American Journal of Pathology, Vol. 165, No. 2, August 2004

Commentary

Caspase-Mediated Degeneration in Alzheimer's Disease

David H. Cribbs, Wayne W. Poon, Robert A. Rissman, and Mathew Blurton-Jones

From the Institute for Brain Aging and Dementia, University of California Irvine, Irvine, California

In Alzheimer's disease (AD) the underlying molecular mechanisms that trigger neurodegeneration and result in the accumulation of plaques and tangles have been a major focus of many laboratories studying the disease. However, many of the pathological markers used to confirm the postmortem diagnosis of AD may not provide direct information regarding the underlying molecular mechanism(s) involved in the pathogenesis of the disease. For example, the β-amyloid (Aβ) peptide, the main component of senile plaques, is cleaved from the amyloid precursor protein (APP) by the β- and γ-secretases, which appear to be constitutively expressed in normal tissues throughout the body. Thus, while secretases do not appear to be overexpressed in AD, these proteases are critical for the production of Aβ. Furthermore, the γ-secretase complex has several other substrates that are involved in important cellular signaling pathways. In addition, other proteases including cathepsins, calpains, proteosomes, and caspases have also been proposed as candidates that may contribute to AD degeneration. In particular, the cleavage of cytoskeletal components, which clearly alters cell morphology may either activate death signals or disrupt survival signals necessary to suppress cell death.[1,2] The primary focus of this commentary is to discuss the possible role of caspases in AD neurodegeneration.

Caspases are cysteine aspartate proteases that are critically involved in apoptosis. These enzymes can be broadly divided into initiator and executioner caspases, with the former functioning to initiate apoptosis by activating executioner caspases and the latter acting on downstream effector substrates that result in the progression of apoptosis and the appearance of hallmark morphological changes such as cell shrinkage, nuclear fragmentation, and membrane blebbing.[3] The detection of active caspases and the accumulation of cleaved substrates, such as fodrin, actin, and APP in postmortem AD brain tissue supports the hypothesis that apoptotic-like mechanisms may contribute to neuronal loss in AD.[4–7] A series of recent studies now support the hypothesis that caspase-mediated cleavage of critical proteins contributes to neuronal degeneration in AD, including and the manuscript by Guo et al[8] describing active caspase-6 and caspase-6-cleaved tau in this issue of *The American Journal of Pathology*.

The First Hints That Apoptosis May Be Involved in AD Neurodegeneration

Based on the observation that neurites surrounding Aβ-deposits show degenerative changes in the postmortem AD brain, various investigators pursued the hypothesis that Aβ is not metabolically inert as was initially believed, but rather possesses biological activity. The initial reports suggested that Aβ may stimulate transient growth of neuronal processes, but when self-assembled into small aggregates and/or β-sheet structures, it acquires the ability to activate degenerative mechanisms.[9–12] Subsequently, fibrillar Aβ was shown to serve as an inducer of neuronal apoptosis.[13,14] In addition to Aβ, many other stimuli, such as oxidative stress, neurotrophic factor deprivation, low energy metabolism, and mitochondrial dysfunction, which can induce apoptosis in cultured neurons are also prominent in the AD brain.

The Search for Evidence of Apoptosis in the AD Brain

Because caspases are activated during apoptosis, data showing caspase activity is commonly used as proof of apoptosis. Direct measurement of caspase activity in tissue samples, particularly in postmortem brain tissue, can be difficult to document because typically only small subsets of cells within particular brain regions are actually undergoing apoptosis at any given time. In addition, analysis of protease activity in tissue lysates does not provide information on which cell types are undergoing apoptosis. Indirect evidence of protease activity can be

Accepted for publication May 25, 2004.

