

DECEMBER 08, 1999

Researchers Find Early Trigger of Brain Cell Death in Alzheimer's Disease

Researchers studying the brain cells of patients with Alzheimer's disease have identified a molecule that triggers the early formation of some of the deadly snarls of protein that damage and kill brain cells.

The discovery offers important insights into the underlying pathology of Alzheimer's disease and also points out promising new targets for drug therapy, say Howard Hughes Medical Institute (HHMI) investigator [Li-Huei Tsai](#) and her colleagues at Harvard Medical School.

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- Li-Huei Tsai

Postmortem studies of the brains of people with Alzheimer's disease have revealed a number of striking details about the disease's pathology. Most notably, researchers believe that brain cell death is caused by the accumulation of the proteins amyloid- β -found in extracellular plaques- and tau, which is a component of the neurofibrillary tangles observed in the brain tissue of patients with the disease.

In the December 9, 1999, issue of the journal *Nature*, Tsai and her colleagues describe how the interaction of several molecules may lead to the early formation of neurofibrillary tangles. According to Tsai and her colleagues, which include Gentry N. Patrick, Lawrence Zukerberg, Margareta Nikolic, Suzanne de la Monte and Pieter Dikkes, people with Alzheimer's disease have high levels of a shortened, malfunctioning version of the protein p35 in

their brains. Normally, p35 regulates cyclin-dependent kinase 5 (Cdk5)-an enzyme that catalyzes the construction and maintenance of neuronal tissue during development-by attaching to Cdk5 and anchoring it in the brain cell's plasma membrane. Enzymes that are kinases, such as Cdk5, activate other enzymes by adding a phosphate group to them a process called phosphorylation.

Tsai's group showed that in the cells of people with Alzheimer's disease, p35 is cleaved into two smaller proteins, p10 and p25. The p25 fragment maintains the activity of p35 and can still switch on Cdk5. Trouble starts because p25 loses a critical targeting segment that is contained in p35, so p25 "turns on" Cdk5 and allows it to wander through the cell's cytoplasm "hyperphosphorylating" other proteins notably a cytoskeletal building-block protein called tau. The altered tau protein becomes less able to attach to cytoskeletal proteins and aggregates into the lethal neurofibrillary tangles seen in brain cells damaged by Alzheimer's disease.

"The full-length p35 protein is normally very tightly regulated, with a very short half-life," explained Tsai. "The degradation of p35 is normally triggered by its association with Cdk5, which phosphorylates it. On the other hand, p25 is completely stable, which is why we found it at levels twenty to forty times higher in Alzheimer's brains." The far higher p25 levels in brain cells overwhelm normal regulation of Cdk5, said Tsai, constantly activating the enzyme.

In the *Nature* article, the scientists compared brain tissue samples from eight patients with Alzheimer's disease to samples from five people without the disease. While their analyses revealed normal levels of Cdk5 and p35 in the Alzheimer's tissue, they found far higher levels of the protein p25, the shortened version of p35. Using antibodies to selectively target and stain p25, p35 and tau, the scientists showed that the high p25 levels were distinctively associated with cells containing neurofibrillary tangles.

Genetic sequencing of the p35 and p25 proteins revealed that the shorter p25 lacks a crucial signal that allows it to anchor to the plasma membrane, where Cdk5 normally triggers other enzymes. When the scientists separated the various components of brain cells and analyzed them, they discovered that while p35 was more abundant in the cells' membrane component, p25 was enriched in the cytoplasmic compartment.

Using genetic engineering techniques, the scientists inserted the genes for p25 or p35, along with those for tau and Cdk5, into cells that lacked them, and found that p25 and Cdk5 did, indeed, hyperphosphorylate tau *in vivo* far more efficiently than p35 and Cdk5.

"This experiment was important, because nearly every kinase under the sun has been shown to phosphorylate tau in the test tube," said Tsai. "So we

wanted to make sure that p25/Cdk5 worked on tau in living cells." Also, when the scientists inserted the genes for p25 and Cdk5 into otherwise normal neuronal cells, they found that the cells showed cytoskeletal disruption, degeneration and death.

In particular, said Tsai, two lines of evidence from her team's studies implicated p25 as an early cause of Alzheimer's disease.

"For one thing, we found more neurons with accumulated p25 than we did neurons with neurofibrillary tangles," she said. "This suggests that p25 precedes the formation of detectable tangles.

"Also, we did not find p25 in one patient who had terminal-stage Alzheimer's disease. Her brain was less than half the weight of a normal brain, and since all of the affected neurons had already died, we would not expect to see p25."

Tsai and her colleagues are now seeking the culprit enzyme, called a protease, responsible for snipping p35 to produce p25 and p10. In a *News and Views* article in the same issue of *Nature*, Eckhard Mandelkow of the Max-Planck-Unit for Structural Molecular Biology in Hamburg, Germany writes that "Tsai and colleagues' results provide potential targets for diagnosis or therapy. These may come either at the level of p35 cleavage or at the level of Cdk5 inhibition, and therapy should be possible with the development of highly specific inhibitors."

Tsai agrees, stating that the protease would make an excellent target for drugs aimed at blocking the formation of neurofibrillary tangles.

"While many researchers have considered using drugs to block Cdk5, that enzyme is absolutely essential for day-to-day neural functioning," said Tsai. "However, blocking p25 itself or the protease that produces it would likely prove much more benign and effective targets for drug therapy for Alzheimer's disease."

The researchers are also seeking any possible connections between p25 formation and amyloid plaques -- the fibrous clots of protein and neuronal debris in brain tissue that are the other principal hallmark of Alzheimer's disease. Finding such connections would begin to link the mechanism of formation of the plaques and neurofibrillary tangles believed to be the two major causes of brain cell death in Alzheimer's disease.