

# Promising protein research for neurodegenerative diseases

Since the discovery that neurofibrillary tangles are a hallmark present in Alzheimer's disease, **Professor Gloria Lee** has been at the forefront of research investigating tau, the protein that is implicated in the formation of these structures. Her work has contributed to elucidating both targets for therapeutic intervention and biomarkers for early diagnostic testing.



## Q&A

**Out of all the research you have worked on through your career on tau, what discovery was the most challenging to produce evidence for?**

Showing the association of endogenous tau with Fyn in neuronal cells was most challenging. This was almost 20 years ago – it was necessary to isolate a complex of tau and Fyn and finding the right conditions to break open the cells and to recover a lysate that contained the complex was time consuming. Looking back, it is likely that the complex was in lipid rafts (unknown at the time) and, for that reason, the use of detergent solubilisation was not straightforward.

**What do you think are the most promising avenues for therapeutic targets and intervention of the progression of Alzheimer's disease?**

I do not believe that the plaques and tangles are the best therapeutic targets. I would rather target an early aspect of the disease, which would mean targeting A $\beta$  oligomers or hyperphosphorylated tau or tau oligomers. I believe that A $\beta$  oligomers are activating abnormal signalling pathways that create abnormal forms of tau, which then go on to cause neurodegeneration. Disrupting the signalling pathway can be achieved by eliminating abnormal A $\beta$  or tau, or by identifying and eliminating other members of the activated pathways.

**How far are we from having early diagnostic testing available for Alzheimer's disease, and how will this relate to your research on tau?**

The ability to image abnormal tau using PET is under development and such a tool would be useful as an early diagnostic test. Various probes are currently being tested. The availability of patients with documented early Alzheimer's would be useful since we could then obtain plasma and other samples for use in testing. In particular, we would be interested to determine if tyrosine phosphorylated tau was an early marker in plasma. Tyrosine phosphorylation is characteristic of signalling events and adult tau is not normally tyrosine phosphorylated. When

we found that tau in Alzheimer's acquired tyrosine phosphorylation, this suggested that tau had acquired new properties, possibly participating in abnormal signalling pathways. We speculate that this happens early in the neurodegenerative process. While the imaging test would definitely be useful, developing an easier and less expensive test for early Alzheimer's would also be useful.

**What, in the context of your research, has been the most exciting technological advance that has occurred during your career and what impact did this have on your research?**

Many technological advances have occurred during my career and it is difficult to pick out the "most exciting" one. For instance, DNA sequencing was discovered when I was a graduate student and my thesis involved DNA and RNA sequencing. Among the many technological advances that have been made, we have extensively used "transfection" which is a technique where one can express an engineered protein (such as a tagged wild type, mutant, or fragment) in mammalian cells. Another advance was the ability to make monoclonal antibodies in mice. This allowed us to make an antibody that recognizes tau with phospho-tyrosine at a specific residue. Confocal microscopy is another advance that has been useful for us. The most recent, exciting technological advance that has aided our research is the development of the proximity ligation assay. This technique allows us to locate protein complexes in cells, using fluorescence microscopy.

**What are your hopes for future outcomes of your current research in the coming years?**

I hope to elucidate the function of the tau–Fyn interaction in the developing brain and in the neurodegenerative process. Given that Fyn is a tyrosine kinase, the interaction is likely to be involved in signalling. Knowing the function of tau–Fyn interaction in the neurodegenerative process would aid in identifying abnormal signalling pathways activated, thus giving us more targets for therapeutics and early diagnostics.

**A**lzheimer's disease, named after Alois Alzheimer who first described the condition in 1906, is the most common cause of dementia, accounting for 60-70% of cases. The development of the disease is associated with the formation of structures in the brain known as tangles and plaques, which are accompanied by the death of neuronal cells and loss of brain tissue.

Dr Gloria Lee, Professor of Internal Medicine at the University of Iowa, Carver College of Medicine, researches the protein tau and its involvement in Alzheimer's disease. The protein is critical for neuronal development and is the primary component found in the neurofibrillary tangles associated with the disease. Mutations in the gene encoding tau have also been linked to other neurodegenerative diseases including frontotemporal dementia. Therefore, understanding the structure and function of tau is of great importance to biomedical research, with far reaching implications for the development of treatments for neurodegenerative conditions.

