The JNK/c-Jun cascade and Alzheimer's disease

Hitohi Okazawa, MD, PhD
Steven Estus, PhD

Abstract

Emerging evidence indicates that the JNK/c-Jun cascade is activated in neurons of the Alzheimer's disease brain and suggests its involvement in abnormal processes, ranging from tau phosphorylation to neuronal death. Substantial new data have accumulated on the functional relevance of causative genes in familial Alzheimer's disease and the pathological processes that occur within neurons. In this review, we summarize reported findings of the JNK/c-Jun cascade in Alzheimer's disease and discuss the relationship between the cascade and other pathological processes. We suggest that the effort to connect amyloid deposition with intracellular activation of the JNK/c-Jun cascade may modify the amyloid theory of Alzheimer's disease. Therapeutic approaches targeting the JNK/c-Jun cascade and other signaling may complement therapeutic strategies directed at reducing amyloid deposition.

Key words: Alzheimer's disease, amyloid, CDK5, cell death, c-Jun, c-Jun N-terminal kinase (JNK), GSK-3, neuron, phosphorylation, presenilin, SAPK, tau, transcription

Introduction

Alzheimer's disease (AD) is pathologically characterized by senile plaques (SP), neurofibrillary tangles (NFT), and neuronal loss. SPs include interstitial aggregates of β amyloid peptide (Aβ) processed from the amyloid precursor protein (APP), while NFTs are intraneuronal aggregates of hyperphosphorylated tau protein. In classic neuritic plaques, a central Aβ deposit is surrounded by dystrophic neurites and reactive astrocytes, and is permeated by activated microglia. Aβ deposits termed diffuse plaques are detected by immunohistochemistry with anti-Aβ antibodies, and lack the neuritic and inflammatory components. Clinically, progressive dementia is caused by either neuronal loss or by synaptic loss in the limbic system with similar events in the cerebral cortex believed to be secondary phenomena. Clinicopathological definition of this disorder was established by Alois Alzheimer in 1907 at Munich,[1] whereas understanding of the molecular pathology has not been completed in spite of enormous efforts by a large number of researchers.

However, during the past 10 years, genetic analyses of familial AD have revealed major factors in the disease pathogenesis. These factors include amyloid precursor protein (APP),[2] presenilin-1 (PS1),[3,4] presenilin-2 (PS2),[5] and apolipoprotein-E (ApoE).[6] The first three factors are strongly linked to early-onset familial AD, while ApoE is considered to be a major risk factor for late-onset AD. Other molecules such as α_2-macroglobulin (α_2-M)[7] and LDL receptor-related protein (LRP)[8] also have weak linkage with AD, at least in some populations. Another locus of late-onset AD on chromosome-10 was reported recently by three groups.[9-11] These discoveries have enabled us to apply molecular biological approaches by using identified molecules and thereby to take a larger perspective of AD pathology. However, in turn, these results clearly suggest that AD is a disorder with heterogeneous etiology, but shares core pathological processes. Therefore, we want to know the precise pathological roles of these molecules and the functional relationship among them to clarify the common pathway leading to AD pathology.

PS functions as γ-secretase

One of the most remarkable advances in AD research is the notion that PS by itself or a protein complex including PS possesses γ-secretase activity, which is essential for cleaving out Aβ from APP. Interaction

Hitohi Okazawa, MD, PhD, Department of Neurology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; Present address: Department of Molecular Therapeutics, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan.

Steven Estus, PhD, Department of Physiology, Sanders-Brown Center on Aging, University of Kentucky, Lexington, Kentucky.

between PS and APP was suggested by immunoprecipitation and two-hybrid assays.[12-14] Aβ1-42, which aggregates more readily than the more common Aβ1-40,[15] was reported to be increased in the AD brain or in cells overexpressing mutant PS.[16] The γ-secretase hypothesis was tested with this background. PS was co-precipitated within the protein complex possessing the γ-secretase activity.[17] Inhibition of presenilins in cell biological experiments and presenilin knock-out mice showed severe disturbance in the cleavage of APP, presenilins as well as notch at the γ-secretase acceptor site.[18-22] Further, a chemical inhibitor of γ-secretase was reported to be cross-linked to PS at its possible catalytic center.[23] These data strongly suggest that PS is a component of γ-secretase complex or γ-secretase itself.[24] Another component of the γ-secretase protein complex seems to be Nicastrin, which was isolated as a binding protein to PS.[25] Deletion of Nicastrin was reported to disturb the γ-secretase activity for Aβ production.[25]

However, some questions have also been raised about the concept that PS is γ-secretase.[26,27] These questions include the structural difference of PS from aspartyl proteases reported so far and the location of the catalytic center as γ-secretase. Recent reports also suggest discrepancies among substrates in the cleavage by PS. Capell *et al.* report that mutation of the aspartate-257 eliminates Notch cleavage without altering γ-secretase activity for APP.[28] Petit *et al.* show that a new protease inhibitor suppresses γ-secretase activity, but neither Notch cleavage nor self-cleavage of PS.[29] According to these data, APP γ-secretase activity is distinct from Notch and PS cleavage activity, although they are believed to be mediated by the same catalytic site of PS. These questions await further examination. In addition, the relationship between the γ-secretase function and multiple-binding partners of PS1 reported to date[30] needs to be clarified.

Changing amyloid theory

Another great advance in AD is the use of Aβ immunization as a therapeutic approach. Active immunization in transgenic mice largely prevents Aβ deposition[31] and inhibits the associated decline in memory function.[32,33] Commercial studies (Elan Pharmaceuticals) also have shown an anti-Aβ effect of passive immunization in an AD mouse model and integration of immunoglobulin into the cerebrum.[34] These findings are extremely attractive in a clinical sense, and this surprising approach awaits the results of ongoing clinical trials. If the vaccine proves effective in human AD, it would clearly support the amyloid theory, which predicates that Aβ deposition is the central event of AD.[35-37] A further important point is that these transgenic models, without neuronal loss, did show behavioral effects, which are presumably related to synaptic changes.

The amyloid theory has recently been modified. A critical notion requiring modification is the discovery of a tauopathy.[38] Specific mutations in tau and subsequent aggregation of abnormal tau proteins in the brain, without Aβ plaque deposition, are now known to cause neuronal death. Furthermore, transgenic mice overexpressing tau show progressive neurodegeneration associated with hyperphosphorylated tau and and argyrophilic inclusions formed by tau-immunoreactive filaments.[38] A growing body of data link tau gene mutations with specific types of neurodegenerative diseases, including frontotemporal dementia and Parkinsonism (FTDP).[39] This finding is generally thought to mean that NFTs are downstream from SP in AD pathology and linked directly to cell death.[40] However, we need to consider carefully whether NFTs by themselves cause cell death, since we do not know the exact mechanisms by which tau aggregation kills neurons. Actually, some people hypothesize that NFT formation and even tau hyperphosphorylation are a consequence of activation of the cell death cascade.[41] One of the reasons supporting this hypothesis is that neuronal loss greatly predominates NFTs in tauopathy. In addition, this idea is compatible with a recent paper showing neurodegeneration without NFTs in transgenic flies.[42] Instead of NFTs themselves, a specific type of abnormal signaling which leads to NFTs may be the cause of cell death.

