Intracellular Amyloid β-Protein and Its Associated Molecules in the Pathogenesis of Alzheimer's Disease

Yasumasa Ohyagi[1,*] and Takeshi Tabira[2,*]

[1]*Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan;* [2]*National Institute for Longevity Sciences, NCGG, Aichi 474-8511, Japan*

Abstract: Amyloid β-protein (Aβ) plays a pivotal role in Alzheimer's disease (AD). Therapeutic strategies inhibiting Aβ aggregation and promoting extracellular Aβ removal are currently advocated. Here, we review recent literature on intracellular Aβ, especially intranuclear Aβ, and its associated molecules. We also discuss alternative therapeutic strategies to inhibit intracellular Aβ-related pathogenesis.

Key Words: Alzheimer's disease, amyloid β-protein, intracellular, intranuclear, Aβ-related Death Inducing Protein, p53, promoter, apoptosis.

GENERAL BACKGROUND

Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with memory loss and various impairments of cognitive function, and is the most common form of dementia in elderly people. At present, many clinical trials of treatment for AD patients are under study, but sufficient therapies for symptomatic cognitive dysfunction and fundamental disease progression are not yet established [1]. Here, we propose a new therapeutic strategy for AD based on our recent findings. Pathologically, there are two major hallmarks in AD, neurofibrillary tangles (NFTs) and senile plaques (SPs). NFTs are composed of hyperphosphorylated tau, and SPs are composed of amyloid β-protein (Aβ) deposits that are associated with glial and neuritic responses. Numerous reports suggest that Aβ aggregation is an upstream event in the pathological mechanism of AD [2]. Aβ ending at 42 (Aβ42) is the major form of Aβ found in deposits [3]. Aβ is normally generated from cleavage of amyloid precursor protein (APP) by β- and γ-secretases in the endoplasmic reticulum (ER) and trans-Golgi network [4,5]. Abnormal increases in extracellular water-soluble Aβ42 [6] and oligomeric Aβ42 [7] are thought to cause neurodegeneration through multiple pathways including apoptosis [8]. These facts have been supported by i) an increase in Aβ42 production by early onset familial AD (FAD)-related mutations in presenilin (PS) 1, PS2 and APP genes [9-12]; ii) the fact that Aβ-degrading enzyme gene polymorphisms, such as those within the gene encoding insulin degrading enzyme (IDE), may account for AD pathology [13]; and iii) therapeutic effects of anti-Aβ antibodies on AD [14,15]. Therefore, clearance of extracellular Aβ deposits and inhibition of extracellular Aβ neurotoxicity are considered to be major treatment strategies for AD [16,17]. Degradation of extracellular Aβ by some endopeptidases, such as IDE, neprilysin (NEP), plasmin, and tissue plasminogen activator (tPA), should thus be enhanced. Fibrillization inhibitors and Aβ immunization are also being trialed as possible AD therapies [1]. However, a recent trial of Aβ immunization, which was interrupted because of meningoencephalitis, has shown only slight efficacy in slowing AD progression [18]. It is therefore conceivable that neuronal degeneration processes in AD are more complicated and an alternative Aβ42-related pathogenesis exists. Ever since we reported an apparent linkage between intracellular Aβ42 and apoptosis in the brains of transgenic AD mice [19] and AD patients [20,21], we have been focusing on intracellular Aβ42. Intracellular Aβ42 has also been suggested to activate intrinsic apoptotic pathways through mitochondrial damage [22] and ER stress [23]. However, we recently reported a neurodegeneration pathway occurring through activation of the p53 promoter by intranuclear Aβ42 [24]. We also recently identified a novel intracellular Aβ-chaperone protein, the Aβ-related death inducing protein (AB-DIP) [25]. In the present article, we focus on our knowledge of the biological significance of intracellular Aβ42 and its chaperone proteins, and therapeutic strategies to inhibit apoptosis-inducing pathogenic pathways in AD.

ABNORMAL Aβ42 LOCALIZATION IN THE CYTOSOL AND THE NUCLEUS OF NEURONS

As Aβ is normally generated in ER/Golgi, and then rapidly secreted to the extracellular space, positive immunostaining with anti-Aβ antibodies is not normally found in neurons. However, it was recently noted that anti-Aβ42 end-specific antibodies stain lots of neurons in AD [20,26,27] and Down syndrome (DS) brains [28,29]. Further rigorous immunocytochemical studies revealed that intracellular Aβ42 is present in multivesicular bodies, and is associated with synaptic pathology in AD brain [30,31]. Moreover, an improved immunostaining method with autoclave pretreatment revealed Aβ42 accumulation in the cytosol, and even in the nucleus in AD and mutant APP-transgenic mice neurons

*Address correspondence to these authors at the Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; Tel: +81-92-642-5340; Fax: +81-92-642-5352;
E-mail: ohyagi@neuro.med.kyushu-u.ac.jp;

National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, 36-3 Gengo, Morioka-cho, Obu City, Aichi 474-8511, Japan; Tel: +81-562-46-2311; Fax: +81-562-48-2373; E-mail: tabira@nils.go.jp

1389-5575/06 $50.00+.00

[24]. However, the kinetics of intraneuronal Aβ42 in AD brain is not fully understood.