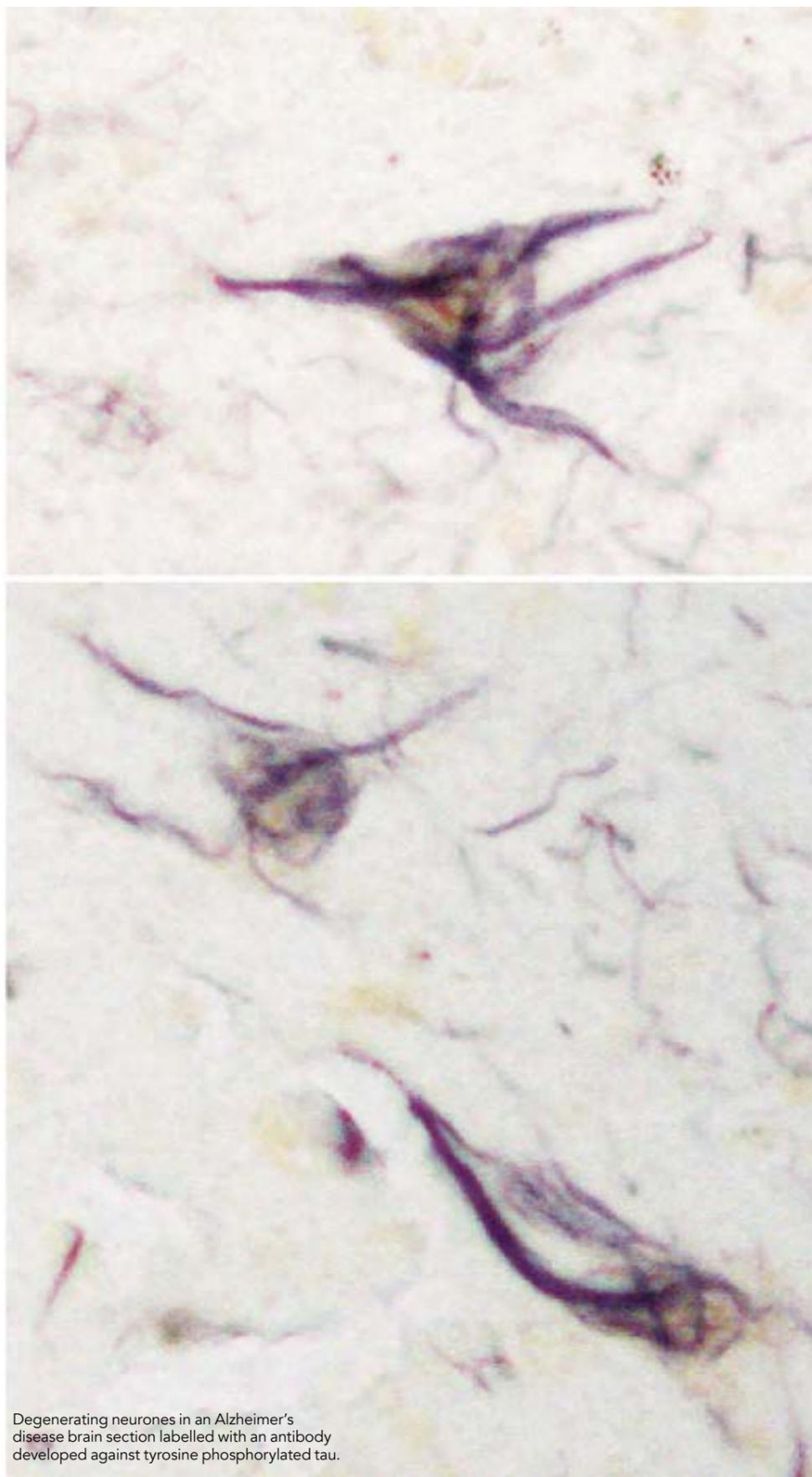
#### EXISTING CHALLENGES

Currently, there is no known cure for Alzheimer's disease, with no treatment available that can halt or reverse its progression. The disease is difficult to diagnose in the early stages, as the symptoms can be subtle, resemble other illnesses or appear similar to normal signs of ageing. As current methods of diagnosis are largely based on documenting clear signs of mental decline, by the time the disease is identified, severe brain damage will have already occurred. Therefore, it is essential that new ways of diagnosing the illness in its very early stages are found, in addition to targets for therapeutic intervention of the disease.

When Professor Lee first embarked on her research investigating tau more than 30 years ago, the protein had not yet been linked to Alzheimer's disease. Once the connection had been made, she says she, 'felt that tau would be important and understanding its uniqueness would give us some clues into its role in Alzheimer's disease'.