Furthermore, several important observations have challenged the amyloid hypothesis. First, a growing number of reports suggest that oligomeric or soluble Aβ peptides rather than SP formation are toxic for neurons.[43] In other words, these data raise a critical question as to whether neuronal damage is initiated by Aβ plaque formation or by soluble and nonaggregated Aβ peptides. Second, recent reports from various groups indicate that Aβ peptides accumulate in neuronal cell bodies in the affected area of the AD brain.[44-47] This newly recognized Aβ localization has raised a question as to whether cell injury is initiated inside or outside of neurons. In the classic amyloid cascade hypothesis, secreted Aβ?? initiates plaque formation and damages the cell from the outside. However, the presence of Aβ within the cell suggests that Aβ oligomers or conformationally unfavorable Aβ peptides may trigger the death signaling from inside the neurons. In other words, the finding proposes an alternative pathway from Aβ to cell death.

Perturbed cell homeostasis and signaling

Substantial data have accumulated regarding abnormal

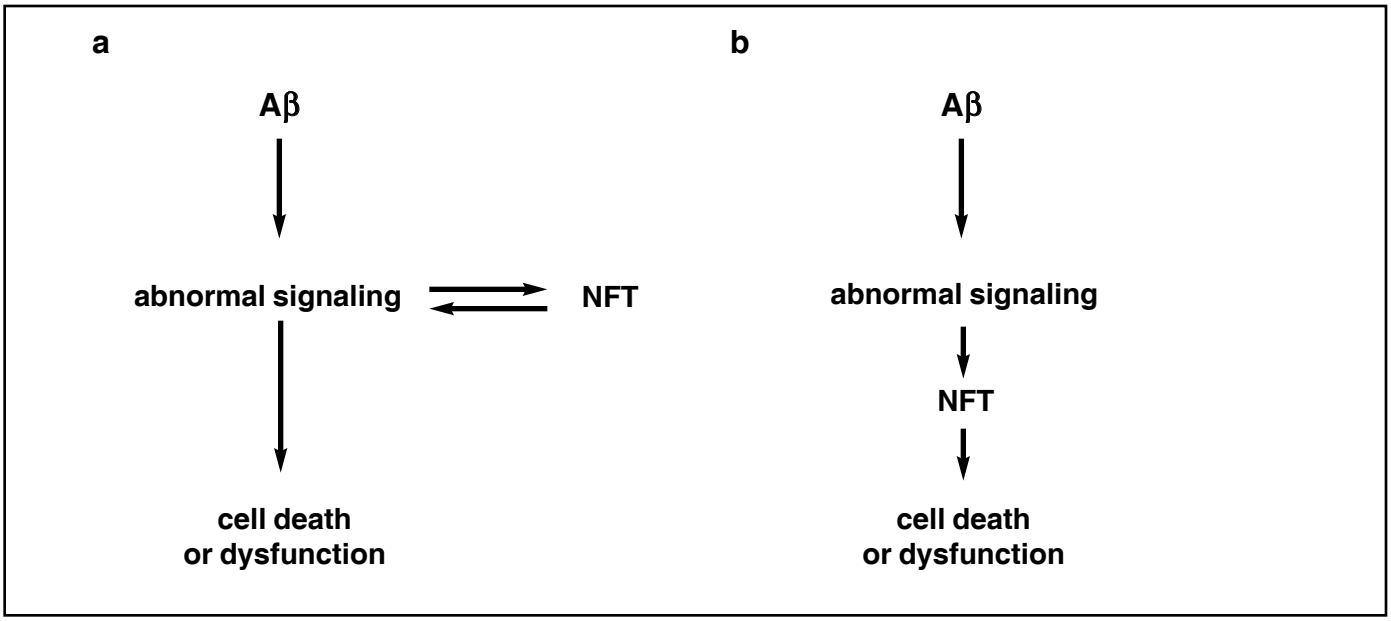

Figure 1. Hypothetical cascades of the Alzheimer's disease pathology.

neuronal signaling as possible mediators of the neuronal death in AD pathology. Representative pathways are oxidative stress response, excess calcium accumulation, and activation of kinases. Exaggerated oxidative stress in the AD brain was suggested by immunohistochemical data with antisuperoxide dismutase and catalase antibodies,[48] increased products of the oxidative stress response,[49,50] and inhibition of Aβ-induced cell death by antioxidants.[51] Alterations of calcium content in AD were initially suggested by the data in cultured skin fibroblasts.[52] Excessive calcium influx was observed on the basis of cell biological experiments where aggregated Aβ was applied from outside the cells.[53] Recently, Mattson's group reports that mutant PS1 "knock-in" mice show increased vulnerability due to perturbed calcium homeostasis, increased oxidative stress and mitochondrial dysfunction,[54] supporting abnormal metabolism in these pathways. Though calcium and mitochondrial dysregulation will lead to abnormal activation of kinases, the relationship between signaling alternations and NFT formation has not been established. Both NFT formation and abnormal signaling may be the results of either extracellular or intracellular Aβ accumulation. In this case, perturbed signaling triggers cell death independently of NFT. Alternatively, perturbed signaling causes NFT, which then induces cell death (see Figure 1).

Although it is not known whether tau phosphorylation is the direct cause of cell death, it is likely that tau hyperphosphorylation is a molecular event close to cell death. In this regard, activation of kinases relevant to tau phosphorylation has attracted attention. The first candidate molecule is cyclin-dependent kinase-5 (CDK5).[55-57] According to the data of Patrick *et al.*, a constitutively active form of CDK5, p25, was found in the neurons of AD brains.[58] The second candidate molecule is glycogen synthetase kinase-3 (GSK3).[59,60] This kinase is involved in complex formation with tau and PS1.[61] c-Jun N-terminal kinase (JNK) is the third kinase that has been implicated in tau phosphorylation. Two groups have reported that JNK phosphorylates tau.[62-64] Recently, we and others have used anti-phospho-JNK antibodies to establish JNK activation in neurons of sporadic and PS1-linked AD brains.[47,65] Extracellular application of aggregated Aβ induces neuronal apoptosis with c-Jun activation.[66] These observations support activation of the JNK/c-Jun cascade in the AD brain and may be related to a previously reported increase of c-Jun immunoreactivity.[67-69] Furthermore, our group and another have found that PS1 prevents c-Jun activation by either c-Jun co-factor QM/Jif-1 or JNK, and that mutation of PS1 perturbs the suppressive effect of PS1 on c-Jun,[70,71] suggesting acceleration of c-Jun-mediated neuronal death by mutant PS1. All these findings suggest the JNK/c-Jun cascade may interact with various processes in AD pathology.