Recently, Bückig *et al*. [32] showed that Aβ42 overproduced in ER was exported to the cytosol, where it formed aggresome-like structures in cultured cells. Since Aβ42 is selectively overproduced by various FAD-related gene mutations [2], increased production of Aβ42 is an important pathogenic factor not only for fostering formation of extracellular Aβ oligomers but also for promoting cytosolic Aβ42 accumulation. As there have been no reports demonstrating Aβ fibrils in AD neurons by immuno-electron microscopy, it is likely that Aβ42 accumulating in the cytosol forms soluble monomeric or oligomeric structures. We previously reported that apoptosis-inducing agents such as oxidative stress-inducing H_2O_2 caused a selective increase in the levels of Aβ42 in primary cultured neurons [33]. Furthermore, we demonstrated the sequential re-localization of Aβ42 from the cytosol to the nucleus in primary neurons following H_2O_2 treatment [24]. However, the nucleus is an unusual compartment for Aβ42 to reside, and mechanisms of Aβ42 transport from the cytosol to the nucleus are still unclear. AB-DIP interacts with Aβ, is involved in apoptosis, and has a nuclear targeting sequence [25] (Fig. (**1**)). Aβ can bind either the N-terminal glutamine-rich region or the C-terminal D1 and D2 conserved regions of AB-DIP (Fig. (**1**)), and the Aβ-AB-DIP complex was shown to clearly mediate intracellular Aβ42 neurotoxicity [25]. Thus, AB-DIP may partly regulate translocation of Aβ42 from the cytosol to the nucleus. These data strongly imply that Aβ42 may fundamentally be an intracellular protein that regulates, at least in part, some signal transduction in the nucleus.

INTRANUCLEAR Aβ42 CAUSES NEURONAL DEATH THROUGH P53 ACTIVATION

What roles does Aβ42 molecule play in the nucleus, and how does it contribute to neuronal death in AD? Although it has been suggested that APP C-terminal intracellular domain (AICD) associated with FE65 and Tip60 may regulate gene transcription [34,35], Aβ itself has not been considered to be a transcription factor. However, Aβ theoretically forms a β-hairpin shape followed by a helix-turn-helix (HTH) motif [36], an essential motif in the DNA-binding domain of heat shock transcription factors (HSF) [37]. The p53 promoter is thought to be one of the targets of nuclear Aβ42, because it may be linked to the induction of neuronal apoptosis in AD and DS [38,39], and the p53 promoter contains heat shock elements (HSE) [40]. Moreover, Zhang *et al*. [41] reported that p53 was an important mediator of intracellular Aβ42 neurotoxicity. Based on these observations, we investigated interactions between Aβ42 and the p53 promoter, and found that Aβ42 directly bound the p53 promoter, and activated it *in vitro* [24] (Fig. (**2**)). In addition, we reported that binding of Aβ42 to AB-DIP was crucial for the induction of intracellular Aβ42-related apoptosis [25]. Accordingly, we suggest a

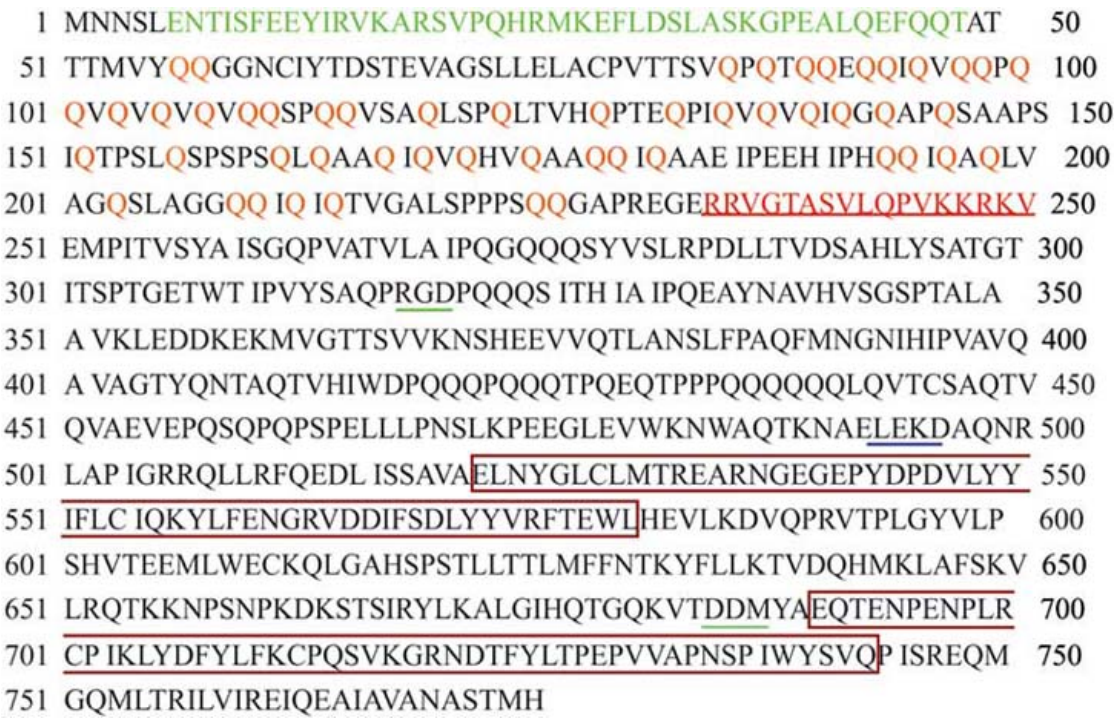

Fig. (1). Amino acid sequence and domain architecture of AB-DIP protein [24].