Address reprint requests to David H. Cribbs, Institute for Brain Aging and Dementia, Irvine CA 92697-4540. E-mail: cribbs@uci.edu.

inferred by the detection of proteolytic fragments generated by a specific protease. This can be particularly powerful if the protease substrate is a major cellular protein and produces a stable cleavage fragment that accumulates within the cell. Initially, Western blotting was used to detect the signature fragment. However, the development of neoepitope antibodies with specificity for the cleavage site, but not the intact protein, have been extremely valuable in dissecting out proteolytic events in cultured cells and tissues. One excellent example of a protein that meets the criteria established above is brain fodrin, which is a sensitive target of calpain and caspases, and represents a critical component in the linkage of the cytoskeleton to the plasma membrane. Not only is there an increase in brain fodrin immunoreactivity and abnormal deposits of fodrin in sprouting neurons in AD,[15,16] but also the increased immunoreactivity has been correlated with accumulation of a stable breakdown product of fodrin. Moreover, an increase in fodrin proteolysis in fibroblasts from aged and AD donors has also been reported,[17] indicating that there is widespread alteration in the proteolytic processing of this protein in aging cells. The calpain cleavage site in the α-subunit of fodrin was identified by several laboratories where calpain cleavage site-directed antibodies were developed.[18,19] However, there is also very good evidence showing that fodrin is a substrate for caspases.[20–22] During the initiation phase of apoptosis, fodrin is one of the first substrates to be cleaved by caspases, and the caspase cleavage of the α-subunit of fodrin strongly correlates with the exposure of phosphatidylserine on the cell surface.[21,23] These distinct protease activities result in at least two unique, clearly defined, and stable cleavage products. Interestingly, fodrin cleavage products generated by calpain and caspase protease activity have similar molecular weights. However, because the cleavage sites for the proteases are different, the cleavage products produced are antigenically distinct. They therefore represent desirable targets for cleavage site-directed antibodies. Unfortunately, the relative contribution of calpain and caspases to fodrin cleavage products that accumulate in AD brain remains to be determined. *In vitro*, several different caspases can produce the 150-kd cleavage products, but to date, only caspase-3 has been shown to produce both the 150- and the 120-kd fragments.[24] This point is particularly relevant to Guo et al (discussed below) which claims that caspase-6 is the principle caspase involved in AD pathogenesis. The identification of the specific caspase cleavage site in fodrin and development of cleavage site-directed antibodies that recognize death products provided a novel cytoplasmic marker for caspase activation in neurons undergoing apoptosis, and also provides a way to discriminate between the contribution of calpain and caspase to the accumulation of fodrin cleavage products in AD.

Another neoepitope antibody that has proved useful as a probe for detecting caspase activity *in situ* was developed by Yang et al[6] against the C terminus of a 32-kd caspase-cleaved actin fragment that specifically labels apoptotic but not necrotic cells. This antibody serves as an excellent marker of apoptosis-related events in the axons and dendrites. Fractin-positive immunostaining was observed in processes and soma of degenerating neurons and plaque-associated microglia in AD tissue.

Caspase Cleavage of APP and Tau in AD

Further evidence of the involvement of caspases in AD was reported by Gervais et al[7] who demonstrated that the cytoplasmic tail of APP was cleaved by caspase-3. They generated a neoepitope antibody to the caspase cleavage site in APP and showed that degenerating neurons and senile plaques were enriched with caspase-cleaved APP. This study provided evidence that caspase activation was linked to increased Aβ production. Collectively, caspase cleavage site-directed antibodies provide powerful diagnostic tools to better assess the contribution of apoptosis in neurodegenerative diseases. Furthermore, these antibodies will also be useful for investigating the temporal and spatial relationship between caspase cleavage of specific substrates and other events associated with apoptosis.

More recently, tau has been implicated as another substrate for caspases and several caspases have been linked to cleavage of tau.[25,26] Although the role of tau caspase cleavage in AD pathology remains unresolved, recent evidence now implicates caspase cleavage of tau in tangle pathology.[27,28] The cleavage of tau has been shown to significantly alter tau function and Aβ has been shown to promote pathological tau filament assembly in neurons *in vitro* by triggering caspase cleavage of tau, which enhances tau filament polymerization kinetics of the tau fragment.[27,28,29]

