

Pathology of the Cleaved Tau Protein in the Context of Toxicity and the Formation of Neurofibrillary Tangles

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Abstract

In Alzheimer's disease, tau protein is abnormally processed to self-aggregate into pathologically paired helical filaments and neurofibrillary tangles. Accumulation of these structures in the somatodendritic compartment of neurons may result in pathological alterations of the cytoskeleton stability, abnormal sorting of molecules and obstruction of the intracellular transport of organelles. Initially, abnormal phosphorylation of tau was considered by many to be the major modification that alters its microtubule-binding capacity. In recent years, however, proteolytic cleavage of tau protein produced by caspases has been shown to promote the abnormal aggregation properties of tau *in vitro* and to produce toxic effects in cell and animal models of Alzheimer's disease. Although some of these results have been debated, truncation of tau associated with neurofibrillary tangle formation has been shown to correlate well with the clinical progression of Alzheimer's disease. Although new alternative mechanisms of tau pathogenesis in a monomeric or oligomeric state have been proposed, the aggregated form of intact or truncated tau into insoluble polymers is still a major indicator of neuronal degeneration.

Keywords

Alzheimer's disease, neurofibrillary tangles, tau protein, caspases, truncation, confocal microscopy, conformational changes, apoptosis, tau polymerisation, neurodegeneration

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Alzheimer's disease (AD) is the most common type of dementia and is characterised by memory loss and cognitive impairment in the elderly. These symptoms are attributed to the accumulation of abnormal structures, namely amyloid- β ($A\beta$) plaques and neurofibrillary tangles (NFTs). The latter structures first appear in the entorhinal cortex and parallel the clinical progression of the disease, as they spread out to the limbic regions and then the isocortex.¹

This hierarchical distribution of NFTs correlates better with AD progression than the deposition of $A\beta$.^{2,3} Structurally, NFTs are made up of insoluble paired helical filaments (PHFs) composed of the microtubule-associated protein tau, found mainly in a hyperphosphorylated state.^{4,5} Polymeric tau has been considered toxic⁶ because of this abnormally phosphorylated state, which potentially reduces its microtubule-binding capacity.^{7,8} It is also toxic because of its abnormal redistribution to the somatodendritic compartment, restricting the physical space and interfering with several processes, such as the sorting of molecules and intracellular transport.⁹⁻¹²

These data have suggested a relevant role for NFTs as the major pathological structures that impose a pathological insult on central nervous system neurons in AD patients. For this reason it becomes crucial to analyse the pathological processing of tau protein to better

understand the mechanisms involved in the genesis of the NFTs in AD. Besides abnormal phosphorylation and conformational changes, proteolysis of the tau protein is a newly emerging research area. Proteolysis contributes to the neuron toxicity and the formation of NFTs in AD.¹³⁻¹⁷ This review discusses and summarises the relevance of tau proteolysis as a new pathological modification that contributes to the formation of NFTs and the toxicity of these structures in AD.

The Cleavage of Tau Protein and Its Relation to Alzheimer's Disease

To identify the minimum component that composes the PHFs, native tau filaments isolated from the brains of AD patients were sonicated in formic acid. They were then treated proteolytically, releasing a 12kDa fragment of the tau molecule as the major component. This minimum component began in the vicinity of histidine-268 and contained the microtubule-binding domains. This fragment ended at the C-terminus position, glutamic acid 391 (Glu³⁹¹), and is referred to as the PHF-core.^{15,18} Thereafter, the monoclonal antibody MN423 was generated, which specifically recognises the Glu³⁹¹-truncated tau *in vitro* and also the polymeric tau forming the neurofibrillary pathology when it is assessed in the brain of AD patients (see *Figure 1*).^{15,18,19} Later, to determine the clinicopathological role of the Glu³⁹¹-cleaved tau in AD, the density of NFTs immunolabelled with MN423 was

correlated with the progression of neurofibrillary pathology determined by Braak staging criteria.¹ More significantly, a positive correlation to the clinical severity of dementia was shown.^{13,14,20,21} Supporting the claim for the relevance of the cleavage of tau protein in AD, recombinant Glu³⁹¹-cleaved tau showed increased rates of polymerisation *in vitro* over full-length tau.²² To explain this result, it was postulated that the C-terminus of tau could interfere with the polymerisation of this protein, caused by the folding of this region to the microtubule-binding repeats.^{22,23} Although Glu³⁹¹ cleavage is an alternative mechanism involved in tau toxicity and aggregation, a candidate enzyme responsible for this cleavage under physiological conditions has not yet been identified.²⁴