#### LAYING THE FOUNDATIONS

Her early research was focused on first elucidating the basic aspects of the protein itself, with her work being the first to describe the DNA sequence that encodes it. Armed with this knowledge, she delved further into studying the function of the protein. Lee felt



Degenerating neurones in an Alzheimer's disease brain section labelled with an antibody developed against tyrosine phosphorylated tau.

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that detailed structure and function studies of tau were essential in order to understand the microtubule binding properties of the protein, with the hope of providing the best chance of finding targets for therapeutic intervention.

Lee and her team identified a region of the protein that is comprised of repeats in the sequence that they demonstrated as having the ability to bind to structural components in cells, known as microtubules. Microtubules are vital cellular components integral to the cell cytoskeleton, which, as well as providing structural support to the cell, are involved in many dynamic cellular processes such as intracellular transport, mitosis and cell migration.

The distribution of microtubules varies between cell type and the highest concentration is found in cells in the brain, with tau proteins responsible for stabilising microtubules and promoting their growth. Lee and her research group identified the region of tau that is involved in binding the protein to microtubules and upon further investigating this region, they discovered that a specific part of this region is required to "nucleate" the formation of new microtubules, in addition to promoting the growth of existing ones. Other researchers used this knowledge to further investigate how the region is fundamental to microtubule binding. In Alzheimer's, the loss of tau's ability to stabilize microtubules has been thought to be critical to the neurodegenerative process. Therefore, understanding the molecular level through which tau acts is useful for developing agents to compensate for the loss of tau.

#### EXPANDING THE RESEARCH

Lee's research also led her to work on the first ever project to look at neurofibrillary tangles in an animal model using lamprey, a type of fish commonly used in biomedical studies. Together with Dr Garth Hall, they showed that genetically modifying the lampreys' cells to produce excessive quantities of human tau protein resulted in the tangle-like structures. This discovery spurred on other researchers to develop a mammalian system in which neurofibrillary tangles could be observed.

Professor Lee's group also investigated what other roles tau plays in cells, in isolation of microtubules. Lee explains that she chose to focus on one of the end regions of the protein as it was unique and the function of the domain was unknown. This led to the finding that tau can associate with the plasma membrane of cells, which occurs independently to what had been found regarding its microtubule binding ability. The plasma membrane of cells is a site of signal transduction for cells, where signals are received from sources outside the cell, interacting with proteins on the plasma membrane, which go on to influence events inside the cell.

#### IDENTIFYING A KEY EARLY PROCESS

Given tau's association with the plasma membrane, they have discovered that tau is also involved in cell signalling and identified several previously unknown interacting proteins. One of these interacting partners is known as Fyn, a tyrosine kinase, and they found that tau was phosphorylated on tyrosine as a result of this interaction. In addition, they found that mutant forms of tau, linked to other neurodegenerative diseases such as frontotemporal dementia, had increased association with Fyn. Her team also developed an antibody that recognises the phosphorylated form of tau created by Fyn. They have utilised this new antibody in their research to show that this modification occurs in both the developing brain and in cases of Alzheimer's. The antibody is now commercially available for research purposes and negotiations are in process regarding its use for immunotherapy.

Lee and her team continue to investigate the role that tau plays in the pathology of Alzheimer's. Currently they are investigating the role of tau in cell signalling during neuronal differentiation, hoping to reveal the mechanism by which this occurs. They are also continuing to delve deeper into the details of the interaction between Fyn and tau, hoping that their work will yield even more avenues for research into therapeutic intervention for neurodegenerative diseases.

## Detail

#### RESEARCH OBJECTIVES

Dr Lee's research focuses on the tau protein and its role in the development of Alzheimer's disease. Over 30 years of work she has helped to elucidate its structure and function as well as its role in neurodegeneration.

#### COLLABORATORS

- Roland Brandt, Professor in Germany
- Kiran Bhaskar, Assistant Professor in New Mexico
- Garth Hall, Associate Professor in Massachusetts

#### BIO

After receiving her PhD from Harvard University, Dr Lee completed her post-doc work at the University of California. When she started to work on tau in Marc Kirschner's lab, the connection to Alzheimer's disease had not yet been made. When the connection was made, she was offered a job in Neurology at Harvard Medical School, despite having no background in neurology or human disease. She continued to work on tau, certain that it would be important and that understanding its uniqueness would give some clues into its role in Alzheimer's.

Dr Lee moved to the University of Iowa in 1998, the same year that mutations in tau were found to cause frontotemporal dementia. She has continued to work on tau and her research has influenced others in the field.

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