Two laboratories independently reported that PS1 interacts with Ire1,[72,73] a mediator of the unfolded protein response (UPR) against accumulation of unfolded proteins in the endoplasmic reticulum. Katayama *et al.* reported that mutant PS1 inhibits the UPR,[72] although there remains some debate about their data.[74,75] Also, it is not yet clear whether Ire1 is a substrate of PS as a proteinase. Meanwhile, Ire1 was reported to activate chaperone genes and JNK in response to ER stress.[76] As some of these data are still controversial,[74,75] we cannot reach a

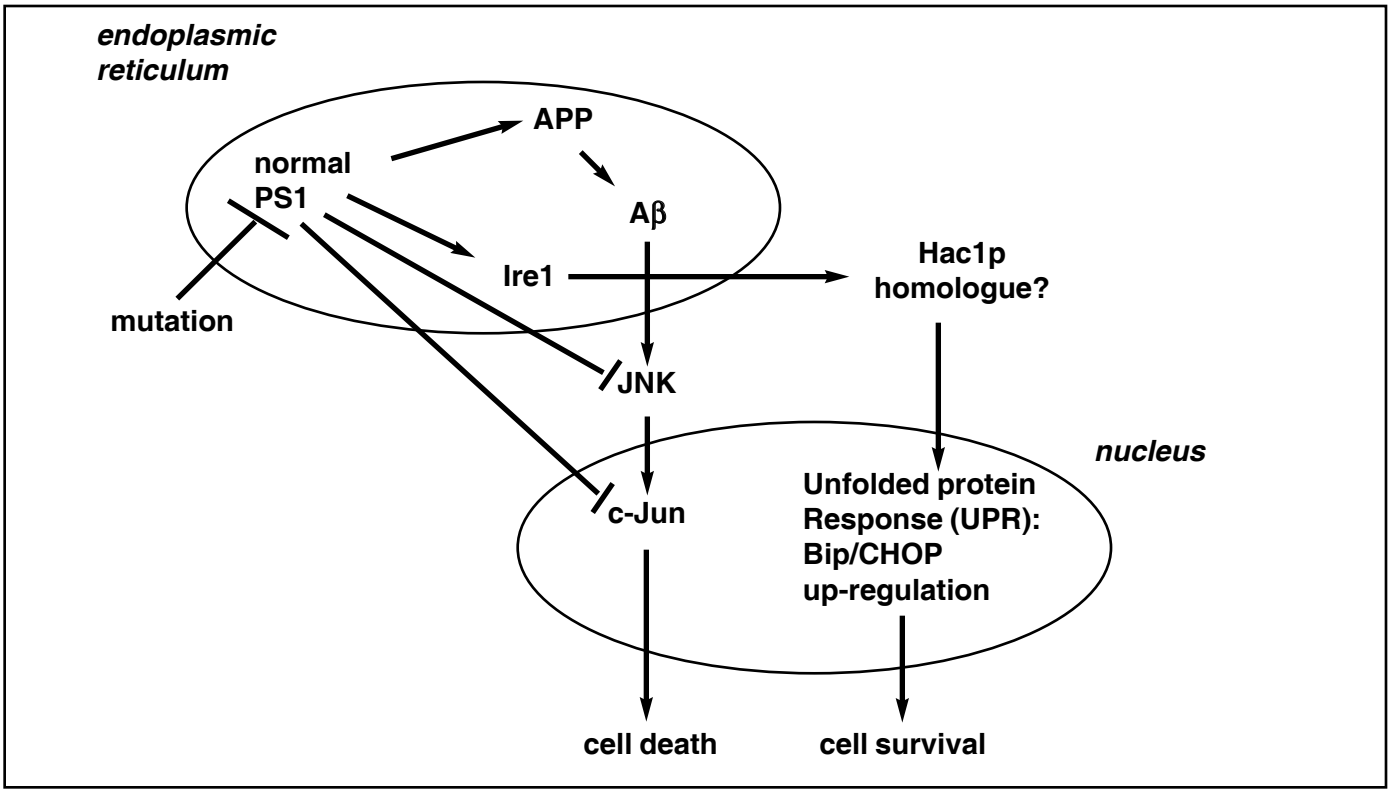

Figure 2. Ire1 has both endonuclease and kinase functions. In yeast, Ire1 converts the mRNA coding Hac1p from an inactive form to an active form and induce UPR. However, mammalian homologue of Hac1p has not been known. PS1 mutation disturbs up-regulation of a chaperone gene, BiP via Ire1,[72] instead abrogates suppression of c-Jun by reducing transport of c-Jun to the nuclei.[70] Therefore, PS1 mutation may shift the signaling which branches at Ire1 from cell survival to cell death.

firm conclusion on how these findings correlate with JNK activation observed in the AD brain.[47,65] However, it may be possible and is very interesting to hypothesize that Aβ accumulated in the endoplasmic reticulum triggers JNK activation and PS1 affects it in some way (Figure 2).

In addition, JNK/c-Jun cascade is relevant to some other molecules in AD pathology. First, the ApoE receptor-2 (ApoER2) binds to JIP1/2 (JNK interacting protein 1/2), and hence forms a complex with JNK. JIP is known to recruit MAP kinases and regulate their activities. Thus, ApoE signaling seems to be at least partially mediated by JNK. Secondly, c-Jun is also implicated in transcriptional regulation of the APP gene. The consensus binding site for AP-1/AP-4 in the upstream region of the APP gene has been shown to promote transcription, although the binding of AP-1 complex to this promoter has not been proven.[77]

Molecular mechanisms of neurodegeneration by JNK/c-Jun cascade

This section reviews the role of JNK and c-Jun in neurodegeneration. We will begin by reviewing the role of JNK and c-Jun in naturally occurring neuronal death during development, proceed to the evidence regarding the role of c-Jun and JNK in pathologic states, and conclude with a few remarks concerning the adequacy of current *in vitro* and *in vivo* models of AD.

Recent work has suggested that the prior assumption of necrosis and apoptosis as two distinct and dominant modes of cell death must be revised to accept that neuronal death may have attributes of apoptosis and necrosis. This is in contrast to normal nervous system maturation, which is characterized by extensive cell death that may be largely apoptotic.[78] Shortly after the period of neuroblast proliferation, many neurons, commonly about 50 percent, die during a limited period of time as functional connections are being made. The assumption that naturally occurring neuronal death includes an apoptotic-like process is supported by both *in vivo* and *in vitro* data. The most studied model of programmed neuronal death (PND) induced by trophic factor deprivation is based on the physiological role of the prototypical neurotrophic factor, NGF. Depriving immature sympathetic neurons of NGF results in massive cell death *in vivo*, even in the adult animal, albeit at a slower rate.[79]

This neonatal sympathetic PND can be reconstructed *in vitro*,[80,81] where apoptosis is dependent on transcription and translation, and apoptotic cells are visible 24 to 48 hours after NGF.[82] Two groups have shown that oxidative stress is an important early trigger in this model, and death can be inhibited by overexpressing Cu/Zn-superoxide dismutase in the neurons.[83,84] Moreover, several years ago, our group and Jonathan Ham's laboratory showed that c-Jun was induced during cell death and was necessary for it in this model.[85,86] The death of cultured sympathetic neurons undergoing NGF deprivation in vitro manifests the hallmarks of apoptosis, including membrane blebbing, chromatin condensation and DNA fragmentation into oligonucleosomes, cytochrome C release from the mitochondria, and caspase activation.[80,81,87-89]

To investigate the role of c-Jun phosphorylation in this paradigm, we and others have performed immunofluorescent analyses of c-Jun phosphorylation as well as quantitative assays of JNK activity at different times after NGF deprivation.[90,91] By using an antibody raised against phospho-Jun (Ser 63) for immunofluorescent studies, we observed a robust increase in c-Jun phosphorylation in NGF-deprived neurons, as compared to NGF maintained neurons.[90,91] To correlate this increase in c-Jun phosphorylation with JNK activity, we used an *in vitro* assay, where JNK was immunoprecipitated and then exposed to a c-Jun fragment for a substrate. Some 2.5 hours after NGF deprivation, JNK activity increased about twofold, and maintained this level until the neurons began to die, approximately 25 hours after NGF deprivation.[90,92] Hence, phosphorylation of c-Jun is increased in sympathetic neurons after NGF deprivation, and this correlates qualitatively with an increase in JNK activity.