The Caspase Activation and Recruitment Domain (CARD, amino acids 6-48) is highlighted in green. There is a caspase cleavage site (LEKD) underlined in blue. Glutamine residues in the glutamine rich region (GRR) are highlighted in orange. The bipartite nuclear targeting sequence (234-250) is highlighted in red, and underlined. There are two unique motifs, a cell attachment sequence (RGD) and a potential RGD binding motif (DDM), both of which are underlined in green. The two conserved domains, D1 (523-581) and D2 (690-743) at the carboxy terminal end are boxed within a red line. Note that Aβ binds either GRR or D1/D2 domains.

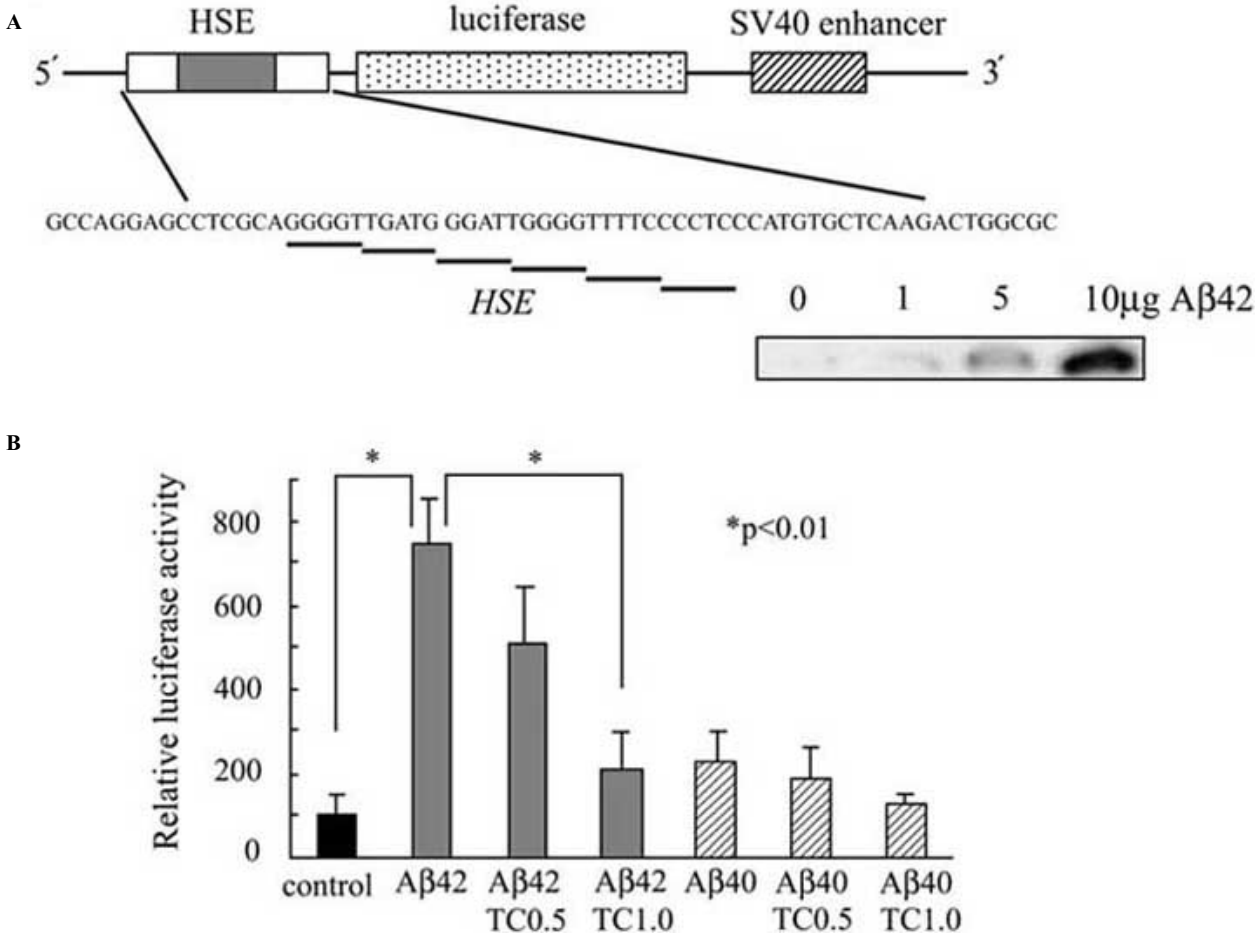

Fig. (2). Luciferase assays and gel-mobility shift assays of Aβ42 and the p53 promoter [23].

