Possible roles of amyloid intracellular domain of amyloid precursor protein

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Amyloid precursor protein (APP), which is critically involved in the pathogenesis of Alzheimer's disease (AD), is cleaved by gamma/epsilon-secretase activity and results in the generation of different lengths of the APP Intracellular C-terminal Domain (AICD). In spite of its small size and short half-life, AICD has become the focus of studies on AD pathogenesis. Recently, it was demonstrated that AICD binds to different intracellular binding partners ('adaptor protein'), which regulate its stability and cellular localization. In terms of choice of adaptor protein, phosphorylation seems to play an important role. AICD and its various adaptor proteins are thought to take part in various cellular events, including regulation of gene transcription, apoptosis, calcium signaling, growth factor, and NF-κB pathway activation, as well as the production, trafficking, and processing of APP, and the modulation of cytoskeletal dynamics. This review discusses the possible roles of AICD in the pathogenesis of neurodegenerative diseases including AD. [BMB reports 2010; 43(10): 656-663]

INTRODUCTION

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder. Typical late-onset AD occurs after age 65, begins with short-term memory loss, continues with more widespread cognitive and emotional dysfunction, and follows a 5- to 15-year course (1). Currently, it is estimated that 50% of people older than age 85 are afflicted with AD (2). Dementia is estimated to affect 24.3 million people today, in addition to 4.6 million new cases every year (one new case every 70 seconds) (2). The number of people affected will double every 20 years to 81.1 million by 2040 (3). The worldwide social cost of dementia has increased from $315 to $422 billion in only a

few years (4). Such conditions also cause trouble to patients and caregivers in terms of financial cost, stress, and agony. Considering the above, effective treatments are greatly needed.

Even today, a definitive diagnosis of AD is only possible through postmortem analysis of the brain (5). Such histopathological analysis of the brain is required to demonstrate the classic triad of AD pathology: amyloid plaques containing amyloid β (Aβ) peptides, neurofibrillary tangles (NFTs) containing hyperphosphorylated tau, and widespread neuronal loss in the cortex and hippocampus (6).

The presence of amyloid plaques, which are composed of Aβ peptides, does not correlate well with the severity of neurodegeneration observed in AD (7, 8). Therefore, recent research has attempted to identify new causative factors in the pathogenesis of AD.

APP processing and production of AICD

Proteolytic processing of amyloid precursor protein (APP) occurs via two alternative cleavage pathways, which are localized to different subcellular compartments and generate different biological effects (Fig. 1).

Ectodomain shedding of APP can be executed by α-secretases, including ADAM9, ADAM10, and ADAM17 (TACE). Such cleavage occurs inside the Aβ domain, thereby precluding the generation of toxic Aβ peptides, but does generate a soluble extracellular fragment (sAPPα) that has neurotrophic and neuroprotective activities (9). Since sAPPα production occurs at the plasma membrane (10) as well as inside intracellular compartments such as the endoplasmic reticulum (ER) and golgi (11), and since γ-secretase is also present at the plasma membrane (12, 13), APP intracellular C-terminal domains (AICDs) can be generated.

