-related protein 1 (LRP1) (*18*). We also find that AICD can bind to the promoter region of the epidermal growth factor receptor (*EGFR*) gene and regulate its expression. Consistent with the notion that dysregulation of EGFR expression and activation is involved in cancers, we found that PS1/γ-secretase deficient mice have increased EGFR levels and increased tumorigenesis, in particular skin cancer. As AICD is generated concurrently with Aβ, which is elevated in AD, our results imply that there is a negative correlation between AD and cancer incidence and that the strategy for inhibiting PS1/γ-secretase activity to treat AD may increase the risk of tumorigenesis. Both implications are supported by recent findings that epidemiological studies have identified an inverse association between cancer and AD (*19*), while AD clinical trials of the γ-secretase inhibitor semagacestat from Eli Lilly have demonstrated that patients receiving the drug have an increased risk of skin cancer compared with those who received a placebo.

REGULATION OF APP INTRACELLULAR TRAFFICKING

Subcellular APP trafficking to divergent sAPPα or Aβ cleavage pathways is critical to neurodegenerative onset, and mechanisms underlying APP trafficking are therefore integral to determining neuropathological AD outcome. We found that SNX17 can interact with APP in the early endosome and that downregulation of SNX17 leads to reduced APP levels with a concomitant increase in Aβ (*20*). In addition, we and others have demonstrated that members of the low-density lipoprotein (LDL) receptor family, including LRP1, LRP1B, SorLA/LR11, and apolipoprotein E receptor 2, interact with APP and regulate its endocytic trafficking and Aβ generation. Moreover, we have shown that one of the γ-secretase components, PS1, also regulates APP trafficking where loss of PS1 results in increased budding/generation of APP-containing vesicles from both the ER and TGN, along with a concomitant increase in cell surface localization of APP. Moreover, familial AD-linked PS1 variants are significantly impaired in vesicle budding, thereby attenuating cell surface delivery of APP (*10*). We also found that PS1 interacts with phospholipase D1 (PLD1), a phospholipid-modifying enzyme regulating membrane trafficking events, and recruits PLD1 to the Golgi/TGN, thus potentially altering APP trafficking. Indeed, PLD1 overexpression promotes budding of APP vesicles from the TGN, concomitantly increasing cell surface levels of APP (*21, 22*).

PERSPECTIVE

Overproduction and aggregation of Aβ in the brain are key patho-

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genic events in AD. Studies from our group and others have revealed novel pathways by which APP function and processing are regulated. Further studies investigating the function and regulation of APP in AD will not only help to elucidate the mechanism underlying disease pathogenesis, but also to identify new targets for disease treatment.

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Role of Tau Hyperphosphorylation in Alzheimer's Disease-Associated Neurodegeneration

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Tau proteins play an important role in maintaining the stability of the neuronal cytoskeleton system. In Alzheimer's disease (AD), tau is abnormally hyperphosphorylated and aggregates into paired helical filaments (PHFs) forming neurofibrillary tangles (NFTs) in neurons (*1*). Clinical investigations have shown that the intracellular accumulation of NFTs is positively correlated with the severity of dementia. The transmission of abnormal tau or NFTs from the entorhinal cortex to the hippocampus and cerebral cortex matches the clinical manifestation, which is the international gold standard at present for assessing AD progression (Braak grading) (*2*). Recent studies suggest that the toxicity of β-amyloid protein (Aβ, another pathogenic factor in AD) needs the tau protein as a mediator (*3*). These data together suggest that abnormal tau plays an important role in the onset and evolution of neurodegeneration and the learning/memory deficits in AD patients.

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To date, no tau gene mutation has been found in the AD patients. The main focus of tau research therefore has been on posttranslational modifications, of which hyperphosphorylation is the most extensively studied. The imbalance of protein kinases (generally upregulation) and protein phosphatases (generally downregulation) is the direct cause for abnormal tau hyperphosphorylation. Among various protein kinases and protein phosphatases, glycogen synthase kinase-3β (GSK-3β) and protein phosphatase-2A (PP2A) are most heavily involved in AD-like tau hyperphosphorylation (*4*–*6*).

ROLE OF GSK-3β IN TAU HYPERPHOSPHORYLATION

GSK-3β was the first tau kinase to be identified; it can phosphorylate tau at multiple sites in vitro (*7*). In vivo activation of GSK-3β causes tau hyperphosphorylation at several sites related to impairment of spatial memory in AD (*8*). Multiple factors can cause tau hyperphosphorylation through GSK-3β activation, including peroxide nitrite (*9*), advanced glycation end products (*10, 11*), persistent light illumination (*12*), endoplasmic reticulum stress (*13, 14*), Aβ (*8, 15*), and proteasome dysfunction (*16, 17*). While activating GSK-3β inhibits long-term potentiation (LTP), inhibiting it promotes LTP through mechanisms involving the presynaptic release of neurotransmitter (*18, 19*). Inhibiting GSK-3β significantly attenuates tau hyperphosphorylation and improves memory (*20, 21*), while overexpression of neuroprotective neuroglobin decreases tau hyperphosphorylation by inhibiting GSK-3β (*22, 23*).