(A) Construct for luciferase assay. The p53 promoter contains heat shock elements (HSE). Gel-mobility shift assay (right panel) showing dose-dependent binding of Aβ42 to p53 promoter oligonucleotides. (B) Luciferase assay showing significant promoter activation by cytosolic Aβ42 expression. Cytosolic Aβ40 has a lesser effect. Addition of tetracycline (TC, 1.0 μg/ml) to inhibit Aβ expression counteracts p53 promoter activation.

novel pathway for neuronal apoptosis related to intranuclear Aβ42 in AD. As shown in Fig. (**3**), cumulative age-associated oxidative stress or Aβ42 overproduction due to genetic or environmental backgrounds (e.g., homocysteic acid [42]) induces cytosolic Aβ42 accumulation. Although aberrant accumulation of Aβ42 in the cytosol may independently induce mitochondrial or synaptic damage (see the following section), the putatively active form of AB-DIP, p62, may promote translocation of Aβ42 to the nucleus resulting in overexpression of p53 mRNA and neuronal apoptosis in AD brain. Supporting our hypothesis, prominent accumulations of nuclear Aβ42 and p53 were observed in degenerating neurons, which were apparently TUNEL positive, in AD and mutant APP-transgenic mouse brains [24]. Thus, nuclear translocation of Aβ42 accompanied by AB-DIP may be one of the critical steps in Aβ42-related p53-dependent apoptosis. Indeed, AB-DIP depletion by siRNA, which may inhibit Aβ42 translocation to the nucleus, clearly counteracted intracellular as well as extracellular Aβ42-related apoptosis [25].

Recently, Esposito *et al*. [43] have shown that i) FAD-related mutant APP, which generates more Aβ42, counteracts the anti-apoptotic function of wild type APP; ii) intracellular Aβ42 primes proapototic pathways including the p53-dependent pathway; but iii) extracellular Aβ42 does not show such effects. Thus, it is possible that Aβ42 accumulating in the cytosol exerts effects on multiple proapoptotic pathways besides just direct activation of p53 mRNA expression.

OTHER MOLECULES RELATED TO INTRACELLULAR Aβ42 PATHOGENESIS

Other important targets of intracellular Aβ neurotoxicity in AD are the mitochondria [22], ER [23] and synapses [30,44]. Mitochondria are essential for energy production (i.e., ATP) through oxidative phosphorylation, and are also involved in the regulation of intracellular Ca^{2+} homeostasis. In addition, mitochondria play key roles in controlling apoptosis. Mitochondrial dysfunction results in overproduction of reactive oxygen species (ROS) and release of cytochrome *c*,

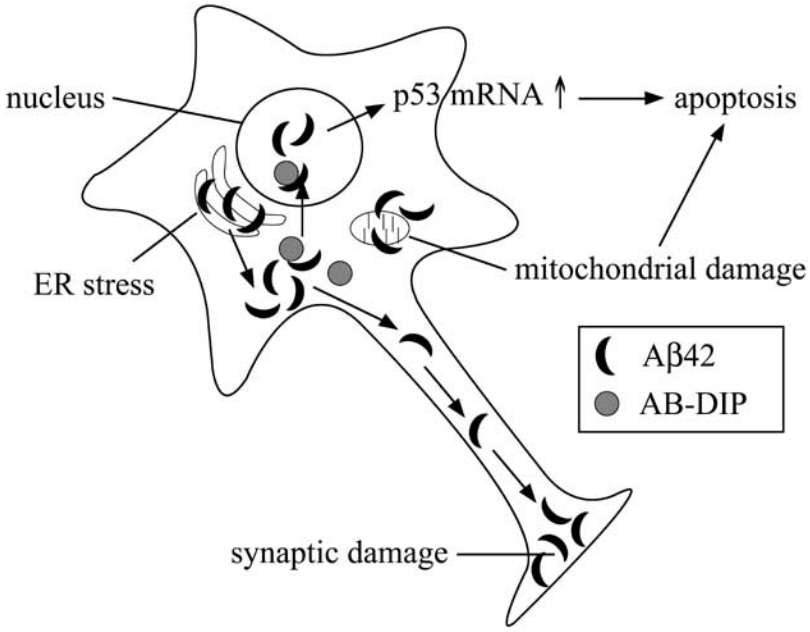

Fig. (3). Scheme of intraneuronal pathogenesis of Aβ42.

Overproduction of Aβ42 or aberrant folding of Aβ42 induces ER stress, and transfer of Aβ42 into the cytosol. Aβ42 accumulating in the cytosol damages mitochondria interacting with ABAD, and induces apoptosis. Simultaneously, some of the accumulated Aβ42 damages the synapse, or is transferred to the nucleus by AB-DIP inducing p53 mRNA overexpression and apoptosis. Reduction of cytosolic Aβ42 levels may thus protect neurons from severe apoptosis in AD.

which binds Apaf-1 and in turn activates caspase-9 [45]. Thus, cytosolic Aβ42 may directly damage mitochondria. Interestingly, more recent reports suggest that Aβ interacts with the Aβ-binding alcohol dehydrogenase (ABAD) in mitochondria [46], and that this interaction induces ROS generation, impairment of mitochondrial membrane potential, a decrease in ATP, and release of cytochrome *c* resulting in caspase-3 activation and apoptosis [47]. Since AB-DIP contains a caspase-9 cleavage site [25], apoptosis mediated by AB-DIP may also be linked to mitochondrial dysfunction.

