Proline-directed kinase systems in Alzheimer’s disease pathology

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Immunohistochemical analysis was used to assess the distribution of the proline-directed kinase, cdc2, in Alzheimer’s disease (AD) pathology. A robust signal was most prominent in the neurofibrillary tangle (NFT) of affected neurons that also contained abnormally phosphorylated tau protein. Biochemical analysis identified a pool of cdc2 in bovine brain microtubules that contain normal tau. These results strongly support the hypothesis that cdc2 is involved in the abnormal phosphorylation of tau in AD pathology and they raise important issues regarding regulation of tau phosphorylation in normal and diseased neurons.

The protein kinase cdc2 plays a critical role in mitosis of dividing cells1,12-14. Homologs of cdc2 are found in a variety of mammalian cells1,12-14 and, curiously, in brain, normally considered a nonproliferating tissue6,7,9. This raises the possibility that cdc2 kinases might play additional roles in differentiated cells such as neurons. The consensus phosphorylation site for cdc2 and related kinases including the mitogen-activated protein (MAP) kinases includes a proline residue so this family has come to be designated proline-directed protein kinases. There is considerable interest in this family with regard to neurodegenerative diseases based on several recent observations regarding their properties in phosphorylating the microtubule-associated protein, tau. Tau is a phosphoprotein whose normal function is to promote the assembly and stability of microtubules15. Abnormally phosphorylated tau is a major component of the cytoskeletal pathology of Alzheimer’s disease (AD), including the neurofibrillary tangle (NFT)1,5,7,8,16. This abnormal phosphorylation compromises tau functional properties and probably contributes to formation of the cytoskeletal pathology seen in AD tissue1,10. Until recently, candidate kinases responsible for abnormally phosphorylating AD tau could not be identified. Several groups have now shown that the proline-directed kinases, especially MAP kinase and cdc2, phosphorylate tau on sites that mimic wholly or in part those sites phosphorylated on AD tau1,2,7. It is not clear, however, how these in vitro observations relate to the actual pathology seen in AD since virtually nothing is known about the cellular distribution of the kinases. In this report, we examine the distribution of the kinase family in AD tissue and show that a homolog of cdc2, in particular, is upregulated in a subset of affected neurons containing abnormally phosphorylated tau.

Hippocampus and frontal cortex from five neuropathologically confirmed cases of AD patients (71–83 yr) and three age-matched nondemented controls were fixed by immersion for 2 h at room temperature in the fixative of McLean and Nakane11. The tissues were embedded in 4% agar and sectioned with a Lancer vibratome into 50-μm slices.

All subsequent steps were carried out with gentle agitation of the sections in 50 mM Tris-buffered physiological saline (pH 7.3). Sections were blocked for 1 h at room temperature with 1% normal goat serum and 0.3% Triton X-100 and then incubated with antibody in blocking buffer overnight at 4°C. Affinity purified anti-peptide polyclonal antibodies or monoclonal antibodies (UBI, Zymed, Pharmingen, Santa Cruz Biotechnology) to various conserved domains of cdc2 and the different isoforms of MAP kinase as well as a paired helical filament (PHF)-specific antibody, PHF 1 (generously supplied by S. Greenberg) were employed at dilutions of 1:50–1:5000. Antibody binding was detected with a Vectastain ABC kit (Vector Laboratories), washed 4–6 times (15 min each) between steps and developed with a 0.05%
Fig. 1. Light micrographs illustrating anti cdc2 (panels a,c,e and f) and PHF-1 (panels b,d and g) immunoreactivity in hippocampus of an AD brain. In these images, label with both antibodies is primarily restricted to NFT of affected neurons. Panel A insert is a Western blot analysis of purified microtubule fractions showing a robust signal for cdc2 (right lane). Molecular weight markers are indicated in left lane. Arrow points to 31-kDa marker.
diaminobenzidine/0.02% hydrogen peroxide solution on ice for 8–10 min.

The panels in Fig. 1 are light microscopic views of AD hippocampus revealing anti N-terminal cdc2 staining of neuronal cell bodies. At higher magnification, a fibrillar nature of the staining is apparent in some of the neurons but in others reaction product appears to be more homogeneously distributed. It should be noted that neurons in control tissue exhibit a low level of specific cell body staining for cdc2, and the same level of staining is apparent in normal neurons in the AD tissue. We have chosen a dilution of antibody at which the staining of normal neurons is barely detectable to emphasize the apparent upregulation of cdc2 kinase in a subpopulation of neurons.

For comparative purposes, adjacent sections were stained with PHF 1 which selectively identifies disease affected neurons containing abnormally phosphorylated tau. Based on morphology and location, there is considerable overlap between anti cdc2 and PHF 1 staining of neuronal populations and preliminary double-label immunocytochemistry supports this impression (data not shown). Thus, those neurons in which cdc2 is upregulated contain abnormally phosphorylated tau proteins. Based on initial morphometric analysis of double-labeled sections, however, it appears that not all neurons containing abnormal tau are positive for cdc2. The significance of this will require additional experimental work to ascertain.

We have also stained AD sections with a panel of antibodies to the MAP kinase isoforms searching for an indication of upregulation of this kinase system in AD pathology. The results to date have been negative but these experiments are ongoing.

These results strongly support the hypothesis that proline-directed kinases, such as cdc2, may be involved in the cytoskeletal pathogenesis seen in AD. The primary substrate for phosphorylation in this pathology is tau. In vitro studies indicate that a homolog of cdc2 can abnormally phosphorylate tau at the site originally recognized as abnormal in sections of AD brain using the Tau 1 monoclonal antibody. This cdc2 kinase can also phosphorylate tau at additional sites. The precise subcellular relationship of tau and cdc2 must be identified as part of elucidating the mechanisms for regulation of tau phosphorylation by cdc2. Since tau is a microtubule-associated protein, we have assessed the association of the cdc2 homolog studied here with purified microtubule fractions. Since AD brain microtubules do not resemble, we have used bovine brain microtubules. Fig. 1 shows a robust signal for cdc2 on Western blots of microtubule fractions probed with the same N-terminal antibody used for immunocytochemistry. This demonstrates that the homolog we have identified in AD pathology can associate with brain microtubules containing tau through repetitive cycles of assembly/disassembly.

These results raise a critical issue regarding normal and abnormal regulation of tau phosphorylation. The data indicate that cdc2 is present in normal neurons and in normal microtubules. Why, then, is tau not abnormally phosphorylated in normal brain and normal microtubules? Several possible reasons are: (1) The kinase is inactive due to the presence of endogenous inhibitors that become less active in affected AD neurons. (2) The kinase is active but its activity is reversed by efficient dephosphorylation of AD tau sites through action of protein phosphatases; in this scenario affected AD neurons would contain less active protein phosphatases. (3) The kinase is active but prefers substrates other than tau and this preference is altered in affected AD neurons. These possibilities are addressable experimentally.

In summary, we have provided direct evidence for an apparent upregulation of a homolog of cdc2 in affected AD neurons which contain abnormally phosphorylated tau proteins. This homolog is a viable candidate for mediating, at least in part, abnormal phosphorylation of tau which results in impaired tau function and eventually in AD cytoskeletal pathology.

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