In the case of the amyloidogenic pathway, APP is cleaved by β-secretase (BACE1) within its large extracellular domain to yield two fragments, sAPPβ (an N-terminal fragment) and CT99 or CT89 (C-terminal fragment of APP) (6). Subsequently, the remaining CT99 fragment inside the membrane is cut by the γ-secretase complex, including nicastrin (NCSTN or APH2), anterior pharynx defective 1 (APH1), presenilin enhancer 2 (PEN2), presenilin 1 (PSEN1), and/or presenilin 2 (PSEN2) (14). Finally, this process generates Aβ peptide and AICDs. Due to
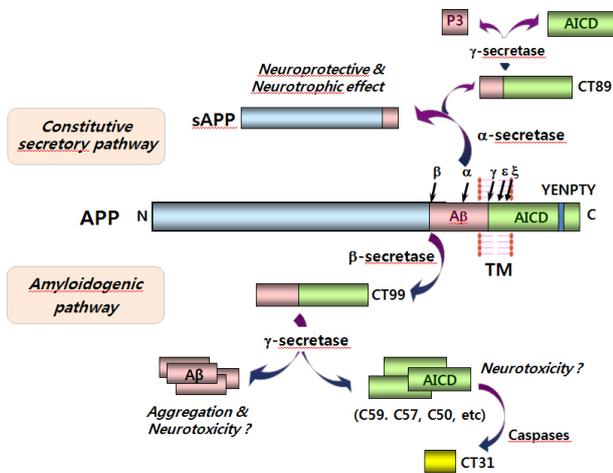
Fig. 1. Schematic representation of APP proteolytic catabolism. APP proteolytic catabolism includes two different pathways: an amyloidogenic pathway and a non-amyloidogenic pathway (constitutive secretary pathway). The different APP fragments are generated after secretase cleavage.
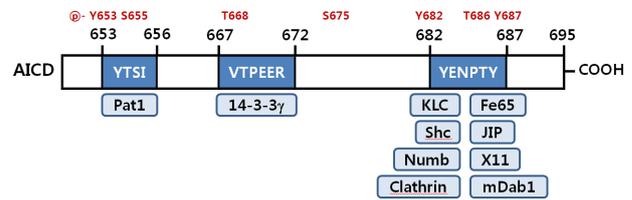

Fig. 2. Binding partners of AICD. The sequence of AICD is generated from APP695. There are seven phosphorylation sites denoted by 'P' within circles. The different binding regions are shown with the corresponding adaptors for each region.

heterogenous cleavage of γ-secretase, the length of the AICD varies from less than 57 to 59 amino acids (15). AICDs (51, 50, 48, and 53 amino acids) can also be generated by ε- or ζ-cleavage activities (16-19). Inside the cell, AICDs soon undergo sequential cleavage at a specific caspase cleavage site to produce the CT31 fragment (20).

In both cases, AICD is generated. However, the exact role of AICD generated by sequential α-γ or β-γ-cleavage in the regulation of gene expression is a subject of much debate in recent times. Nuclear signaling by endogenous AICD in primary neurons could similarly be blocked by inhibition of β-cleavage but not α-cleavage, suggesting that amyloidogenic cleavage is predominantly responsible for AICD-mediated nuclear signaling (21).

Degradation of AICD

AICD is difficult to study due to its small size and short half-life (22). AICD59 is rapidly degraded when overexpressed in baby hamster kidney cells or primary cultured neurons (22).

As responsible factor for the degradation of AICD, cytoplasmic metalloprotease insulin degrading enzyme (IDE), an Aβ degrading enzyme, was shown to be responsible for the degradation of AICD (23, 24). This result is supported by the increased amounts of AICD in IDE knockout mice (25). In contrast, proteasome-dependent AICD degradation also seems to take part in degradation of AICD (26). When APP C-terminal peptides (CT) were incubated with recombinant 20S proteasome, CT was cleaved at several different sites, including at the YENPTY motif which interacts with several APP-binding proteins (26).

Structure of AICD

AICD possesses a few conserved regions capable of interacting with intracellular adaptor proteins (Fig. 2). The 682-YENPTY-687 sequence (amino acid numbering according to human APP695 isoform) is a consensus motif for clathrin-mediated endocytosis and contains the consensus sequence for phosphotyrosine binding (PTB) domain interactions. The 653-YTSI-656 motif, localized near the cell membrane, is essential for basolateral targeting in polarized epithelial cells. The combination of -YTSI- & -YENPTY- motifs is essential for rapid degradation of APP in lysosomal compartments (19, 27). AICD exhibits transient structural features consisting of a hydrophobic cluster (656I-667V), an N-terminal helix capping box (667-VTPEER-672), a type I β-turn (684-NPXY-687), and a nascent helix for residues (664-DAA-666, 675-SKMQQNGYE-683, and 688-KFFEQM-693) (28).

In tissue samples, AICD is obtained in phosphorylated or unphosphorylated form, with two threonine residues (654 and 668) and a serine residue (655) acting as the sites of phosphorylation (29). The actual phosphorylation event, which is cell cycle-dependent, may act as a conformational switch in the cytoplasmic tail of APP that alters the specificity and affinity of binding to a cytosolic partner (29-31).

Adaptor proteins of AICD

AICD binds with various physiological partners ('adaptor proteins'), some of which are well studied kinases and others which are involved in intracellular transduction.