A new cleavage site in tau protein was later described^{25,26} and involved cytoplasmic proteases, named caspases, that are associated with programmed cell death or apoptosis. This cell suicide programme, which normally regulates cell growth and proliferation during development, has also been associated with the cell loss occurring in both normal ageing and neurodegenerative disorders.^{27–30} Caspases are from a cysteine-aspartyl-protease family with increased activity in the brain of AD patients.^{31,32} Caspase-3 was proved to cleave tau *in vitro* at the position aspartic acid-421 (Asp⁴²¹), which also increased its polymerisation rate *in vitro* over that observed for the C-terminus-intact tau protein.^{25,26,33} The truncation by these 20 amino acids was fundamental for causing a polymeric state. This was because a reduction in the normal levels of polymerisation was attained again when the complementary 422–441 peptide was included in the polymerisation assay.²³

A new immunological probe named Tau-C3 was developed to monitor the existence of Asp⁴²¹-truncated tau in the brain of AD patients. It confirmed that this truncated protein was conforming to the major structures that composed the neurofibrillary pathology in AD.^{25,34} The genesis of Asp⁴²¹-cleaved tau as a caspase–proteolytic product was indirectly confirmed at a cellular level when primary hippocampal neurons were grown in the presence of extracellular A β . This A β is known to cause apoptosis by activation of cell-death receptors.^{25,35}

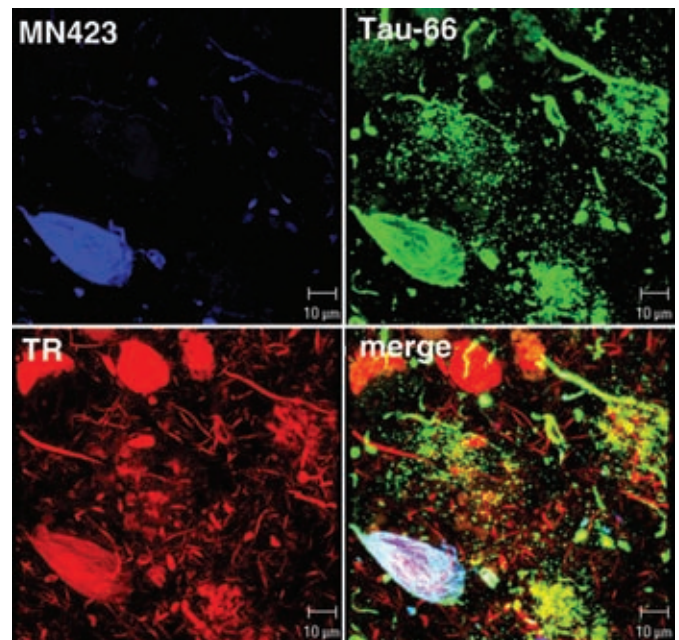
The cleavage of tau seems to be an important post-translational modification that contributes to the abnormal self-assembly of tau. However, its pathological role leading to neuronal degeneration and tau aggregation remains controversial.³⁶ The revised information that points to the controversial role of the cleaved tau in AD is further discussed in this review.