The ER regulates not only protein synthesis, folding, and trafficking but also cellular responses to stress and intracellular Ca^{2+} levels. Disruptions of Ca^{2+} homeostasis, protein glycosylation or gene mutations cause accumulation of unfolded proteins in the ER, resulting in ER stress [48]. ER stress activates the unfolded protein response (UPR) pathway, which induces ER-localized chaperones and promotes protein degradation. When ER stress is prolonged, caspase-4 in humans and caspase-12 in mice are activated, leading to apoptosis. ER stress-mediated apoptosis might play an important role in AD pathogenesis; FAD-linked PS1 gene mutations [49] and aberrantly spliced isoforms of PS2 [50] may affect the ER stress response, enhancing neuronal vulnerability to ER stress-related apoptosis. On the other hand, accumulation of Aβ42 in the ER might directly promote ER stress responses and apoptosis. An ER stress-inducible membrane protein Herp binds PS and enhances PS-mediated Aβ generation [51], indicating the importance of ER stress in exacerbating the vicious cycle of intracellular Aβ pathogenesis. Extracellular Aβ42 also activates caspase-12 [52] and caspase-4 [53], in mouse and human respectively, indicating ER stress. Therefore, the ER is a major target of both intracellular and extracellular Aβ neurotoxicity.

In addition, the synapse is an interesting site related to intracellular Aβ pathogenesis. Findings that Aβ42 accumulates in synapses in AD brains [30,31] and in triple transgenic mouse (3x-Tg) [54] brains, might indicate a pathological role for Aβ at this stage, but molecular mechanisms of synaptic damage remain to be elucidated. As extracellular Aβ oligomers also induce synaptic dysfunction [55] through affecting calcium homeostasis [56], synapses may be damaged by both intracellular and extracellular Aβ.

THERAPEUTIC STRATEGIES TO INHIBIT PATHOLOGICAL MECHANISMS MEDIATED BY INTRACELLULAR Aβ42 AND P53-DEPENDENT NEURONAL APOPTOSIS

Altogether, evidence suggests that intracellular Aβ42 may affect four major targets −the ER, mitochondria, the synapse, and the nucleus− to cause neuronal damage/apoptosis (Fig. (**3**)). Based on the theory of intracellular Aβ42-related pathogenesis, appropriate therapeutic strategies are i) inhibition of Aβ production, ii) inhibition of cytosolic Aβ42 accumulation, iii) inhibition of AB-DIP function to prevent Aβ42 translocation to the nucleus, and iv) inhibition of p53 apoptotic function. First, inhibitors of β- or γ-secretases to attenuate Aβ generation are currently under development [1,2]. Second, lowering cytosolic Aβ42 levels may be the preferred strategy. For this, two manipulations are rational, i.e., inhibition of Aβ42 accumulation and enhancement of Aβ42 degradation. Since Aβ42 accumulation may be triggered by age-related oxidative stress, some anti-oxidant drugs may fundamentally be useful. To date, little attention has been devoted to drugs enhancing intracellular Aβ42 degradation. Interestingly, recent reports showed that i) 3x-Tg mice revealed remarkable AD-specific pathology including early

intraneuronal Aβ accumulation [54]; ii) anti-Aβ antibody therapy inhibited even intraneuronal accumulation [57]; and iii) such immunotherapy rescued early cognitive deficits [58]. Thus, anti-Aβ immunotherapies may be effective in part, but their mechanisms of action remain obscure. On the other hand, as cytosolic Aβ42 can be degraded by the proteasome [59], which may be affected in AD [60,61], drugs enhancing proteasome activity could also be used as therapeutic candidates. More recently, it has also been reported that IDE degrades cytosolic Aβ42 [62], indicating that drugs that activate IDE might also be useful. We are currently investigating drugs that accelerate cytosolic Aβ42 degradation systems, and these could represent additional potential therapeutic candidates. Such novel drugs may ultimately inhibit mitochondrial and synaptic damage as well as p53-dependent neuronal apoptosis. The third strategy is to inhibit AB-DIP function, so that nuclear translocation of Aβ42 and induction of intranuclear Aβ42-related apoptosis can be attenuated. We are screening drugs that inhibit AB-DIP-related apoptosis. As caspase-9 cleaves AB-DIP to produce the active form, inhibition of caspase-9 might inhibit AB-DIP function. Finally, inhibition of p53 function could be the fourth strategy. Some inhibitors of p53 actually suppress production of Bax, a p53 target protein and were shown to protect dopaminergic neurons from MPTP-induced apoptosis in mice [63]. Such drugs may also inhibit intracellular Aβ42-related p53-dependent neurodegeneration in AD.

At present, the majority of the Alzheimer researchers are developing anti-Aβ treatments to inhibit Aβ aggregation, neurotoxicity, and deposition in the extracellular space. Although inhibition of Aβ generation, using treatments such as β-/γ-secretase inhibitors and anti-Aβ immunotherapy, may attenuate intracellular Aβ accumulation, more specific and safer anti-intracellular Aβ treatments should also be developed. Indeed, serious adverse effects such as meningoencephalitis by Aβ vaccination should be prevented [64]. We are also developing a novel oral vaccine using an adeno-associated virus vector and Aβ cDNA to reduce the risk of encephalitis [65].

CONCLUSION

Many recent reports suggest a variety of pathogenic mechanisms for intracellular Aβ42, but the central pathogenesis in AD is still under debate. However, it is plausible that lowering intraneuronal Aβ42 accumulation may be beneficial by inhibiting neuronal damage and apoptosis. Recently, we have demonstrated a novel neurodegeneration pathway occurring through nuclear translocation of Aβ42 leading to p53-mediated apoptosis, and involving the putative nuclear targeting chaperone protein, AB-DIP. Drugs that inhibit this novel process might represent an alternative strategy to attenuate intracellular Aβ42-related neurodegeneration in AD.