As mentioned above, the -YENPTY- motif interacts with adaptors possessing a PTB domain, namely the X11 family (X11a, X11L, X11L2) (32), Fe65 family (Fe65, Fe65L1) (33), cJun-N-terminal kinase interacting protein (JIP) family (JIP1b, JIP2) (34, 35), the Shc family (ShcA and ShcC), mammalian disabled-1 (mDab1), Numb and Numb-like proteins, kinesin light chain (KLC), Abl-non-receptor tyrosine kinase (36), clathrin (37), and growth factor receptor protein-binding protein 2 (Grb2) (38). The interactions take place both in phosphotyrosine-dependent or -independent manners and different interaction seems to result in different and more specific biological

effects.

Role of AICD in transcription

Although Fe65-independent signaling might be due to direct binding of AICD to Tip60 (39), Fe65 seems to be essential for the nuclear translocation of AICD based on several pieces of evidence (40-42). The interaction between Fe65 and AICD/APP is modulated by APP Thr^{668} phosphorylation as well as its subsequent nuclear translocation (43, 44). In addition, phosphorylation by c-Abl could regulate the AICD and Fe65 interaction (36, 45).

Two different transcriptionally-active AICD complexes, AICD-Fe65-Tip60 and AICD-Fe65-CP2/LSF/LBP1, have been reported (46, 47). Tip60 is a histone acetyltransferase that is a part of a large nuclear complex having DNA binding, ATPase and DNA helicase activities (48). Fe65 was shown to associate with Tip60, and both of these proteins colocalize with AICD in the nucleus by forming the AICD-Fe65-Tip60 complex (termed AFT complex) (42, 46, 49). In Gal4 reporter gene experiments, co-expression of APP (or AICD), Fe65, and Gal4-Tip60 leads to dramatically enhanced expression of Gal4 in the presence of CP2, a transcription factor that interacts with Fe65 (46). Moreover, Cao and Sudhof (50) reported that γ-cleavage of APP along with nuclear translocation of Fe65 are required for transactivation. Meanwhile, the Fe65 adaptor protein has been described to interact with the transcription factor CP2 (CP2/LSF/LBP1) through its PID1 domain (51). The involvement of AICD in the complex was confirmed by co-immunoprecipitation of AICD with CP2 in the presence of Fe65 (47). This second ternary complex (AICD-Fe65-CP2/LSF/LBP1) was suggested to modulate the expression of glycogen synthase kinase-3β (GSK-3β) (47).

During the regulation of this complex, many adaptor proteins are involved. JIP1b overexpression increases transcriptional activation mediated by Gal4-APP or AICD (52). Overexpression of 14-3-3γ by Gal4-AICD fusion protein enhances Fe65-dependent gene transactivation by two-fold in mouse neuroblastoma-2a cells (53). Recently, Sp1 was reported to play a crucial role in transcriptional regulation of human Fe65 (54). In contrast, X11α has an inhibitory effect on AICD-mediated gene transactivation by trapping AICD in the cytoplasm (42). Further, Dexras1, a Ras family small G protein, also functions as a suppressor of Fe65-APP-mediated transcription (55). Recent work suggests that Aβ impairs APP gene transcriptional activation by decreasing AICD production as well as the formation of AICD/Fe65 transactivation complexes (56).

Although the role of AICD as a gene transcription inducer is still controversial (57, 58), several targets of AICD- or APP-mediated transcriptional activation are reportedly regulated by APP nuclear signaling, including *APP itself, BACE, Tip60, GSK-3β, p53, Mn-SOD, KAI1, Neprilysin, transgelin, α2actin, S100a9,* and other genes (42, 47, 59-65).

Putative role of AICD as a modulator of apoptosis

Previously, Passer *et al.* (66) identified AICD-like peptides in brain tissues of normal control and sporadic AD patients and demonstrated that AICD acts as a positive regulator of apoptosis. Several researchers have suggested that AICD plays an active role in triggering the apoptotic response (39, 44, 65, 67). Recently, we found that various lengths CTs, including AICD and CT31, can translocate to the nucleus and exert apoptosis, and mutant studies have suggested that the YENPTY domain is most important for inducing neurotoxicity (unpublished data).