The manuscript by Guo et al entitled "Active Caspase-6 and Caspase-6-Cleaved Tau in Neuropil Threads, Neuritic Plaques, and Neurofibrillary Tangles of Alzheimer's Disease"[8] promotes the hypothesis that caspases are intimately involved in the neurodegeneration driving AD. LeBlanc and co-workers[8] show caspase-6 activation in AD frontal and temporal cortex. The presence of activated caspase-6 in pre-tangles suggests that it occurs early and supports previous studies demonstrating caspase activation in mild cognitively impaired (MCI) but not AD subjects. While caspase-6-cleaved tau was found in neuritic plaques, neuropil threads, and NFTs, active caspase-6 localized primarily to neurites. This is consistent with the hypothesis that apoptotic-like mechanisms can damage synapses, axons, and dendrites, without causing overt neuronal death. These results also lend support to the hypothesis that the activation of apoptosis-like mechanisms may be involved in AD pathogenesis. Since tau is a component of NFT, and a target of several caspases, caspase activation would link neuronal loss to tau pathology. Guo et al argue[8] that caspase-6, but not caspase-3 is integral in AD pathology, because active caspase-3 is primarily found in GVDs. However, caspase-3 and other caspases cannot be excluded since caspase-9, an upstream activator of caspase-3, is activated in AD. In addition, activated caspase-3 rapidly degrades itself "in vitro".[30] This

finding may explain why active caspase-3 is primarily observed within GVDs where this self-degrading activity may be neutralized. Dr. LeBlanc[8] has been a pioneer in using cultured fetal human neurons to study apoptosis, and has demonstrated that there are significant differences between rodent and human neuronal culture models regarding the involvement of specific caspases. The novel finding in this report is that the putative culprit caspase in AD is caspase-6. A compelling piece of evidence is the finding that active caspase-6 is associated with degenerating neurons and caspase-cleaved tau. If this is correct, a new, although similar, therapeutic target has been identified in AD. If caspase-6 is the primary caspase activated in the AD brain, then previous reports of caspase-3-cleaved substrates such as fodrin, actin, and APP may also be the product of caspase-6 cleavage due to redundancy in the activities of the various caspases. This is a likely possibility considering the similarities in the caspase recognition sites in protein substrates and the known cross-reactivity of various inhibitors designed to inhibit a specific caspase. The development of caspase cleavage site-directed antibodies has provided a powerful diagnostic tool to better assess the contribution of caspases to neurodegeneration. While the present study strongly implicates caspase-6, additional studies are needed to determine the relative contribution of the various caspases to neuronal demise in Alzheimer's disease.