ABBREVIATIONS

Aβ = Amyloid β-protein

AD = Alzheimer's disease

NFTs = Neurofibrillary tangles

SPs = Senile plaques

Aβ42 = Aβ ending at 42

APP = Amyloid precursor protein

ER = Endoplasmic reticulum

IDE = Insulin degrading enzyme

FAD = Familial AD

PS = Presenilin

AB-DIP = Aβ-related death inducing protein

AICD = APP C-terminal intracellular domain

HTH = Helix-turn-helix

HSF = Heat shock transcription factor

ROS = Reactive oxygen species

ABAD = Aβ-binding alcohol dehydrogenase

UPR = Unfolded protein response

REFERENCES

[1] Jacobsen, J.S.; Reinhart, P.; Pangalos, M.N. *NeuroRx* **2005,** *2*, 612.
[2] Golde, T.E. *Int. Brain Path.* **2005,** *15*, 84.
[3] Younkin, S.G. *Ann. Neurol.* **1995,** *37*, 287.
[4] Evin, G.; Weidemann, A. *Peptides* **2002,** *23*, 1285.
[5] Greenfield, J.P.; Tsai, J.; Gouras, G.K.; Hai, B.; Thinakaran, G.; Checler, F.; Sisodia, S.S.; Greengard, P.; Xu, H. *Proc. Natl. Acad. Sci. USA* **1999,** *96*, 742.
[6] Tabaton, M.; Piccini, A. *Int. J. Exp. Path.* **2005,** *86*, 139.
[7] Walsh, D.M.; Klyubin, I.; Fadeeva, J.V.; Rowan, M.J.; Selkoe, D.J. *Biochem. Soc. Trans.* **2002,** *30*, 552.
[8] Small, D.H.; Mok, S.S.; Bornstein, J.C. *Nat. Rev. Neurosci.* **2001,** *2*, 595.
[9] Suzuki, N.; Cheung, T.T.; Cai, X.D.; Odaka, A.; Otvos, L. Jr.; Eckman, C.; Golde, T.E.; Younkin, S.G. *Science* **1994,** *264*, 1336.
[10] Scheuner, D.; Eckman, C.; Jensen, M.; Song, X.; Citron, M.; Suzuki, N.; Bird, T.D.; Hardy, J.; Hutton, M.; Kukull, W.; Larson, E.; Levy-Lahad, E.; Viitanen, M.; Peskind, E.; Poorkaj, P.; Schellenberg, G.; Tanzi, R.; Wasco, W.; Lannfelt, L.; Selkoe, D.; Younkin, S. *Nat. Med.* **1996,** *2*, 864.
[11] Borchelt, D.R.; Thinakaran, G.; Eckman, C.B.; Lee, M.K.; Davenport, F.; Ratovitsky, T.; Prada, C-M.; Kim, G.; Seekins, S.; Yager, D.; Slunt, H.H.; Wang, R.; Seeger, M.; Levey, A.I.; Gandy, S.E.; Copeland, N.G.; Jenkins, N.A.; Price, D.L.; Younkin, S.G.; Sisodia, S.S. *Neuron* **1996,** *17*, 1005.
[12] Citron, M.; Westaway, D.; Xia, W.; Carlson, G.; Diehl, T.; Levesque, G.; Johnson-Wood, K.; Lee, M.; Seubert, P.; Davis, A.; Kholodenko, D.; Motter, R.; Sherrington, R.; Perry, B.; Yao, H.; Strome, R.; Lieberburg, I.; Rommens, J.; Kim, S.; Schenk, D.; Fraser, P.; St George-Hyslop, P.; Selkoe, D.J. *Nat. Med.* **1997,** *3*, 67.
[13] Blomqvist, M.E.; Chalmers, K.; Andreasen, N.; Bogdanovic, N.; Wilcock, G.K.; Cairns, N.J.; Feuk, L.; Brookes, A.J.; Love, S.; Blennow, K.; Kehoe, P.G.; Prince, J.A. *Neurobiol. Aging* **2005,** *26*, 795.
[14] Hock, C.; Konietzko, U.; Streffer, J.R.; Tracy, J.; Signorell, A.; Müller-Tillmanns, B.; Lemke, U.; Henke, K.; Moritz, E.; Garcia, E.; Wollmer, M.A.; Umbricht, D.; de Quervain, D.J.; Hofmann, M.; Maddalena, A.; Papassotiropoulos, A.; Nitsch, R.M. *Neuron* **2003,** *38*, 547.
[15] Monsonego, A.; Weiner, H. L. *Science* **2003,** *302*, 834.
[16] Tanzi, R.E.; Moir, R.D.; Wagner, S.L. *Neuron* **2004,** *43*, 605.
[17] Gandy, S. *J. Clin. Invest.* **2005,** *115*, 1121.
[18] Gilman, S.; Koller, M.; Black, R.S.; Jenkins, L.; Griffith, S.G.; Fox, N.C.; Eisner, L.; Kirby, L.; Boada Rovira, M.; Forette, F.; Orgogozo, J.-M. *Neurology* **2005,** *64*, 1553.
[19] Chui, D.H.; Tanahashi, H.; Ozawa, K.; Ikeda, S.; Checler, F.; Ueda, O.; Suzuki, H.; Araki, W.; Inoue, H.; Shirotani, K.; Takahashi, K.; Gallyas, F.; Tabira, T. *Nat. Med.* **1999,** *5*, 560.

