Processing Brain Tissues from Post-mortem Patients for Alzheimer's Research

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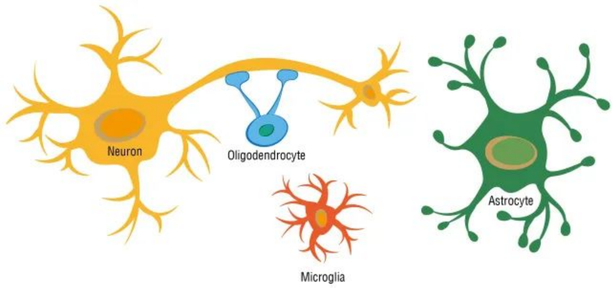
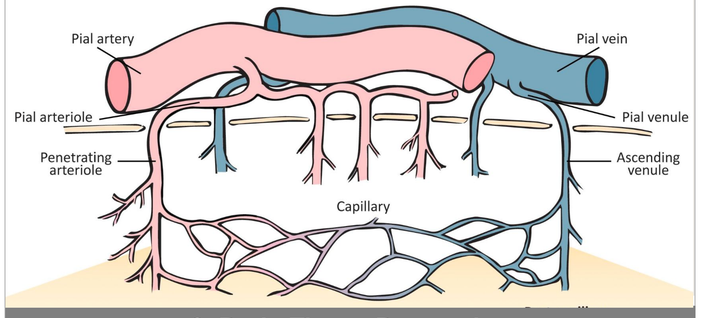
1. Abstract

Alzheimer's disease, the most common form of dementia, impairs memory and cognitive abilities. The apolipoprotein E4 gene (APOE4) is one of the biggest risk factors for AD. Studies have shown a strong correlation that those with the APOE4 allele have a significant risk of getting AD in later life. Research is currently attempting to find specifically how APOE4 contributes to the development of AD. One of the projects at the Yassine Lab focuses on the impact of high doses of omega 3 as a potential preventative measure of AD, and if inhibition of cPLA2 can prevent neuroinflammation in E4 carriers. cPLA2 is an enzyme that can produce lipid mediators of neuroinflammation, leading to AD. Understanding how cPLA2 activation relates to the APOE4 gene is vital to engineering new treatments for AD.

2. Objectives

- Preparing and processing brain tissue samples for AD research.





3. Brain Tissue Processing

Brain tissue samples → Samples are homogenized → Centrifuged at 1000 x g

after centrifuge: Tissue sample → 1st pellet / Supernatant

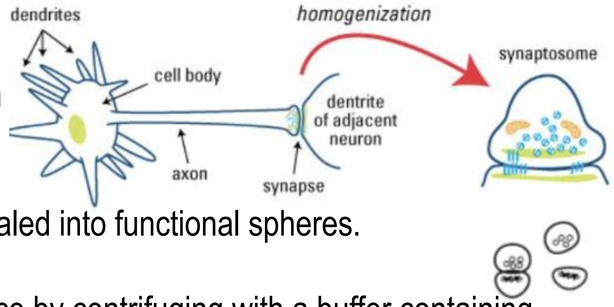
4. Processing 1st Pellet

1. The pellet from the first spin is separated from the supernatant and resuspended in microvessel isolation buffer (MIB).
1. Centrifuge again.
1. Supernatant is separate and collected as myelin-enriched fraction. (A protective lipid layer formed around nerves.)
1. The remaining pellet is again resuspended in MIB, and then filtered through a nylon filter.
1. The substance collected on filter is made up of cerebral microvessels.
1. This collection is washed off the filter with a buffer containing protease and phosphatase inhibitors.

Resulting Products: Myelin & Microvessels

5. Processing Supernatant

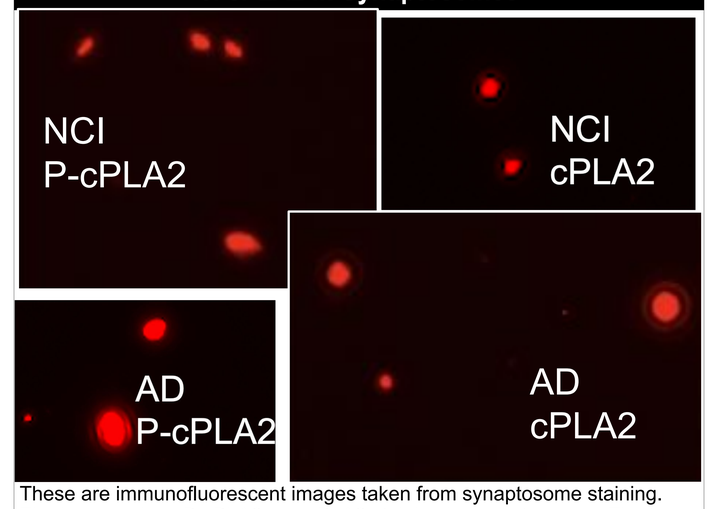
1. The supernatant from the first spin is collected and centrifuged again.
1. The supernatant from the second spin is collected as gliosome-enriched fraction (Major brain cells that perform metabolic and homeostatic tasks).
1. The pellet collected from the second spin is made up of crude synaptic terminals that have resealed into functional spheres.



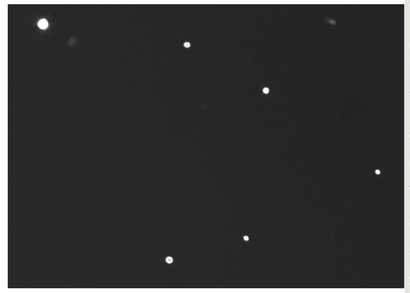
1. The pellet is washed twice by centrifuging with a buffer containing protease and phosphatase inhibitors.
1. The pellet is resuspended in a KR solution.

Resulting Products: Gliosomes & Synaptosomes

6. cPLA2 in Synaptosomes



These are immunofluorescent images taken from synaptosome staining. The images show P-cPLA2 and cPLA2 staining in synaptosomes. The two images on the top are synaptosomes from a patients without AD and the bottom two are from a patient with AD. This is relevant to understanding cPLA2 activation and signaling in synaptosomes in order to create a drug that can inhibit the cPLA2 enzyme.



This image is a from a cPLA2 staining from a patient with AD. At the Yassine lab, data analysis found the correlation that those with impaired cognitive abilities have higher amounts of cPLA2 in synaptosomes.

7. Blood and CSF Sample Processing

At the Yassine Lab, blood and cerebrospinal fluid processing is also conducted. This is vital to diagnosing AD in patients early on.

Conclusion

Processing tissue samples and blood samples is vital for furthering research and treatments for Alzheimer's disease. By processing brain tissue samples we are able to study the mechanisms of the synaptosomes in memory loss, allowing us further insight into neuronal function in the brain. Processing blood samples is important for finding new AD biomarkers which can be used for AD diagnosis.

References

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