Toxicity of Truncated Tau in Cell and Animal Models

To better understand the potential toxicity of the truncated tau, several cell and animal models have been developed to generate the cellular condition that allows tau to polymerise and cause physiological alterations as they might occur in the authentic disease. In some of these experimental approaches, the toxic properties of normal and mutated tau species have been confirmed.^{6,37–42}

In transfected neuronal and non-neuronal cells, the overexpression of either Asp⁴²¹- or Glu³⁹¹-cleaved tau variants produced toxic effects closely associated with the induction of apoptosis.^{33,43–46} Similar data were found in primary cultures of rat hippocampal neurons, a result with particular significance because of the vulnerability of hippocampal neurons reported in AD.⁴⁵

Figure 1: Neurofibrillary Pathology of the Alzheimer's Disease Brain



Triple-labelled neurofibrillary tangle (white colour in the merge channel) is composed of Glu³⁹¹-truncated tau (MN423-positive) in a conformationally altered state (Tau-66-positive). These alterations of the tau protein are absent in neuropil threads, which are predominantly observed in the red channel. TR = thiazin red, which recognises β -pleated sheet conformation structures.

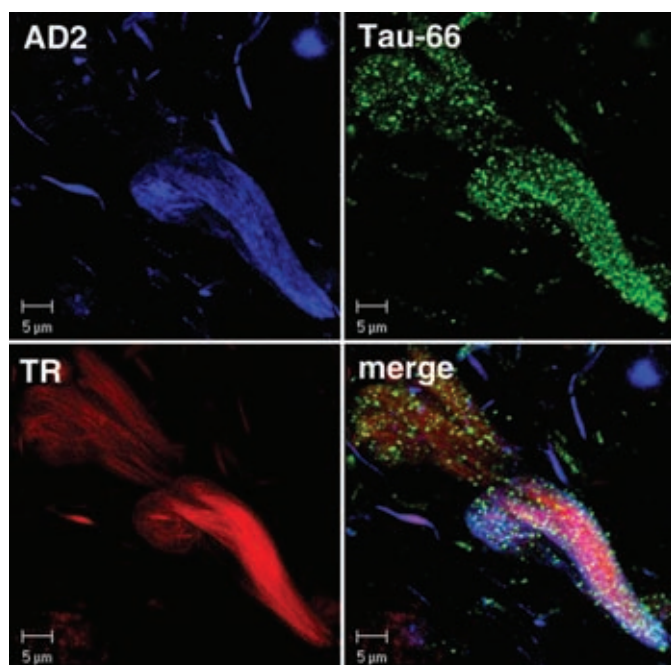
When the toxic effects of tau protein expressed in different types of cells have been analysed, most of the effects were caused by the co-expression or activation of intracellular enzymes, such as diverse kinases.⁴³ These analyses led to controversial results that caused a debate about the contribution of normal, mutated or truncated tau to crucial causes of pathological effects. It was determined that Asp⁴²¹-cleaved tau is able to form an inclusion identified by thioflavine-S; however, it did not result in a potential enhancement of cell death.⁴³ Similar results were obtained under the co-expression of GSK-3 β and Asp⁴²¹-truncated tau in Chinese hamster ovary cells.⁴³ In contrast to these results, a protective role against apoptosis was observed for abnormally phosphorylated tau protein co-expressed with GSK-3 β in N2a cells.⁴⁰

Comparable effects of normal and mutated tau have been found in tau-transgenic animals in which an approximation to physiological conditions is more likely.^{36,47,48} Under the expression of a human truncated-tau variant in a transgenic rat model, increased toxicity was measured in association with the accumulation of reactive oxygen species and oxidative stress.⁴⁷ The lifespan of transgenic rats expressing high levels of a misfolded, truncated tau-protein variant was also reduced.⁴⁸

The appearance of fibrillary aggregates inside neurons of a double (Tet-GSK and b-VLW)^{49,50} and single⁵¹ transgenic mouse composed of Asp⁴²¹-truncated tau has also been reported. This indicates that truncation may be a common mechanism associated with the aggregation and toxicity of tau in several pathologies, including AD.