Several groups, including ours, have asked the next question in this paradigm, *i.e.*, does JNK activity contribute to neuronal death? This line of investigation was initiated by Michael Greenberg's laboratory several years ago. Working with sympathetic neuron-like PC12 cells, this group examined the contributions to NGF-deprivation-induced cell death of MAP kinase family members, including ERK, JNK, and p38. Apoptosis was preceded by sustained JNK and p38 activation, and ERK inhibition. These authors proceeded to show that overactivation of JNK led to apoptosis, whereas activation of the MAPK pathway protected from apoptosis.[93] In the sympathetic neuron model, the Ham laboratory has used a dominant negative JNK-interacting protein (JIP) to investigate whether JNK is necessary for this neuronal death,[91] while Maroney and co-workers have used CEP1347, a pharmacologic inhibitor of the JNK pathway, but not JNK itself, to arrive at a similar conclusion.[94] One of the three JNKs is specific to neurons, *i.e.*, JNK3. We examined neuronal death in neurons isolated from JNK3 deficient neonatal mice and found that c-Jun phosphorylation and neuronal death were both inhibited. Interestingly, the oxidative stress that is also a hallmark of this model was unaffected by JNK3 deficiency.[95] The c-Jun AA knock-in mouse is also defective in kainic-acid-induced apoptosis in the hippocampus, indicating that c-Jun N-terminal phosphorylation is important in this model of pathological neuronal death.[96] Hence, JNK, especially JNK3, appears to contribute to neuronal apoptosis in this classic model of PND.

For several years, the predominant hypothesis in this work was that JNK activation leads to the activation of c-Jun, which, in turn, leads to the altered transcription of genes that are more proximal to neuronal death. In support of this possibility, the Ham and Johnson laboratories have recently reported that the induction of BIM, a pro-apopotosis BAX homolog, is JNK-dependent[97,98] (Figure 3). Very recently, the Johnson laboratory reported that induction of DP5/HRK, a pro-apoptotic Bcl-2 family member, is also JNK-dependent in neurons.[99]

An alternative and additional role for JNK also must be considered relevant to this review. Another recent finding from the Johnson laboratory suggests an additional pro-apoptotic mechanism of JNK action. To appreciate the relevance of this finding, one needs to know that several years ago, these investigators found that sympathetic neurons maintained in the presence of NGF were refractory to apoptosis after cytochrome C injection into the cytoplasm. When neurons were deprived of NGF for approximately 12 hours, the neurons were induced to be competent to die in response to cytochrome C injection.[100] Recently, these investigators extended their findings by showing that CEP1347, but not protein synthesis inhibitors, inhibited neurons from gaining NGF-deprivation-induced sensitivity to cytoplasmic cytochrome C.[88] This remarkable finding suggests that JNK activates some mechanisms leading to apoptosis after cytochrome C release, and this action of JNK does not require new protein synthesis. This further suggests that nontranscription factor substrates for JNK are critical to neurons gaining the competence to undergo apoptosis in response to cytochrome C in the cytoplasm. These substrates and their role in apoptosis are presumably the object of investigation.

c-Jun, JNK, and neuronal death in pathologic conditions

As discussed before, Aβ may induce neuronal death inside as well as outside cells. So far, extracellular Aβ is known to induce apparent apoptosis in cortical and

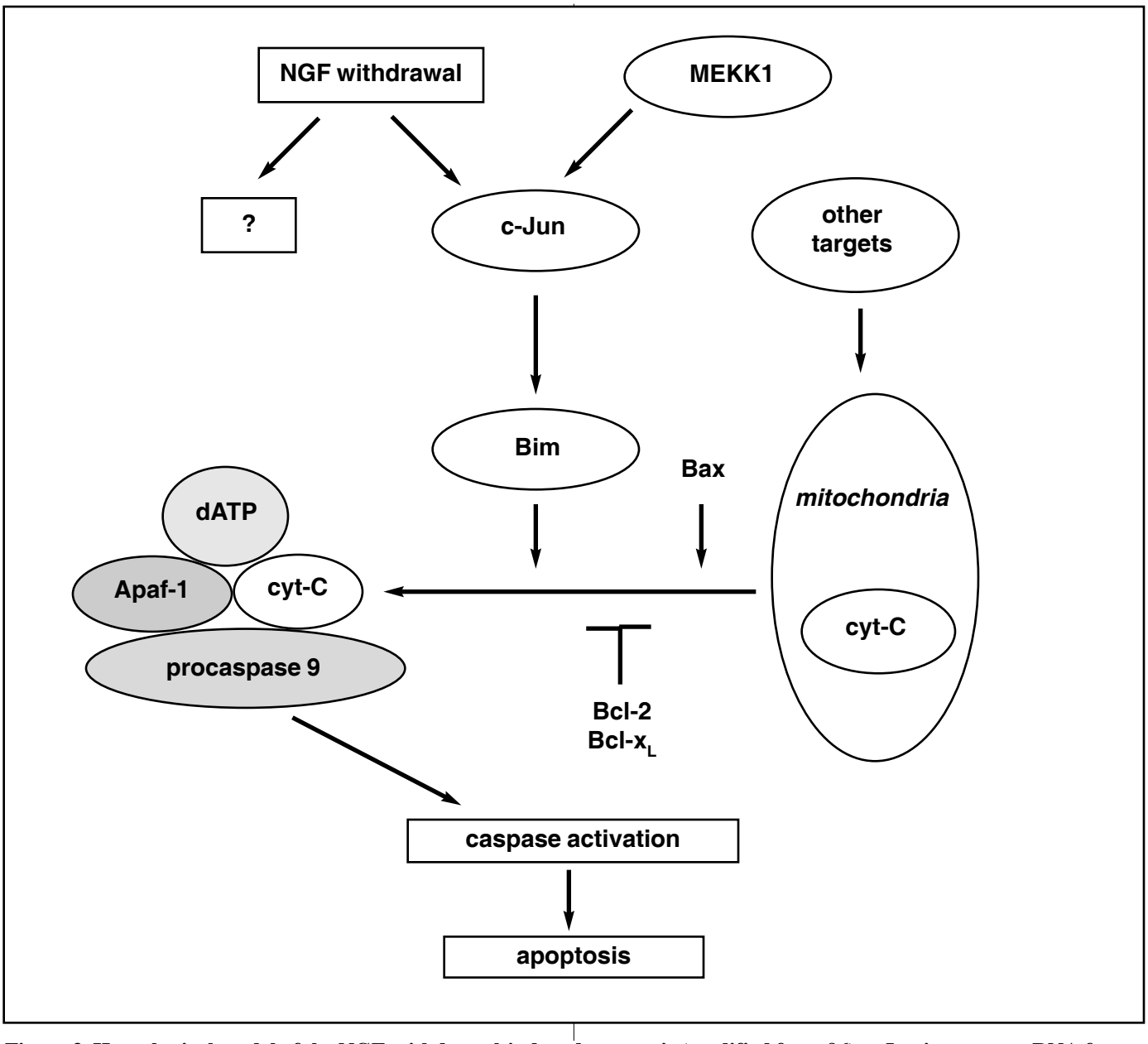

Figure 3. Hypothetical model of the NGF-withdrawal-induced apoptosis (modified from 96). c-Jun increases mRNA for Bim, a proapoptotic member of Bcl family.