[20] Chui, D.H.; Dobo, E.; Makifuchi, T.; Akiyama, H.; Kawakatsu, S.; Petit, A.; Checler, F.; Araki, W.; Takahashi, K.; Tabira, T. *J. Alz. Dis.* **2001,** *3*, 231.
[21] Tabira, T.; Chui, D.H.; Kuroda, S. *Front. Biosci.* **2002,** *7*, a44.
[22] Yan,S.D.; Stern, D.M. *Int. J. Exp. Pathol.* **2005,** *86*, 161.
[23] Borghi, R.; Pellegrini, L.; Lacanà, E.; Diaspro, A.; Pronzato, M.A.; Vitali, A.; Roncarati, R.; Strocchi, P.; Zaccheo, D.; D'Adamio, L.; Tabaton, M. *J. Alz. Dis.* **2002,** *4*, 31.
[24] Ohyagi, Y.; Asahara, H; Chui, D.H.; Tsuruta, Y.; Sakae, N.; Miyoshi, K.; Yamada, T.; Kikuchi, H.; Taniwaki, T.; Murai, H.; Ikezoe, K.; Furuya, H.; Kawarabayashi, T.; Shoji, M.; Checler, F.; Iwaki, T.; Makifuchi, T.; Takeda, K.; Tabira, T. *FASEB J.* **2005,** *19*, 255.
[25] Lakshmana, M.K.; Araki, W.; Tabira, T. *FASEB J.* **2005,** *19*, 1362.
[26] Gouras, G.K.; Tsai, J.; Naslund, J.; Vincent, B.; Edgar, M.; Checler, F.; Greenfield, J.P.; Haroutunian, V.; Buxbaum, J.D.; Xu, H.; Greengard, P.; Relkin, N.R. *Am. J. Pathol.* **2000,** *156*, 15.
[27] D'Andrea, M.R.; Nagele, R.G.; Wang, H.Y.; Peterson, P.A.; Lee, D.H. *Histopathology* **2001,** *38*, 120.
[28] Gyure, K.A.; Durham, R.; Stewart, W.F.; Smialek, J.E.; Troncoso, J.C. *Arch. Pathol. Lab. Med.* **2001,** *125*, 489.
[29] Mori, C.; Spooner, E.T.; Wisniewski, K.E.; Wisniewski, T.M.; Yamaguchi, H.; Saido, T.C.; Tolan, D.R.; Selkoe, D.J.; Lemere, C.A. *Amyloid* **2002,** *9*, 88.
[30] Takahashi, R.H.; Milner, T.A.; Li, F.; Nam, E.E.; Edgar, M.A.; Yamaguchi, H.; Beal, M.F.; Xu, H.; Greengard, P.; Gouras, G.K. *Am. J. Pathol.* **2002,** *161*, 1869.
[31] Takahashi, R.H.; Almedia, C.G.; Kearney, P.F.; Yu, F.; Lin, M.T.; Milner, T.A.; Gouras, G.K. *J. Neurosci.* **2004,** *24*, 3592.
[32] Bückig, A.; Tikkanen, R.; Herzog, V.; Schmits, A. *Histochem. Cell Biol.* **2002,** *118*, 353.
[33] Ohyagi, Y.; Yamada, T.; Nishioka, K.; Clarke, N.J.; Tomlinson, A.J.; Naylor, S.; Nakabeppu, Y.; Kira, J.; Younkin, S.G. *Neuroreport* **2000,** *11*, 167.
[34] Cao, X.; Sudhof, T.C. *Science* **2001,** *293*, 115.
[35] Cao, X.; Sudhof, T.C. *J. Biol. Chem.* **2004,** *279*, 24601.
[36] Durell, S.R.; Guy, H.R.; Arispe, N.; Rojas, E.; Pollard, H.B. *Biophys. J.* **1994,** *67*, 2137.
[37] Wu, C. *Ann. Rev. Cell Dev. Biol.* **1995,** *11*, 441.
[38] de la Monte, S.M.; Sohn, Y.K.; Wands, J.R. *J. Neurol. Sci.* **1997,** *152*, 73.
[39] Seidl, R.; Fang-Kircher, S.; Bidmon, B.; Cairns, N.; Lubec, G. *Neurosci. Lett.* **1999,** *260*, 9.
[40] Sun, X.; Shimizu, H.; Yamamoto, K. *Mol. Cell Biol.* **1995,** *15*, 4489.
[41] Zhang, Y.; McLaughlin, R.; Goodyer, C.; LeBlanc, A. *J. Cell Biol.* **2002,** *156*, 519.
[42] Hasegawa, T.; Ukai, W.; Jo, D.-G.; Xu, X.; Mattson, M.P.; Nakagawa, M.; Araki, W.; Saito, T.; Yamada, T. *J. Neurosci. Res.* **2005,** *80*, 869.
[43] Esposito, L.; Gan, L.; Yu, G.-Q.; Essrich, C.; Mucke, L. *J. Neurochem.* **2004,** *91*, 1260.
[44] Boyd-Kimball, D.; Castegna, A.; Sultana, R.; Fai Poon, H.F.; Petroze, R.; Lynn, B.C.; Klein, J.B.; Butterfield, D.A. *Brain. Res.* **2005,** *1044*, 206.