However, some researchers believe that cell and animal models of degeneration are far from clinical interpretations and extrapolations of the disease. Whether or not the overexpression of exogenous tau

Figure 2: Conformational Changes and Abnormal Phosphorylation in Tau Are Associated with the Maturation of Neurofibrillary Tangles



Conformational changes recognised by Tau-66 antibody (neurofibrillary tangles [NFTs] in green) are associated with the phosphorylation of tau at pSer³⁹⁶ in matured NFTs (NFTs in blue).

is comparable to an aggregation process that develops slowly over many years in the brain of those with AD is still an open question. Therefore, more accurate information related to the toxicity and aggregation of truncated tau in the brains of authentic patients with AD should be preferentially considered.

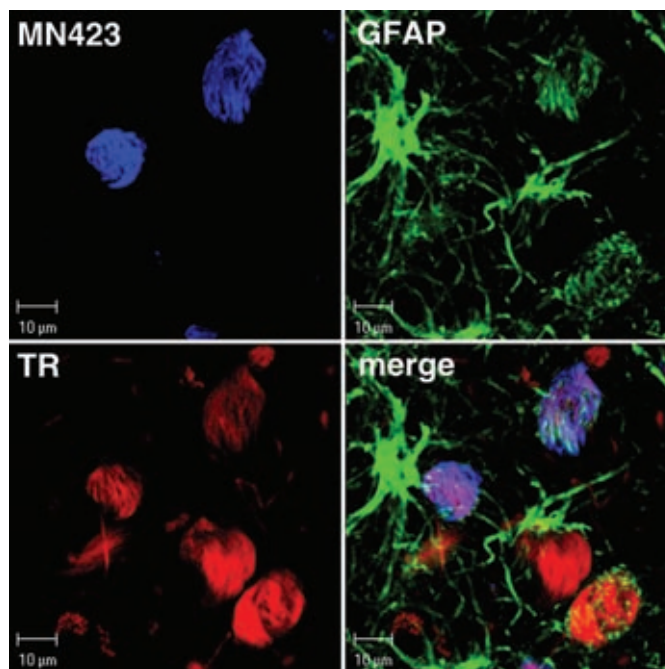
Tau Cleavage Associated with the Formation of Neurofibrillary Tangles and Toxicity

The biological systems described above could only be considered models that attempt to reproduce the physiological conditions in which tau exerts its toxicity in the brains of patients with AD. For many, aggregation of tau in distinct pathologies of the brain is a more complex process. It involves interactions of multiple factors that may affect the functioning of interconnected neurons related to the development of cognitive functions of the patients.⁵²

In situ, proteins are located and expressed in sufficient amounts to ensure the appropriate sorting and redistribution of intracellular components. One primary concern is the mechanism governing trafficking of tau from the axon to the somatodendritic compartment and whether the formation of a complex network of polymers can truly interfere with normal cell function. Recent evidence pointed out this effect of tau when polymers of this protein, introduced into the giant axon of the squid, caused interference with intracellular transport.⁵³ It is known that tau polymers in the authentic disease are the result of several post-translational modifications of the tau protein. These modifications include phosphorylation,⁵⁴⁻⁵⁶ conformational changes^{24,57-59} and truncation.^{13,16,17,34,60} Together, the modifications eventually lead to the formation of PHFs and, ultimately, NFTs (see Figure 2).

In the context of toxicity, NFTs are associated with neurons not functioning well,^{1,61,62} indicating a correlation between the progression

Figure 3: Toxicity of Neurofibrillary Tangles Composed of Truncated Tau in Alzheimer's Disease Brains



High activity of astrocytes (glial fibrillary acidic protein positive) induced by the presence of neurofibrillary tangles composed of Glu³⁹¹-truncated tau (MN423-positive) causes a toxic environment in the brain of Alzheimer's disease patients.

of tau pathology and the disease. This idea is supported by observations indicating that neural loss and NFT density increase in parallel with progression of the disease.^{63,64}

It has been suggested that NFTs are more dynamic structures and during their genesis the tau protein is progressively transformed from a linear non-folded protein to a conformationally altered entity that suffers specific truncations along its extreme C-terminus.^{8,24,34,65} The sequence in which conformation changes and truncation occur in the pathological processing of tau may predict the stage of evolution of the disease. The maturation of NFTs mainly depends on the state of proteolysis of the C-terminus having, as an early event, the truncation at Asp⁴²¹ caused by multiple caspases. The prevalence of this truncated protein in a polymeric state, which has been reported to be toxic in cell models, may be one method of continuous damage in NFT-carrying neurons.