hippocampal neurons maintained *in vitro*. Specifically, Aβ treatment induces neuronal death accompanied by apoptotic DNA fragmentation[101,102] as well as chromatin condensation, membrane blebbing, and polyribosome breakdown.[103] Protein synthesis inhibitors very modestly ameliorate this death,[102] suggesting that Aβ-induced apoptosis may involve at least a portion of PND as well. In this model, Aβ neurotoxicity is dependent on Aβ aggregation.[104-106] We and others have documented c-Jun induction according to this paradigm.[104,107] We have found that neurons from c-Jun- deficient mice were resistant to Aβ toxicity.[66] This work was extended by Troy and co-workers, who have reported that the CEP1347 compound strongly inhibits Aβ toxicity.[108] Indeed, the ability of CEP1347 to block Aβ toxicity is much more robust than the neuroprotection afforded by c-Jun deficiency, suggesting that additional substrates may contribute to JNK-mediated toxicity. Whether this reflects tau phosphorylation is unclear.

One example of pathologic neuronal death *in vivo* modulated by JNK has been observed. When mice are treated with kainic acid, they undergo seizures and a

delayed neuronal death in the hippocampus. Yang *et al.* tested the hypothesis that, since JNK3 is selectively expressed in neurons, neurons lacking JNK3 would be protected from this apoptosis-inducing stress.[109] These researchers reported that JNK3-deficient mice require higher concentrations of kainate to induce seizures, relative to mice in nature. Moreover, these investigators correlated JNK3 deficiency with a decrease in kainate-induced AP-1 activity and apoptosis in hippocampal neurons. In contrast, JNK1- and JNK2-deficient mice lacked this protection. Hence, JNK3 activity at least appears important for stress-induced neuronal death *in vivo*.

Finally, we would like to consider the ability of *in vitro* and *in vivo* models in adequately replicating the hallmarks of AD, which include synapse loss, neuronal loss, NFT, SP, and memory deficits. The goal of the researcher is to prevent, diagnose, or treat the disease. The predominant symptomology of the disease is memory loss, which likely reflects primarily synapse loss, and, perhaps secondarily, neuronal loss. Whether NFTs and senile plaques contribute to or reflect synapse loss and neuronal loss is not yet determined. However, models that recapitulate synapse and neuronal loss in an overall milieu similar to that of the AD brain may be helpful. The predominant *in vitro* model of AD is treating neurons with Aβ, which leads to Aβ aggregation reminiscent of Aβ fibrils in AD, asynapse loss, and culminates in neuronal death. This is in contrast to the results obtained from transgenic mice that overexpress APP. These mice often have exceedingly robust Aβ deposits and apparently can develop NFTs if expressing human tau linked to tauopathy.[110] However, these mice in general have very little neuronal death.[111] This difference may reflect the fact that Aβ toxicity is enhanced in the *in vitro* model because of the lack of extracellular matrix and other possible factors that ameliorate direct Aβ toxicity *in vivo*, perhaps by minimizing direct interaction between Aβ and the neuronal surface. The potential significance of this is twofold. First, the lack of neuronal death *in vivo* is suggestive that the Aβ *in vitro* toxicity models do not accurately reflect the this aspect of AD. Second, both models reflect synapse loss, which may be the primary cause of symptoms. In summation, while we must always interpret the data from *in vitro* and *in vivo* models with an openness as to their relevance to AD, the mechanisms underlying neuronal degeneration in *in vitro* and *in vivo* models may provide crucial insight into the pathogenic mechanisms in AD.

Therapeutic approaches by using JNK/c-Jun cascade

Therapeutic approaches for AD can be classified into several categories. The first category is suppression of Aβ synthesis or deposition. Immunization with Aβ belongs to this category. The recent observation that a copper-zinc chelator inhibits Aβ deposition in transgenic mice also belongs to this group.[112] The second category is inhibition of signaling relevant to cell death.

Two recent reports showed the effect of a JNK inhibitor, CEP-1347/KY-7515 on Aβ-induced cell death.[108,113] Troy *et al.* added CEP-1347/KY-7515 to a culture medium and examined the effect on Aβ-induced JNK activation and cell death of PC12 cells. As expected, CEP-1347/KY-7515 inhibited JNK activation by an aggregated form of Aβ and suppressed cell death. These researchers also used primary sympathetic neurons and obtained similar results. Importantly, protection by CEP-1347/KY-7515 requires treatment of cells within two hours of Aβ addition. This suggests that JNK activation acts relatively proximally in death signaling induced by Aβ. Bozyczko-Coyne *et al.* further tested the effect of CEP-1347/KY-7515 on primary cortical neurons. These researchers also found protection against Aβ-induced cell death. In addition, they showed inhibition of cytochrome-c release. Both groups observed inhibition of caspases activated by Aβ. All these observations suggest inhibition of the JNK/c-Jun cascade will be therapeutic against AD.

In addition to JNK inhibitors, Cross *et al.* tested the effect of the GSK-3 inhibitors, SB-216763 and SB-415286, on cell death induced in primary cerebellar granule neurons by potassium withdrawal or LY-294002 treatment.[114] Although their experiments were not directly relevant to AD, these compounds were shown to be effective neuroprotectants. While it is not clear whether tau phosphorylation is a consequence or cause of cell death, these researchers observed inhibition of tau phosphorylation by these compounds.

Conclusion

Both *in vitro* and *in vivo* evidence suggests that the JNK/c-Jun cascade is a critical event in the neuronal death pathway in AD. Whether JNK/c-Jun activation is a disease-specific phenomenon or reflective of a generalized neuronal apoptotic pathway is unclear. JNK/c-Jun activation may be a general hallmark of neurodegeneration. For this question, we have few results from studies on the relationship between PS1 and JNK/c-Jun.[70,71] It is essential to clarify how PS, APP, and Ire1 interact in the endoplasmic reticulum and trigger the JNK/c-Jun cascade. As for AD therapy, inhibitors of this pathway are candidates in either case. Before applying these inhibitors for patients, we need to test such chemicals with animal models *in vivo*. Furthermore, it is absolutely necessary to have complete knowledge of unfavorable effects of inhibition of this general signaling pathway.

Acknowledgments

We thank Dr. Yasuo Ihara, Graduate School of Medicine, University of Tokyo, and Dr. Jonathan Ham, Institute of Child Health, University College London, for critical reading and helpful comments.