[45] Chen, M.; Wang, J. *Apoptosis* **2002,** *7*, 313.
[46] Lustbader, J.W.; Cirilli, M.; Lin, C.; Xu, H.W.; Takuma, K.; Wang, N.; Caspersen, C.; Chen, X.; Pollak, S.; Chaney, M.; Trinchese, F.; Liu, S.; Gunn-Moore, F.; Lue, L.-F.; Walker, D.G.; Kuppusamy, P.; Zewier, Z.L.; Arancio, O.; Stern, D.; Yan, S.S.; Wu, H. *Science* **2004,** *304*, 448.
[47] Takuma, K.; Yao, J.; Huang, J.; Xu, H.; Chen, X.; Luddy, J.; Trillat, A.-C.; Stern, D.M.; Arancio, O.; Yan, S.S. *FASEB J.* **2005,** *19*, 597.
[48] Schroder, M.; Kaufman, R.J. *Mutat. Res.* **2005,** *569*, 29.
[49] Katayama, T.; Imaizumi, K.; Sato, N.; Miyoshi, K.; Kudo, T,; Hitomi, J.; Morihara, T.; Yoneda, T.; Gomi, F.; Mori, Y.; Nakano, Y.; Takeda, J.; Tsuda, T.; Itoyama, Y.; Murayama, O.; Takashima, A.; St George-Hyslop, P.; Takeda, M.; Tohyama, M. *Nat. Cell Biol.* **1999,** *1*, 479.
[50] Katayama, T.; Imaizumi, K.; Sato, Manabe, T.; Hitomi, J.; Kudo, T,; Toyama, M. *J. Chem. Neuroanat.* **2004,** *28*, 67.
[51] Sai, X.; Kawamura, Y.; Kokame, K.; Yamaguchi, H.; Shiraishi, H.; Suzuki, R.; Suzuki, T.; Kawaichi, M.; Miyata, T.; Kitamura, T.; De Strooper, B.; Yanagisawa, K.; Komano, H. *J. Biol. Chem.* **2002,** *277*, 12915.
[52] Nakagawa, T.; Zhu, H.; Morishima, N.; Li, E.; Xu, J.; Yankner, B.A.; Yuan, J. *Nature* **2000,** *403*, 98.
[53] Hitomi, J.; Katayama, T.; Eguchi, Y.; Kudo, T.; Taniguchi, M.; Koyama, Y.; Manabe, T.; Yamaguchi, S.; Bando, Y.; Imaizumi, K.; Tsujimoto, Y.; Tohyama, M. *J. Cell Biol.* **2004,** *165*, 347.
[54] Oddo, S.; Caccamo, A.; Shepherd, J.D.; Murphy, M.P.; Golde, T.E.; Kayed, R.; Metherate, R.; Mattson, M.P.; Akbari, Y.; LaFerla, F.M. *Neuron* **2003,** *39*, 409.
[55] Selkoe, D.J. *Science* **2002,** *298*, 789.
[56] Xie, C.W. *Neuromol. Med.* **2004,** *6*, 53.
[57] Oddo, S.; Billings, L.; Kesslak, J.P.; Cribbs, D.H.; LaFerla, F.M. *Neuron* **2004,** *43*, 321.
[58] Billings, L.M.; Oddo, S.; Green, K.N.; McGaugh, J.L.; LaFerla, F.M. *Neuron* **2005,** *45*, 675-688.
[59] Lopez Salon, M.; Pasquini, L.; Besio Moreno, M.; Pasquini, J.M.; Soto, E. *Exp. Neurol.* **2003,** *180*, 131.
[60] Lopez Salon, M.; Morelli, L.; Castano, E.M.; Soto, E.F.; Pasquini, J.M. *J. Neurosci. Res.* **2000,** *62*, 302.
[61] De Vrij, F.M.; Sluijs, J.A.; Gregori, L.; Fischer, D.F.; Hermens, W.T.; Goldgaber, D.; Verhaagen, J.; Van Leeuwen, F.W.; Hol, E.M. *FASEB J.* **2001,** *15*, 2680.
[62] Schmitz, A.; Schneider, A.; Kummer, M.P.; Herzog, V. *Traffic* **2004,** *5*, 89.
[63] Duan, W.; Zhu, X.; Ladenheim, B.; Yu, Q.S.; Guo, Z.; Oyler, J.; Cutler, R.G.; Cadet, J.L.; Greig, N.H.; Mattson, M.P. *Ann. Neurol.* **2002,** *52*, 597.
[64] Goni, F.; Sigurdsson, E.M. *Curr. Opin. Mol. Ther.* **2005,** *7*, 17.
[65] Hara, H.; Monsonego, A.; Yuasa, K.; Adachi, K.; Xiao, X.; Takeda, S.; Takahashi, K.; Weiner, H.L.; Tabira, T. *J. Alz. Dis.* **2004,** *6*, 483.

Received: May 17, 2006 **Revised: June 23, 2006** **Accepted: July 12, 2006**