Proteolysis of tau is extended along the C-terminus during the evolution of AD, reaching a truncation event at the site Glu³⁹¹ at advanced stages of the disease, again contributing permanent toxicity to the neurons⁸ (see Figure 3). Validation of the clinicopathological association between the progression of the disease and accumulation of NFTs composed by either Asp⁴²¹ or Glu³⁹¹-truncated tau variants was recently described by the authors' group.^{13,65} Moreover, other researchers have proposed that toxicity associated with NFTs may also be based on the abnormal trapping properties of these structures to sequester some cellular components that are important for normal cell function. Thus, PHFs are able to sequester prolyl isomerase-1, a chaperone protein that binds to phosphoproteins containing phosphoserine or phosphothreonine followed by proline.⁶⁶

Another protein with similar features is MAP1B, which in the aberrant phosphorylation state produced by a proline-dependent protein kinase

also co-purifies, like PHFs.⁶⁷ These abnormal associations may affect the stability of microtubules leading to disruption of the intracellular transport and maintenance of synaptic contacts.^{64,68,69} All of these data support a pathological role for polymeric tau in the form of NFTs by interfering with the normal functioning of the neurons. This, in combination with the loss of the ability of tau to stabilise microtubules, may also exert continuous toxicity during the progression of AD.^{24,34,60,65}

In spite of the evidence suggesting NFTs are one of the major toxic candidates in AD, contradictory information has suggested that tau species have no toxicity and aggregates are just an epiphenomenon resulting from the toxic properties of other pathological hallmarks of AD.^{70,71} Some reports have demonstrated that neurodegeneration in transgenic models occurs without NFT formation,⁷² and those neurons bearing fibrillary lesions can survive for a long time.⁷³ These observations conversely suggested a more protective role for tau aggregates instead of a toxic effect produced for monomeric tau.⁶⁴ In transgenic mice, similar results were obtained, with memory impairment and neuronal loss occurring in the absence of tau aggregates.⁷⁴⁻⁷⁶

Conclusion

The implication of the toxicity of tau aggregates in neurodegeneration has been extensively discussed.^{8,64,71,77-79} At present the precise

mechanisms involved in the genesis of AD are still unclear. Further investigation of the truncation of tau as a contributor to AD may therefore generate new insights, leading to a better understanding of this important neurological disorder. ■