References

1. Alzheimer A: Uber eine eigenartige Erkrankung der Hirnrinde. *Zbl Neurol. Psychiat*. 1907; 18: 177-179.
2. Goate A, Chartier-Harlin MC, Mullan M, *et al.*: Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*. 1991; 349: 704-706.
3. Schellenberg GD, Bird TD, Wijsman ED, *et al.*: Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science*. 1992; 258: 668-671.
4. Scherrington R, Rogaev EI, Liang Y, *et al.*: Cloning of a novel gene bearing missense mutations in early onset familial Alzheimer disease. *Nature*. 1995; 375: 754-760.
5. Levy-Lahad E, Wasco W, Poorkaj P, *et al.*: Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*. 1995; 269: 973-977.
6. Strittmatter WJ, Saunders AM, Schmechel D, *et al.*: Apolipoprotein E: High-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Nat Acad Sci USA*. 1993; 90: 1977-1981.
7. Blacker D, Wilcox MA, Laird NM, *et al.*: Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nature Genet*. 1998; 19: 357-360.
8. Kang DE, Saito T, Chen X, *et al.*: Genetic association of the low-density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. *Neurology*. 1997; 49: 56-61.
9. Bertram L, Blacker D, Mullin K, *et al.*: Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science*. 2000; 290: 2302-2303.
10. Ertekin-Taner N, Graff-Radford N, Younkin LH, *et al.*: Linkage of plasma Aβ 42 to a quantitative locus on chromosome 10 in late-onset Alzheimer's disease pedigrees. *Science*. 2000; 290: 2303-2304.
11. Myers A, Holmans P, Marshall H, *et al.*: Susceptibility locus for Alzheimer's disease on chromosome 10. *Science*. 2000; 290: 2304-2305.
12. Xia W, Zhang J, Perez R, *et al.*: Interaction between amyloid precursor protein and presenilins in mammalian cells: Implication for the pathogenesis of Alzheimer's disease. *Proc Nat Acad Sci USA*. 1997; 94: 8208-8213.
13. Waragai M, Imafuku I, Takeuchi S, *et al.*: Presenilin-1 binds to amyloid precursor protein directly. *Biochem Biophys Res Commun*. 1997; 239: 480-482.
14. Weidemann A, Paliga K, Durrwang U, *et al.*: Formation of stable complexes between two Alzheimer's disease products: Presenilin-2 and beta-amyloid precursor protein. *Nature Med*. 1997; 3: 328-332.
15. Iwatsubo T, Okada A, Suzuki N, *et al.*: Visualization of Aβ 42(43) and Aβ 40 in senile plaques with end-specific Aβ monoclonals: Evidence that an initially deposited species is Aβ 42(43). *Neuron*. 1994; 13: 45-53.
16. Selkoe D: Alzheimer's disease: Genes, proteins, and therapy. *Physiol Rev*. 2001; 81: 741-766.
17. Li YM, Lai MT, Xu M, *et al.*: Presenilin-1 is linked with γ-secretase activity in the detergent solublized state. *Proc Nat Acad Sci USA*. 2000; 97; 6138-6143.
18. DeStrooper B, Saftig P, Craessaerts K, *et al.*: Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*. 1998; 391: 387-390.
19. DeStrooper B, Annaert W, Cupers P, *et al.*: A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. *Nature.* 1999; 398: 518-522.
20. Wolfe MS, Xia W, Ostaszewski BL, *et al.*: Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature*. 1999; 398: 513-517.
21. Herreman A, Serneels L, Annaert W, *et al.*: Total inactivation of γ-secretase activity in presenilin-deficient embryonic stem cells. *Nature Cell Biol*. 2000; 2: 461-462.
22. Zhang Z, Nadeau P, Song W, *et al.*: Presenilins are required for γ-secretase cleavage of β-APPP and transmembrane cleavage of Notch-1. *Nature Cell Biol*. 2000; 2: 463-465.
23. Li YM, Xu M, Lai MT, *et al.*: Photoactivated γ-secretase inhibitors directed to the active site covalently label presenilin-1. *Nature*. 2000; 405: 689-694.
24. Wolfe MS: Presenilins and γ-secretase: Structure meets function. *J Neurochem*. 2001; 76: 1615-1620.
25. Yu G, Nishimura M, Arakawa S, *et al.*: Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and APP processing. *Nature*. 2000; 407: 48-54.
26. Small D: The role of presenilins in γ-secretase activity: catalyst or cofactor? *J Neurochem*. 2001; 76: 1612-1614.
27. Cechler F: The multiple paradozes of presenilins. *J Neurochem*. 2001; 76: 1621-1627.
28. Capell A, Steiner H, Romig H, *et al.*: Presenilin-1 differentially facilitates endoproteolysis of the β-amyloid precursor protein and Notch. *Nature Cell Biol.* 2000; 2: 205-211.
29. Petit A, Bihel F, daCosta AV, *et al.*: New protease inhibitors prevent γ-secretase-mediated production of Aβ 40/42 without affecting Notch cleavage. *Nature Cell Biol*. 2001; 3: 507-511.
30. VanGassen G, Annaert W, VanBroeckhoven C: Binding partners of Alzheimer's disease proteins: Are they physiologically relevant? *Neurobiol Dis*. 2000; 7: 135-151.
31. Schenk D, Barbour R, Dunn W, *et al*.: Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*. 1999; 400: 173-177.
32. Janus C, Pearson J, McLaurin J, *et al.*: Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature*. 2000; 408: 979-982.
33. Morgan D, Diamond DM, Gottschall PE, *et al*.: Aβ vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature. 2000; 408: 982-985.
34. Bard F, Cannon C, Barbour R, *et al.*: Peripherally administered antibodies against amyloidβ- peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nature Med*. 2000; 6: 916-919.
35. Hardy J, Allsop D: Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci*. 1991; 12: 383-388.
36. Hardy JA, Higgins GA: Alzheimer's disease: The amyloid cascade hypothesis. *Science*. 1992; 256: 184-185.
37. Selkoe DJ: Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci*. 1997; 20: 154-159.
38. Ishihara T, Hong M, Zhang B, *et al.*: Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron*. 1999; 24: 751-762.
39. Lee VM, Goedert M, Trojanowski JQ: Neurodegenerative tauopathies. *Annu Rev Neurosci*. 2001; 24: 1121-1159.
40. Hardy T, Duff K, Hardy J, *et al.*: Genetic dissection of Alzheimer's disease and related dementias: Amyloid and its relationship to tau. *Nature Neurosci*. 1998; 1: 355-358.
41. Ihara Y: PHF and PHF-like fibrils—Cause or consequence? *Neurobiol Aging*. 2001; 22: 123-126.
42. Wittmann CW, Wszolek MF, Shulman JM, *et al.* Tauopathy in drosophila: Neurodegeneration without neurofibrillary tangles. *Science*. 2001; 293: 711-714.