References

1. Raff MC: Social controls on cell survival and cell death. Nature 1992, 356:397–400
2. Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD: Programmed cell death and the control of cell survival: lessons from the nervous system. Science 1993, 262:695–700
3. Kerr JF, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972, 26:239–257
4. Cotman CW, Anderson AJ: A potential role for apoptosis in neurodegeneration and Alzheimer's disease. Mol Neurobiol 1995, 10:19–45
5. Rohn TT, Head E, Su JH, Anderson AJ, Bahr BA, Cotman CW, Cribbs DH: Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease. Am J Pathol 2001, 158:189–198
6. Yang F, Sun X, Beech W, Teter B, Wu S, Sigel J, Vinters HV, Frautschy SA, Cole GM: Antibody to caspase-cleaved actin detects apoptosis in differentiated neuroblastoma and plaque-associated neurons and microglia in Alzheimer's disease. Am J Pathol 1998, 152:379–389
7. Gervais FG, Xu D, Robertson GS, Vaillancourt JP, Zhu Y, Huang J, LeBlanc A, Smith D, Rigby M, Shearman MS, Clarke EE, Zheng H, Van Der Ploeg LH, Ruffolo SC, Thornberry NA, Xanthoudakis S, Zamboni RJ, Roy S, Nicholson DW: Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-β precursor protein and amyloidogenic A β peptide formation. Cell 1999, 97:395–406
8. Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC: Active caspase-6 and caspase-6-cleaved tau in neutropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. Am J Pathol 2004, 165:523–531
9. Whitson JS, Selkoe DJ, Cotman CW: Amyloid β protein enhances the survival of hippocampal neurons in vitro. Science 1989, 243:1488–1490
10. Yankner BA, Dawes LR, Fisher S, Villa-Komaroff L, Oster-Granite ML, Neve RL: Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. Science 1989, 245:417–420
11. Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW: In vitro aging of β-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res 1991, 563:311–314
12. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW: Neurodegeneration induced by β-amyloid peptides in vitro: the role of peptide assembly state. J Neurosci 1993, 13:1676–1687
13. Loo DT, Copani A, Pike CJ, Whittemore ER, Walencewicz AJ, Cotman CW: Apoptosis is induced by β-amyloid in cultured central nervous system neurons. Proc Natl Acad Sci USA 1993, 90:7951–7955
14. Watt JA, Pike CJ, Walencewicz-Wasserman AJ, Cotman CW: Ultrastructural analysis of β-amyloid-induced apoptosis in cultured hippocampal neurons. Brain Res 1994, 661:147–156
15. Masliah E, Iimoto DS, Saitoh T, Hansen LA, Terry RD: Increased immunoreactivity of brain spectrin in Alzheimer disease: a marker for synapse loss? Brain Res 1990, 531:36–44
16. Masliah E, Hansen L, Mallory M, Albright T, Terry RD, Abnormal brain spectrin immunoreactivity in sprouting neurons in Alzheimer disease. Neurosci Lett 1991, 129:1–5
17. Peterson C, Vanderklish P, Seubert P, Cotman C, Lynch G: Increased spectrin proteolysis in fibroblasts from aged and Alzheimer donors. Neurosci Lett 1991, 121:239–243
18. Bahr BA, Tiriveedhi S, Park GY, Lynch G: Induction of calpain-mediated spectrin fragments by pathogenic treatments in long-term hippocampal slices. J Pharmacol Exp Ther 1995, 273:902–908
19. Saido TC, Yokota M, Nagao S, Yamaura I, Tani E, Tsuchiya T, Suzuki K, Kawashima S: Spatial resolution of fodrin proteolysis in postischemic brain. J Biol Chem 1993, 268:25239–25243
20. Martin SJ, O'Brien GA, Nishioka WK, McGahon AJ, Mahboubi A, Saido TC, Green DR: Proteolysis of fodrin (non-erythroid spectrin) during apoptosis. J Biol Chem 1995, 270:6425–6428
21. Vanags DM, Porn-Ares MI, Coppola S, Burgess DH, Orrenius S: Protease involvement in fodrin cleavage and phosphatidylserine exposure in apoptosis. J Biol Chem 1996, 271:31075–31085
22. Wang KK, Posmantur R, Nath R, McGinnis K, Whitton M, Talanian RV, Glantz SB, Morrow JS: Simultaneous degradation of αII- and βII-spectrin by caspase 3 (CPP32) in apoptotic cells. J Biol Chem 1998, 273:22490–22497
23. Martin SJ, Finucane DM, Amarante-Mendes GP, O'Brien GA, Green DR: Phosphatidylserine externalization during CD95-induced apoptosis of cells and cytoplasts requires ICE/CED-3 protease activity. J Biol Chem 1996, 271:28753–28756
24. Nath R, Raser KJ, Stafford D, Hajimohammadreza I, Posner A, Allen H, Talanian RV, Yuen P, Gilbertsen RB, Wang KK: Non-erythroid α-spectrin breakdown by calpain and interleukin 1 β-converting-enzyme-like protease(s) in apoptotic cells: contributory roles of both protease families in neuronal apoptosis. Biochem J 1996, 319:683–690
25. Chung CW, Song YH, Kim IK, Yoon WJ, Ryu BR, Jo DG, Woo HN, Kwon YK, Kim HH, Gwag BJ, Mook-Jung IH, Jung YK: Proapoptotic effects of tau cleavage product generated by caspase-3. Neurobiol Dis 2001, 8:162–172
26. Rohn TT, Rissman RA, Davis MC, Kim YE, Cotman CW, Head E: Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. Neurobiol Dis 2002, 11:341–354
27. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI, Cryns VL: Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. Proc Natl Acad Sci USA 2003, 100:10032–10037
28. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, La Ferla FM, Rohn TT, Cotman CW: Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. J Clin Invest 2004, 114:121–130
29. Berry RW, Abraha A, Lagalwar S, LaPointe N, Gamblin TC, Cryns VL, Binder LI: Inhibition of tau polymerization by its carboxy-terminal caspase cleavage fragment. Biochemistry 2003, 42:8325–8331
30. Tawa P, Hill K, Giroux A, Grimm E, Han Y, Nicholson DW, Xanthondakis J: Catalytic activity of caspase-3 is required for its degradation: stabilization of the active complex by synthetic inhibitors. Cell Growth Differ 2004, 11:439–447