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- Braak H, Braak E, *Acta Neuropathol (Berl)*, 1991;82:239-59.
- Arriagada PV, Growdon JH, Hedley-Whyte ET, et al., *Neurology*, 1992;42:631-9.
- Arriagada PV, Marzloff K, Hyman BT, *Neurology*, 1992;42:1681-8.
- Alonso AD, Grundke-Iqbal I, Barra HS, et al., *Proc Natl Acad Sci U S A*, 1997;94:298-303.
- Alonso Adel C, Li B, Grundke-Iqbal I, et al., *Proc Natl Acad Sci U S A*, 2006;103:8864-9.
- Bandyopadhyay B, Li G, Yin H, et al., *J Biol Chem*, 2007;282:16454-64.
- Braak H, Braak E, Grundke-Iqbal I, et al., *Neurosci Lett*, 1986;65:351-5.
- García-Sierra F, Mondragón-Rodríguez S, Basurto-Islas G, *J Alzheimers Dis*, 2008;14:401-9.
- Ebner A, Godemann R, Stamer K, et al., *J Cell Biol*, 1998;143:777-94.
- Niewiadomska G, Baksalerska-Pazera M, *Neuroreport*, 2003;14:1701-6.
- Niewiadomska G, Baksalerska-Pazera M, Riedel G, *Ann N Y Acad Sci*, 2005;1048:287-95.
- Stokin GB, Goldstein LS, *Annu Rev Biochem*, 2006;75:607-27.
- García-Sierra F, Wischik CM, Harrington CR, et al., *J Chem Neuroanat*, 2001;22:65-77.
- Mena R, Edwards PC, Harrington CR, et al., *Acta Neuropathol (Berl)*, 1996;91:633-41.
- Novak M, Kabat J, Wischik CM, *EMBO J*, 1993;12:365-70.
- Wischik CM, Novak M, Edwards PC, et al., *Proc Natl Acad Sci U S A*, 1988;85:4884-8.
- Wischik CM, Novak M, Thogersen HC, et al., *Proc Natl Acad Sci U S A*, 1988;85:4506-10.
- Novak M, Jakes R, Edwards PC, et al., *Proc Natl Acad Sci U S A*, 1991;88:5837-41.
- Wischik CM, Edwards PC, Lai RY, et al., *Neurobiol Aging*, 1995;16:409-17, discussion 418-31.
- Bondareff W, Harrington C, Wischik CM, et al., *J Neuropathol Exp Neurol*, 1994;53:158-64.
- Harrington CR, Mukaetova-Ladinska EB, et al., *Proc Natl Acad Sci U S A*, 1991;88:5842-6.
- Abraha A, Ghoshal N, Gamblin TC, et al., *J Cell Sci*, 2000;113(Pt 21):3737-45.
- Berry RW, Abraha A, Lagalwar S, et al., *Biochemistry*, 2003;42:8325-31.
- García-Sierra F, Ghoshal N, Quinn B, et al., *J Alzheimers Dis*, 2003;5:65-77.
- Gamblin TC, Chen F, Zambrano A, et al., *Proc Natl Acad Sci U S A*, 2003;100:10032-7.
- Rissman RA, Poon WW, Blurton-Jones M, et al., *J Clin Invest*, 2004;114:121-30.
- Cotman CW, Poon WW, Rissman RA, et al., *J Neuropathol Exp Neurol*, 2005;64:104-12.
- Dickson DW, *J Clin Invest*, 2004;114:23-7.
- Newman J, Rissman RA, Sarsoza F, et al., *Acta Neuropathol*, 2005;110:135-44.
- Rubinsztein DC, *Nature*, 2006;443:780-86.
- Bamberger ME, Landreth GE, *Neuroscientist*, 2002;8:276-83.
- Behl C, *J Neural Transm*, 2000;107:1325-44.
- Chung CW, Song YH, Kim IK, et al., *Neurobiol Dis*, 2001;8:162-72.
- Guillozet-Bongaarts AL, García-Sierra F, Reynolds MR, et al., *Neurobiol Aging*, 2005;26:1015-22.
- Gamblin TC, Berry RW, Binder LI, *Biochemistry*, 2003;42:2252-7.
- Delobel P, Lavenir I, Fraser G, et al., *Am J Pathol*, 2008;172:123-31.
- Chun W, Johnson GV, *J Biol Chem*, 2007;282:23410-17.
- Iliev AI, Ganesan S, Bunt G, et al., *J Biol Chem*, 2006;281:37195-204.
- Khlitunova I, Biernat J, Wang Y, et al., *J Biol Chem*, 2006;281:1205-14.