43. Klein WL, Krafft GA, Finch CE, *et al.*: Targeting small Aβ oligomers: The solution to an Alzheimer's conundrum? *Trends Neurosci*. 2001; 24: 219-224.
44. Gouras GK, Tsai J, Naslund J, *et al.*: Intraneural Aβ 42 accumulation in human brain. *Am J Pathol*. 2000; 156: 15-20.
45. Walsh DM, Tseng BP, Rydel RE, *et al.*: The oligomerization of amyloidβ-protein begins intracellularly in cells derived from human brain. *Biochemistry*. 2000; 39: 10831-10839.
46. Mochizuki A, Tamaoka A, Shimohata Y, *et al.*: Aβ 42-positive non-pyramidal neurons around amyloid plaques in Alzheimer's disease. *Lancet*. 2000; 355: 42-43.
47. Shoji M, Iwakami N, Takeuchi S, *et al.*: JNK activation is associated with intracellular β-amyloid accumulation. *Mol Brain Res*. 2001; 85: 221-223.
48. Papolla MA, Omar RA, Kim KS, *et al.*: Immunological evidence of oxidative stress in Alzheimer's disease. *Am J Pathol.* 1992; 140: 621-628.
49. Smith MA, Taneda S, Richey PL, *et al.*: Advanced Maillard reaction products are associated with Alzheimer disease pathology. *Proc Nat Acad Sci USA*. 1994; 91: 5710-5714.
50. Vitek MP, Bhattacharya K, Glendening JM, *et al.*: Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proc Nat Acad Sci USA*. 1994; 91: 4766-4770.
51. Behl C, Davis JB, Lesley R, *et al.*: Hydrogen peroxide mediates amyloidβ protein toxicity. *Cell*. 1994; 77: 817-827.
52. Peterson C, Goldman JE: Alterations in calcium content and biochemical processes in cultured skin fibroblasts from aged and Alzheimer donors. *Proc Nat Acad Sci USA*. 1986; 83: 2758-2762.
53. Mattson MP, Cheng B, Davis D, *et al.*: β-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J Neurosci*. 1992; 12: 379-389.
54. Guo Q, Fu W, Sopher BL, *et al.*: Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. *Nature Med*. 1999; 5: 101-106.
55. Kobayashi S, Ishiguro K, Omori A, *et al.*: A cdc2-related kinase PSSALRE/cdk5 is homologous with the 30kDa subunit of tau protein kinase II, a proline-directed protein kinase associated with microtuble. *FEBS Lett.* 1993; 335: 171-175.
56. Baumann K, Mandelkow EM, Biernat J, *et al.*: Abnormal Alzheimer-like phosphorylation of tau-protein by cyclin-dependent kinases cdk2 and cdk5. *FEBS Lett*. 1993; 336: 417-424.
57. Uchida T, Ishiguro K, Ohnuma J, *et al.*: Precursor of cdk5 activator, the 23 kDa subunit of tau protein kinase II: Its sequence and developmental change in brain. *FEBS Lett*. 1994; 355: 35-40.
58. Patrick GN, Zukerberg L, Nikolic M, *et al*.: Conversion of p35 to p24 deregulates Cdk5 activity and promotes neurodegeneration. *Nature*. 1999; 402: 615-622.
59. Hanger DP, Hughes K, Woodgett JR, *et al.*: Glycogen synthetase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localization of the kinase. *Neurosci Lett*. 1992; 147: 58-62.
60. Yang SD, Song JS, Yu JS, *et al.*: Protein kinase FA/GSK-3 phosphorylates tau on Ser 235-Pro and Ser 404-Pro that are abnormally phosphorylated in Alzheimer's disease brain. *J Neurochem*. 1993; 61: 1742-1747.
61. Takashima A, Murayama M, Murayama O, *et al.*: Presenilin 1 associates with glycogen synthetase kinase 3beta and its substrate tau. *Proc Nat Acad Sci USA*. 1998; 96: 9637-9641.
62. Goedert M, Hasegawa M, Jakes R, *et al.*: Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. *FEBS Lett.* 1997; 409: 57-62.
63. Reynolds CH, Utton MA, Gibb GM, *et al.*: Stress-activated protein kinase/c-Jun N-terminal kinase phosphorylates tau protein. *J Neurochem*. 1997; 68: 1736-1744.
64. Goedert M, Hasegawa M, Jakes R, *et al.*: Phosphorylation of microtuble-associated protein tau by stress-activated protein kinases. *FEBS Lett*. 1997; 409: 57-62.
65. Zhu X, Raina AK, Rottkamp CA, *et al.*: Activation and redistribution of c-Jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease. *J Neurochem*. 2001; 76: 435-441.
66. Kihiko ME, Tucker HM, Rydel RE, *et al.*: c-Jun contributes to amyloidβ-induced neuronal apoptosis but is not necessary for amyloidβ-induced c-jun induction. *J Neurochem*. 1999; 73: 2609-2612.
67. Anderson AJ, Cumming BJ, Cotman CW: Increased immunoreactivity for Jun- and Fos-related proteins in Alzheimer's disease: Association with pathology. *Exp Neurol*. 1994; 126: 286-295.
68. MacGibbon GA, Lawlor PA, Walton M, *et al.*: Expression of Fos, Jun, and Krox family proteins in Alzheimer's disease. *Exp Neurol*. 1995; 147: 316-332.
69. Marcus DL, Strafaci JA, Miller DC, *et al.*: Quantitative neuronal c-fos and c-jun expression in Alzheimer's disease. *Neurobiol Aging*. 1998; 5: 393-400.
70. Imafuku I, Masaki T, Waragai M, *et al.*: Presenilin-1 suppresses the function of c-jun homodimers via intgeraction with QM/Jif-1. *J Cell Biol.* 1999; 147: 121-133.
71. Kim JW, Chang T-S, Lee JE, *et al.*: Negative regulation of SAPK/JNK signaling pathway by presenilin 1. *J Cell Biol*. 2001; 153: 457-463.
72. Katayama T, Imaizumi K, Sato N, *et al.*: Presenilin-1 mutations down-regulate the signalling pathway of the unfolded-protein response. *Nature Cell Biol*. 1999; 8: 479-485.
73. Niwa M, Sidrauski C, Kaufman RJ, *et al.*: A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response. *Cell*. 1999; 99: 691-702.
74. Sato N, Urano F, Yoon Leem J, *et al.*: Up-regulation of BiP and CHOP by the unfolded protein response is independent of presenilin expression. *Nature Cell Biol*. 2000; 12: 863-870.
75. Imaizumi K, Katayama T, Tohyama M: Presenilin and the UPR. *Nature Cell Biol*. 2001; 3: E104.
76. Urano F, Wang X, Bertolotti A, *et al.*: Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRS1. *Science*. 2000; 287: 664-666.
77. Kovacs DM, Wasco W, Witherby J, *et al.*: The upstream stimulatory factor functionally interacts with the Alzheimer amyloidβ-protein precursor gene. *Hum Mol Genet*. 1995; 4: 1527-1533.
78. Oppenheim RW: Cell death during development of the nervous system. *Ann Rev Neurosci*. 1991; 14: 453-501.
79. Gorin PD, Johnson EM, Jr.: Effects of long-term nerve growth factor deprivation on the nervous system of the adult rat: an experimental approach. *Brain Res*. 1980; 198: 27-42.
80. Deckwerth TL, Johnson EM, Jr.: Temporal analysis of events associated with programmed cell death (apoptosis) of sympathetic neurons deprived of nerve growth factor (NGF). *J Cell Biol*. 1993; 123: 1207-1222.
81. Edwards SN, Tolkovsky AM: Characterization of apoptosis in cultured rat sympathetic neurons after nerve growth factor withdrawal. *J Cell Biol*. 1994; 124: 537-546.
82. Martin DP, Schmidt RE, Distefano PS, *et al.*: Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. *J Cell Biol*. 1988; 106: 829-844.
83. Greenlund LJS, Deckwerth TL, Johnson EM, Jr.: Superoxide dismutase delays neuronal apoptosis: a role for reactive oxygen species in programmed neuronal death of protects sympathetic neurons from NGF deprivation induced apoptosis. *Neuron*. 1995; 14: 303-315.
84. Jordan J, Ghadge GD, Prehn JH, *et al.*: Expression of human copper/zinc-superoxide dismutase inhibits the death of rat sympathetic