Confocal microscopy and fluorescence resonance energy transfer analysis showed that AICD58 translocates to the nucleus and forms a complex with Tip60 in order to mediate AICD-induced cell death (39). Ozaki *et al.* (65) found that AICD also interacts with p53 and enhances its transcriptional activity and pro-apoptotic functions, suggesting that AICD may enhance p53-mediated apoptosis. AICD induced neuron-specific apoptosis is likely mediated by GSK3β and p53 (47, 68, 69). In addition, the kinase c-Abl can modulate AICD-dependent cellular responses, inducing apoptosis and transcription of its target genes such as Neprilysin (70).

In addition, Ha *et al.* (71) reported that Hirano bodies, intracellular inclusions found in a number of neurodegenerative diseases, have inhibitory effects on AICD-induced cell death and AICD/Fe65-dependent transcriptional activity, suggesting that the formation of Hirano bodies may play a protective role in the progression of neurodegenerative diseases like AD, although further study is required.

Putative role of AICD as a modulator of cytoskeletal dynamics

In APP transgenic (Tg) flies and mice, axonal defects in the form of swellings that consist of abnormal amounts of microtubule-associated and molecular motor proteins, organelles, and vesicles are present (72, 73). This suggests that overexpression of APP has the potential to impair axonal transport. Based on the observation that APP lacking a C-terminal cytoplasmic domain does not have a harmful effect on axonal transport (73), APP-perturbed axonal transport might result from the activation or inhibition of a number of kinases and phosphatases regulated by APP signalling via binding partners such as JIP1b or Fe65 (34, 74). By studying APP Tg flies under control of the GAL4 promoter with different deletions in the carboxy terminus, Rusu *et al.* (75) also suggested that JIP1b or Fe65 may cause defective APP-induced axonal transport.

AICD was shown to induce the expression of genes having functional roles in actin organization and dynamics, including transgelin (SM22) and alpha2-actin, resulting in a loss of organized filamentous actin structures within the cell (64, 76). In fact, overexpression of transgelin, a proposed AICD target

gene, causes destabilization of actin filaments, depolarization of mitochondrial membrane potential (DeltaPsim), and significant alteration of mitochondrial distribution and morphology in human SHEP neuroblastoma cells and primary neurons (76).

These data demonstrate that induction of AICD/APP significantly alters cytoskeletal dynamics and mitochondrial function in neuronal cells by interacting with JIP1b or Fe65.

Effects of AICD on Ca^{2+} signaling and inflammation

Hamid et al. (77) observed that AICD modulates cellular calcium homeostasis and ATP content, whereas alteration of Ca^{2+} homeostasis in cells lacking AICD is reciprocally linked to mitochondrial bioenergetic function.

Recently, we found that S100a9, known as an inflammation-associated calcium-binding protein belonging to the S100 family (78, 79), was induced in an AD animal model, APPV$_{717I}$-CT100 tg mice and Tg2576 mice brains, and in human AD brains (63). Induction of S100a9 increases the intracellular calcium level and upregulates the expression of inflammatory cytokines (IL-1β and TNFα) and iNOS, causing inflammation (63). Single injection of S100a9 lentiviral short hairpin RNA (sh-S100a9) greatly improves cognitive decline of Tg2576 mice and significantly reduces neuropathology and amyloid plaque formation, suggesting S100a9 as a possible target in the pathogenesis of AD (63).

In addition, Sondag and Combs (80) have suggested that APP mediates proinflammatory activation of monocytic lineage cells. Ghosal et al. (81) also showed that NSAID treatment has a protective effect against neurogenetic defects, suggesting that AICD overexpression results in sustained neuroinflammation. Neuroinflammation is recognized as a significant pathological event in AD, along with insoluble protein deposition and neurodegeneration (82, 83).

Effect of AICD on production, processing, and trafficking of APP

Von Rotz clearly showed that AICD upregulates the expression of APP genes as well as the gene encoding β-site APP cleaving enzyme (BACE) but not that of γ-secretase-complex protein PSEN1 or ADAM10, a proposed α-secretases (42). The life cycle of APP, therefore, encompasses a feedback mechanism for synthesis, subcellular sorting, proteolytic cleavages, and nuclear signaling and transcriptional activation of APP by AICD (42).