- Li HL, Wang HH, Liu SJ, et al., *Proc Natl Acad Sci U S A*, 2007;104:3591-96.
- Santa-Maria I, Hernandez F, Del Rio J, et al., *Mol Neurodegener*, 2007;2:17.
- Wang YP, Biernat J, Pickhardt M, et al., *Proc Natl Acad Sci U S A*, 2007;104:10252-7.
- Cho JH, Johnson GV, *J Biol Chem*, 2004;279:54716-23.
- Fasulo L, Ovecka M, Kabat J, et al., *Alzheimer's Res*, 1996;2:195-200.
- Fasulo L, Ugolini G, Cattaneo A, *J Alzheimers Dis*, 2005;7:3-13.
- Fasulo L, Ugolini G, Visintin M, et al., *J Neurochem*, 2000;75:624-33.
- Cente M, Filipcik P, Pevalova M, et al., *Eur J Neurosci*, 2006;24:1085-90.
- Koson P, Zilka N, Kovacs A, et al., *Eur J Neurosci*, 2008;28:239-46.
- Engel T, Lucas JJ, Gómez-Ramos P, et al., *Neurobiol Aging*, 2006;27:1258-68.
- Mondragón-Rodríguez S, Basurto-Islas G, Santa-Maria I, et al., *Int J Exp Pathol*, 2008;89:81-90.
- Zhang Q, Zhang X, Sun A, *Acta Neuropathol*, 2009;117:687-97.
- Falke E, Nissanonov J, Mitchell TW, et al., *Am J Pathol*, 2003;163:1615-21.
- LaPointe NE, Morfini G, Pigino G, et al., *J Neurosci Res*, 2009;87:440-51.
- Avila J, *FEBS Lett*, 2006;580:2922-7.
- Biernat J, Mandelkow EM, Schroter C, et al., *EMBO J*, 1992;11:1593-7.
- Grundke-Iqbal I, Iqbal K, Tung YC, et al., *Proc Natl Acad Sci U S A*, 1986;83:4913-17.
- Carmel G, Mager EM, Binder LI, et al., *J Biol Chem*, 1996;271:32789-95.
- Luna-Muñoz J, García-Sierra F, Falcón V, et al., *J Alzheimers Dis*, 2005;8:29-41.
- Luna-Muñoz J, Chávez-Macías L, García-Sierra F, et al., *J Alzheimers Dis*, 2007;12:365-75.
- Binder LI, Guillozet-Bongaarts AL, García-Sierra F, et al., *Biochim Biophys Acta*, 2005;1739:216-23.
- Delacourte A, David JP, Sergeant N, et al., *Neurology*, 1999;52:1158-65.
- Royall DR, Palmer R, Mulroy AR, et al., *Exp Aging Res*, 2002;28:143-62.
- Gómez-Isla T, Hollister R, West H, et al., *Ann Neurol*, 1997;41:17-24.
- Hernández F, Avila J, *J Alzheimers Dis*, 2008;14:449-52.
- Basurto-Islas G, Luna-Muñoz J, Guillozet-Bongaarts AL, et al., *J Neuropathol Exp Neurol*, 2008;67:470-83.
- Lu PJ, Wulf G, Zhou XZ, et al., *Nature*, 1999;399:784-88.
- Ulloa L, Montejo de Garcini E, Gómez-Ramos P, et al., *Brain Res Mol Brain Res*, 1994;26:113-22.
- Pollak D, Cairns N, Lubec G, *J Neural Transm Suppl*, 2003;58:188-97.
- Dixit R, Ross JL, Goldman YE, et al., *Science*, 2008;319:1086-9.
- Hardy J, Selkoe DJ, *Science*, 2002;297:353-6.
- Bretteville A, Planel E, *J Alzheimers Dis*, 2008;14:431-6.
- Wittmann CW, Wszolek MF, Shulman JM, et al., *Science*, 2001;293:711-14.
- Morsch R, Simon W, Coleman PD, *J Neuropathol Exp Neurol*, 1999;58:188-97.
- Duff K, Planel E, *Nat Med*, 2005;11:826-27.
- Santacruz K, Lewis J, Spire T, et al., *Science*, 2005;309:476-81.
- Ding H, Johnson GV, *J Alzheimers Dis*, 2008;14:441-7.
- Mondragón-Rodríguez S, Basurto-Islas G, Binder LI, et al., *Future Neurology*, 2009;4:39-53.
- Götz J, Ittner LM, Fandrich M, et al., *J Alzheimers Dis*, 2008;14:423-9.
- Castellani RJ, Nunomura A, Lee HG, et al., *J Alzheimers Dis*, 2008;14:377-83.