TAU's cease and de-*cis*-t letter

By Lauren Martz, Staff Writer

Although it is well established that phosphorylated microtubule-associated protein-τ contributes to the pathology of Alzheimer's disease, antibodies against the target have so far been ineffective and have thus been relegated to research-only use. Now, researchers at **Harvard Medical School** have developed isoform-specific antibodies to target the protein and have shown that these can help detect, treat and prevent AD in mice.[1]

Microtubule-associated protein-τ (MAPT; TAU; FTDP-17) is a microtubule-binding protein that promotes microtubule assembly in healthy neurons. In AD patients, TAU becomes hyperphosphorylated, loses its normal physiological function and forms toxic TAU aggregates. In addition to β-amyloid (Aβ) plaques, AD is characterized by the accumulation of neurofibrillary tangles of hyperphosphorylated-τ 231 (p-τ 231), which also contributes to neurodegeneration.

Previously, a group led by Kun Ping Lu, professor of medicine at Harvard Medical School's **Beth Israel Deaconess Medical Center**, had found that protein peptidylprolyl *cis*/*trans* isomerase NIMA-interacting 1 (PIN1) binds and isomerizes p-τ 231, converting it from *cis* to *trans*.[2] The result was restoration of p-τ 231's ability to regulate microtubule assembly.

Based on those results, Lu and colleagues hypothesized that the *cis* form of p-τ 231 could be responsible for the toxic neurofibrillary tangle formation. The problem is that when p-τ 231 is synthesized chemically it exists almost entirely in the *trans* conformation.[3] Thus, antibodies developed using synthetic p-τ 231 as an antigen specifically recognize and eliminate the *trans* form.

Lu told *SciBX* that previous attempts to design antibodies that eliminate p-τ 231 pathology were ineffective because it was not known that each isoform had a different pathological function and that only the *trans* conformation of the protein was being targeted by the antibodies.

Now, Lu's team has manipulated the synthesis of p-τ 231 to lock up to 74% in the *cis* conformation. By doing so, the group was able to generate isoform-specific antibodies that targeted either the *cis* or the *trans* isoform without cross-reactivity.

In frontal cortical sections from nine healthy human brains, the antibodies detected very low levels of either *cis* or *trans* p-τ 231. In contrast, levels of *cis* p-τ 231 were elevated in brain samples from 11 AD patients and in 4 of 6 samples from patients with mild cognitive impairment. The latter findings suggested increased *cis* p-τ 231 could be an early indicator of AD.

Cis p-τ 231 had a longer half-life, greater stability and more resistance to dephosphorylation—all of which can lead to toxic aggregation—than the *trans* isoform.

Using *in vitro* assays, the group showed that adding PIN1 to p-τ 231 increased levels of *trans* p-τ 231 and decreased levels of *cis* p-τ 231. The isomerization by PIN1 further restored TAU-mediated microtubule assembly, confirming that TAU dysfunction is specifically caused by *cis* p-τ 231.

In a mouse model of AD with Tau overexpression and in Tau-overexpressing mouse neurons, Pin1 overexpression increased levels of *trans* p-τ 231 and decreased levels of *cis* p-τ 231 compared with normal Pin1 expression. Also in the AD model, Pin1 knockout caused an increase in pathogenic *cis* p-τ 231 and a decrease in *trans* p-τ 231.

"We have a way to develop new antibody technology that can be used to understand the very early stages of AD. Our antibody technology could be used for diagnostic or therapeutic applications," said Lu.

Early detection and intervention

Lu said his group's next steps include validating that the antibodies can be used to treat or prevent AD in animal models and testing whether they can identify patients with early disease.

"As Alzheimer's disease takes at least a decade to develop, early diagnosis and treatment of Alzheimer's patients before the onset of severe memory loss could offer doctors a much better chance of halting or even preventing" the disease, he added.

For example, he said, "we can use antibodies to detect *cis* p-τ 231 in spinal fluid and to determine whether the protein is a risk factor or indicator for early disease."

"Some indicators that a patient has AD include measures of phosphorylated TAU and β-amyloid above certain threshold levels. Detecting an increase in *cis* p-τ 231 could indicate that something is going wrong very early on and could be a complementary measure to the diagnostic tools that we already have," noted Gerard Griffioen, CSO of **reMYND N.V.**

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reMYND is developing therapeutics that prevent TAU toxicity. ReS19-T, one such compound, is in preclinical testing to treat AD and is being developed through a partnership with **Roche**.

Gerhard Koenig, SVP of research and CSO of **EnVivo Pharmaceuticals Inc.** agreed and added, "It will be key to establish the dynamic range in human cerebrospinal fluid. Additionally, it will be important to determine whether the levels of *cis* isoform are truly driving the TAU pathology as the authors suggest."

EnVivo has EVP-6124, a nicotinic acetylcholine receptor α7 (CHRNA7) agonist, in Phase II testing to treat AD. The company has exclusive worldwide rights to the compound from **Bayer AG**, and EnVivo has granted rights to **Mitsubishi Tanabe Pharma Corp.** in several Asian markets.

In addition to using anti–*cis* p-τ 231 antibodies as therapeutics, Lu thinks another approach could be to go upstream and increase PIN1 activity. "AD is complicated by other disease mechanisms as well, so blocking TAU pathology is an essential component but might not be a complete solution," he said. "PIN1 prevents TAU pathology, but in two earlier papers we also showed that it prevents β-amyloid pathology by converting APP [amyloid precursor protein] from a *cis* to a *trans* isoform."

The problem, according to Lu, "is that it isn't clear how to specifically increase PIN1 activity in neurons. We can do this in animal models genetically, but directly targeting a PIN1 activator in humans would be very difficult."

This is because "increasing PIN1 in proliferating cells can cause cancer, while increasing its activity in nondividing cells like neurons prevents neurodegeneration."

Lu told *SciBX* that Beth Israel has filed patent applications for the diagnostic and therapeutic applications of the antibodies as well as for *cis* p-τ 231–targeting vaccines made from the team's manipulated *cis* p-τ 231 protein for treating AD at early stages. The IP is available for licensing.

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