neurons caused by withdrawal of nerve growth factor. *Mol Pharmacol*. 1995; 47: 1095-1100.
85. Estus S, Zaks W, Freeman R, *et al.*: Altered gene expression in neurons during programmed cell death: Identification of c-Jun as necessary for neuronal apoptosis. *J Cell Biol*. 1994; 127: 1717-1727.
86. Ham J, Babij C, Whitfield J, *et al.*: A c-Jun dominant negative mutant protects sympathetic neurons against programmed cell death. *Neuron*. 1995; 14: 927-939.
87. Deshmukh D, Vasilakos J, Deckwerth TL, *et al.*: Genetic and metabolic status of NGF-deprived sympathetic neurons saved by an inhibitor of ICE-family protease. *J Cell Biol*. 1997; 135: 1341-1354.
88. Deshmukh M, Kuida K, Johnson EM, Jr.: Caspase inhibition extends the commitment to neuronal death beyond cytochrome c release to the point of mitochondrial depolarization. *J Cell Biol*. 2000; 150: 131-143.
89. Putcha GV, Deshmukh M, Johnson EM, Jr.: Inhibition of apoptotic signaling cascades causes loss of trophic factor dependence during neuronal maturation. *J Cell Biol*. 2000; 149: 1011-1018.
90. Eilers A., Whitfield J, Babij C, *et al.*: Role of the Jun kinase pathway in the regulation of c-Jun expression and apoptosis in sympathetic neurons. *J Neurosci*. 1998; 18: 1713-1724.
91. Eilers A., Whitfield J, Shah B, *et al.*: Direct inhibition of c-Jun N-terminal kinase in sympathetic neurones prevents c-Jun promoter activation and NGF withdrawal-induced death. *J Neurochem*. 2001; 76: 1439-1454.
92. Tammariello SP, Landreth GE, Estus S: The role of Jun-kinases in apoptosis. In Mattson MP, Estus S, Rangnekar V (eds.): *Programmed Cell Death: Cellular and Molecular Mechanisms.* New York: Humana Press, 2001: 197-214.
93. Xia Z, Dickens M, Raingeaud J, *et al.*: Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science*. 1995; 270: 1326-1331.
94. Maroney AC, Finn JP, Bozyczko-Coyne D, *et al.*: CEP-1347 (KT7515), an inhibitor of JNK activation, rescues sympathetic neurons and neuronally differentiated PC12 cells from death evoked by three distinct insults. *J Neurochem*. 1999; 73: 1901-1912.
95. Bruckner SR, Tammariello SP, Kuan CY, *et al.*: JNK3 contributes to c-Jun activation and apoptosis but not oxidative stress in nerve growth factor-deprived sympathetic neurons. *J Neurochem*. 2001; 78: 298-303.
96. Behrens A, Sibilia M, Wagner EF: Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. *Nature Genet.* 1999; 21: 326-329.
97. Putcha GV, Moulder KL, Golden JP, *et al.*: Induction of BIM, a proapoptotic BH3-only Bcl-2 family member, is critical for neuronal apoptosis. *Neuron*. 2001; 29: 615-628.
98. Whitfield J, Neame SJ, Paquet L, *et al.*: Dominant-negative c-Jun promotes neuronal survival by reducing BIM expression and inhibiting mitochondrial cytochrome c release. *Neuron*. 2001; 29: 629-643.
99. Harris CA, Johnson EM, Jr.: BH3-only Bcl-2 family members are coordinately regulated by the JNK pathway and require Bax to induce apoptosis in neurons. *J Biol Chem*. 2001; 8 [published on line].
100. Deshmukh M and Johnson EM, Jr.: Evidence of a novel event during neuronal death: Development of competence-to-die in response to cytoplasmic cytochrome C. *Neuron*. 1998; 21: 695-705.
101. Forloni G, Chiesa R, Smiroldo S, *et al.*: Apoptosis mediated neurotoxicity induced by chronic application of β-amyloid fragment 25-35. *Neuroreport*. 1993; 4: 523-526.
102. Loo DT, Copani A, Pike CJ, *et al.*: Apoptosis is induced by Aβ-amyloid in cultured central nervous system neurons. *Proc Nat Acad Sci USA*. 1993; 90: 7951-7955.
103. Watt JA, Pike CJ, Walencewicz-Wasserman AJ, *et al.*: Ultrastructural analysis of beta-amyloid-induced apoptosis in cultured hippocampal neurons. *Brain Res*. 1994; 661: 147-156.
104. Estus S, Tucker HM, Van Rooyen C, *et al.*: Aggregated amyloid-β protein induces cortical neuronal apoptosis and concomitant apoptotic pattern of gene induction. *J Neurosci*. 1997; 17: 7736-7745.
105. Pike C, Walencewicz A, Glabe C, *et al.*: *In vitro* aging of β-amyloid protein causes peptide aggregation and neurotoxicity. *Brain Res*. 1991; 563: 311-314.
106. Simmons LK, May PC, Tomaselli KJ, *et al.*: Secondary structure of amyloid beta peptide correlates with neurotoxic activity *in vitro*. *Mol Pharmacol*. 1994; 45: 373-379.
107. Anderson AJ, Pike CJ, Cotman CW: Differential induction of immediate early gene proteins in cultured neurons by β-amyloid (Aβ): Association of c-Jun with Aβ induced apoptosis. *J Neurochem*. 1995; 65: 1487-1498.
108. Troy CM, Rabacchi SA, Xu Z, *et al.*: β-amyloid-induced neuronal apoptosis requires c-Jun N-terminal kinase activation. *J Neurochem*. 2001; 77: 157-64.
109. Yang DD, Kuan CY, Whitmarsh AJ, *et al.*: Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature*. 1997; 389: 865-870.
110. Lewis J, Dickson DW, Lin WL, *et al.*: Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science*. 2001; 293: 1487-91.
111. Irizarry MC, McNamara M, Fedorchak K, *et al.*: APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol*. 1997; 56: 965-973.
112. Cherny RA, Atwood CS, Xilinas ME, *et al.*: Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron*. 2001; 30: 665-676.
113. Bozyczko-Coyne D, O'Kane TM, Wu Z-L, *et al.*: CEP-1347/KT-7515, an inhibitor of SAPK/JNK pathway activation, promotes survival and blocks multiple events associated with Aβ-induced cortical neuron apoptosis. *J Neurochem*. 2001; 77: 849-863.
114. Cross DAE, Culbert AA, Chalmers KA, *et al.*: Selective small-molecule inhibitors of glycogen synthetase kinase-3 activity protect neurones from death. *J Neurochem*. 2001; 77: 94-102.