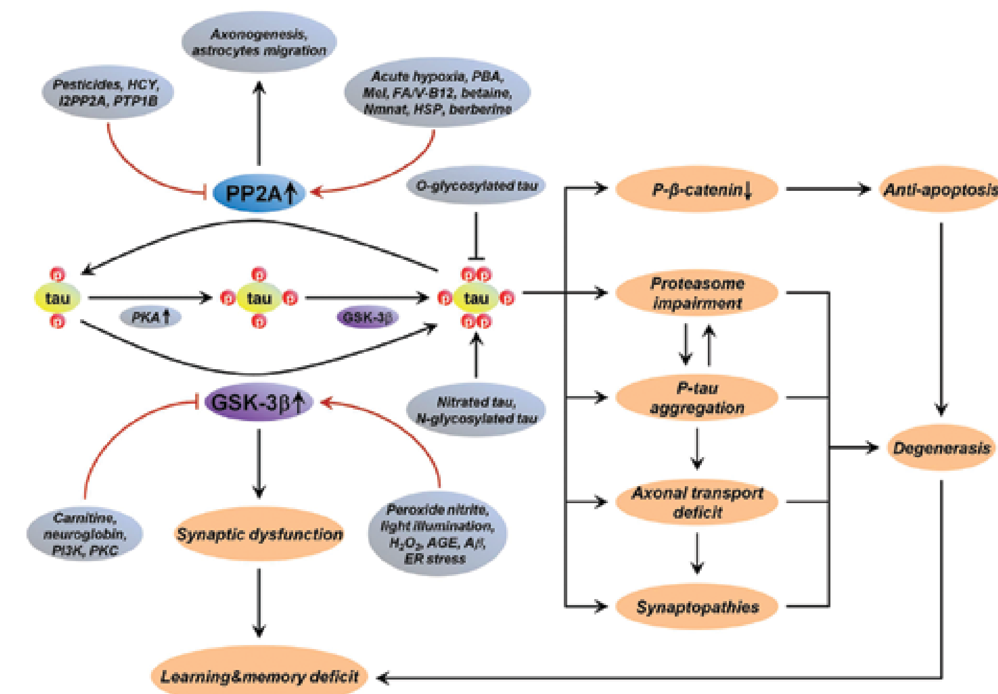

Figure 1. **Schematic summarizing our current understanding of the various signaling pathways through which tau acts within the cell.**

In the brains of rats, inhibition of phosphatidylinositol 3-kinase (PI3K) alone induces a transient activation of GSK-3β, while simultaneous inhibition of PI3K and protein kinase C (PKC) results in a sustained activation of GSK-3β, leading to prolonged tau hyperphosphorylation and spatial memory deficits with reduction of acetylcholine (*8, 24, 25*). Phosphorylation of GSK-3β at serine-9 is recognized to be negatively correlated with GSK-3β activation, while cleavage of GSK-3β by calpain counteracts the inhibitory effect of serine-9 phosphorylation on GSK-3β activity induced by hydrogen peroxide (*26*).

Phosphorylation of tau by protein kinase A (PKA) primes tau, making it a better substrate for GSK-3β, and at least partially explaining why the GSK-3β-preferred sites on tau can be hyperphosphorylated even after transient activation by PKA (*27, 28*). Interestingly, we demonstrated that tau hyperphosphorylation by GSK-3β seemed to be required for hippocampal neurogenesis in the dentate gyrus. Further, tau phosphorylation and GSK-3β activation are essential for the adult neurogenesis in the subventricular zone (SVZ), another niche of neurogenesis in the adult brains (*29, 30*), suggesting that the neurogenesis in the brain may be tightly regulated by local microenvironments.

Due to its role in tau phosphorylation, GSK-3β has been considered as a drug target for inhibiting neurodegeneration. However, GSK-3β has other functions in the cell, so complete inhibition would likely be detrimental. Recent studies that attempted spatiotemporal targeting of abnormal GSK-3β activation found that pathologies and memory deficits in an AD mouse model could be effectively attenuated (*31*). Further studies are needed to clearly define the conditions, but this work shows promise.

ROLE OF PP2A IN TAU HYPERPHOSPHORYLATION

Tau proteins are dephosphorylated by protein phosphatases such as PP2A; therefore, inactivation of phosphatases results in tau hyperphosphorylation. PP2A is the most effective at dephosphorylating abnormally hyperphosphorylated tau isolated from the AD brains (*32, 33*). In vitro, PP2A can dephosphorylate abnormal tau at multiple sites and thus restore its biological activity (*34*). Inhibiting PP2A in vivo by injection of okadaic acid or homocystine into the brain, or in vitro by incubating cells with PP2A inhibitors, induces AD-like tau hyperphosphorylation, intracellular accumulation, axoplasmic transport deficits, and learning/memory dysfunction (*35, 36*). PP2A is activated in the astrocytes of tg2567 mice—a widely used amyloidogenic model of AD—and activation of PP2A stimulates the migration of astrocytes to the amyloid plaques through p38 MAPK inhibition, indicating that PP2A deficits observed in AD brains may cause Aβ accumulation by hindering astrocyte migration (*37*).

Since PP2A activity is significantly inhibited in AD brains, upregulating PP2A seems a promising therapeutic strategy. However, currently no specific activator of PP2A exists, making the search for an upstream regulator of PP2A a critical mission. We have demonstrated that GSK-3β activation can inhibit PP2A by upregulating protein tyrosine phosphatase 1B, which phosphorylates