Since AICD possesses the clathrin-mediated endocytosis signal (-GYENPTY-), its interaction and subsequent complex formation with adaptor proteins would influence APP trafficking and eventually the rate of Aβ production (84). The additional cellular effects of an AICD-induced increase in APP turnover remain to be determined.

AICD Tg mice

AID Tg mice can be a valuable tool to understanding the role of AICD. Recently, two groups examined whether or not AICD can contribute to AD using a AICD Tg mice model.

Pimplikar's group demonstrated that double Tg mice co-expressing AICD59 and its binding partner Fe65 show abnormal activation of GSK-3β and phosphorylation of CRMP-2 (62), age-dependent tau phosphorylation and aggregation, memory deficits, and neurodegeneration without altering APP metabolism or increasing Aβ levels (85). Moreover, these mice show aberrant electrical activity and silent seizures beginning at 3-4 months of age and also display abnormal mossy fiber sprouting beginning at about the same time (86). Elevated levels of AICD render neurons hypersensitive to stress and induce hippocampal circuit reorganization (86). In addition, AICD Tg mice exhibit impair adult hippocampal neurogenesis in an Aβ-independent manner (81). Impaired adult neurogenesis in these Tg mice can be prevented by NSAID treatment, suggesting that neuroinflammation is critically involved in this deficit and that AICD can exacerbate memory defects in AD by impairing adult neurogenesis (81).

In contrast, D'Adamio's group reported that AICD-target genes and the mouse brain basal transcriptoma are not influenced by transgenic expression of AICD alone or in the absence of Fe65 overexpression, whereas transgenic AICD expression does increase neuronal sensitivity to toxic and apoptotic stimuli (87). Furthermore, in AICD Tg mice crossbred with human Tau-bearing mice, they found no evidence that AICD overexpression induces AD-like characteristics, such as activation of GSK-3β, hyperphosphorylation of Tau or formation of neurofibrillary pathology (88).

CONCLUSION

Gamma-secretase cleavage of APP may contribute to neurodegeneration in AD in two ways: release of Aβ and generation of AICDs. Together with Aβ, these bioactive APP intracellular C-terminal domains have been studied in the AD research field for over 10 years. However, it is still unclear whether or not Aβ is involved in AD-related pathophoysiology, although numerous studies have demonstrated the relevance of AICD in the pathogenesis of AD.

Until now, several intracellular binding partners ('adaptor proteins') have been identified. It is widely acknowledged that such proteins might regulate the stability and cellular localization of AICD. Through many experiments with cultured cells or mouse models, AICD and its various adaptor proteins are thought to take part in various cellular events, including the regulation of gene transcription (Fig. 3). Upon its transcriptional modulation, AICD is suggested to induce apoptosis, alter calcium signaling, cytoskeletal dynamics, and growth factor and NF-κB pathway activation, and modulate the production, trafficking, and processing of APP.
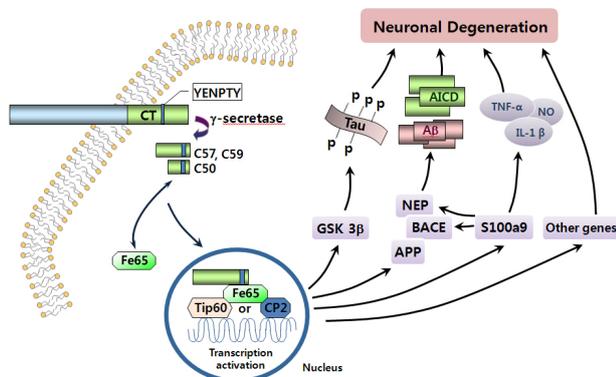
Fig. 3. Putative neurodegenerative roles of AICD. The figure shows possible AICD mechanisms in neurodegeneration. The AICD/Fe65 complex translocates to the nucleus where, in association with Tip60 or CP2, it regulates the gene transcription of various genes such as GSK-3β or S100a9, and others.

Although the role of AICD in the pathogenesis of AD remains controversial, AICD-related mechanisms seem to be strongly involved in the neurodegenerative process associated with AD pathology.

Acknowledgements

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