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Department of Biology

Could viral proteins lead to treatments for Alzheimer's Disease?

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Background

Alzheimer's Disease(AD) is the most common form of dementia, causing symptoms of memory loss and decline of cognitive functions. The pathology is correlated with beta-amyloid plaques and tau protein tangles. A well documented function of tau protein is to stabilize microtubules; a foundational component of the cytoskeleton. However, there is growing evidence tau may have other functions in the cell such as stabilizing DNA and affecting ribosome production. Thus, the mechanism of pathogenesis due to tau modification is not well understood. When tau is hyperphosphorylated, its ability to carry out such functions can be altered (example shown in Figure 1). Previous experiments in our lab show cytomegalovirus affects tau protein phosphorylation(1). Cytomegalovirus (CMV), is a herpes virus found in 40-90% of the human population worldwide(2). Like other herpes viruses, there has been clinical correlation between CMV infection and increased risk of Alzheimer's Disease. Although at later times post CMV infection there is increase in high molecular weight tau, we saw earlier time points show a possible decrease in some tau isoforms. My project aims to confirm this apparent early decrease in additional cell types and characterize the mechanism. Such an understanding could lead to therapeutic treatment for this complication of Alzheimer's Disease.

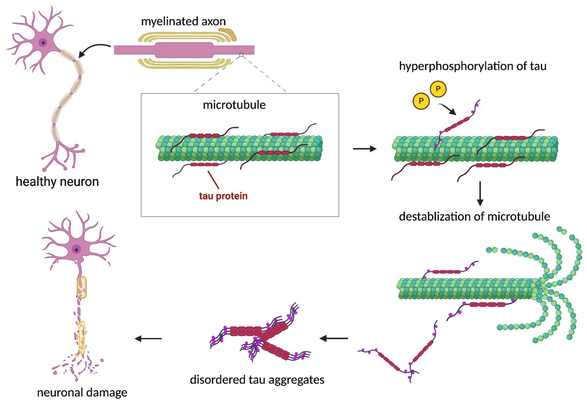

Figure 1: Mechanism of Tau Hyperphosphorylation

Western Blot Results

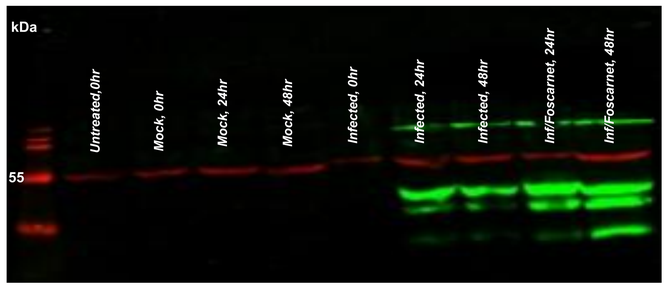

Figure 2: Confirmation of viral infection
Western blot was performed as described in methods; the red is mouse anti-α tubulin and the green in rabbit anti-E1. E1 is an early viral protein, with at least 4 isoforms, and is expressed within 4 hours of viral infection. Immunoblot confirmed viral infection in MCMV-infected cells.

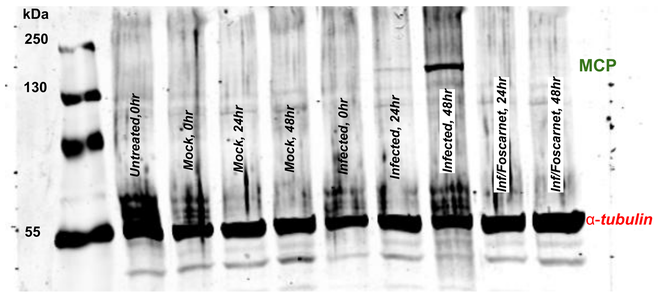

Figure 3: Foscarnet treatment
Western blot was performed as in Figure 2 except rabbit anti-major capsid protein (MCP) was used instead of rabbit anti-E1. MCP is a late protein expressed after viral DNA replication. Because foscarnet inhibits DNA replication, no late genes should be expressed in treated samples. Immunoblot confirmed MCP presence only in the MCMV-infected B35s without foscarnet.

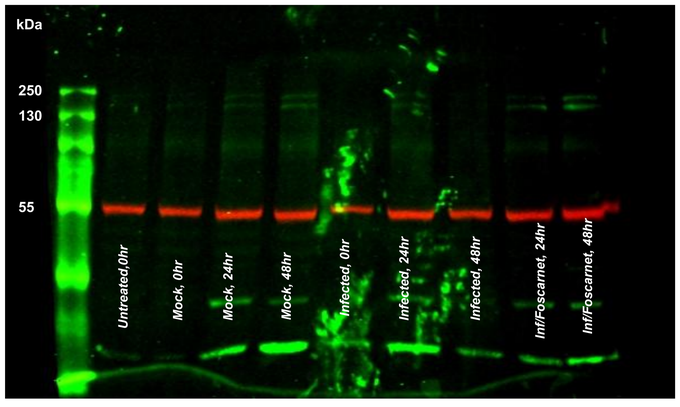

Figure 4: Total tau levels
Western blot was performed as in Figure 2, except used rabbit anti-total tau instead of rabbit anti-E1. In this initial western blot we did not see the initial increase in high molecular weight tau like we had seen in previous experiments.

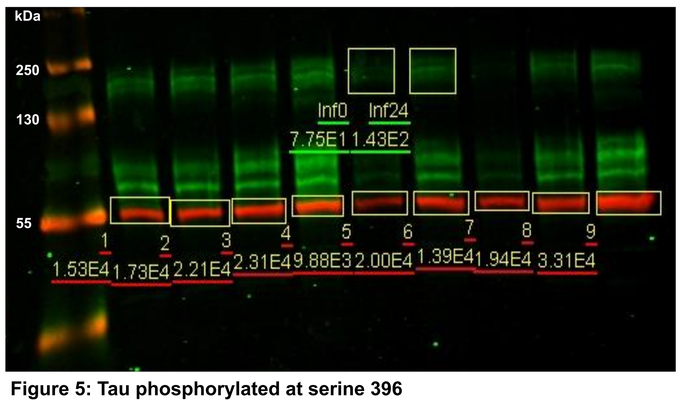

Figure 5: Tau phosphorylated at serine 396
This blot, that was subsequently probed for MCP in Figure 3, was first probed for serine 396 on tau as shown here. Tau protein has a known phosphorylation site at the amino acid serine, at position 396. The red is mouse anti-α tubulin and the green is rabbit anti-tau S396.
The infected sample at 48 hours was contrary to previous experiments as lower relative phosphorylation is observed instead of higher phosphorylation.

Methods

B35 cells were seeded into 60mm tissue culture dishes at $7x10^5$ cells/dish in standard B35 media. Twenty four hours later, the media was removed and replaced with murine cytomegalovirus (MCMV) at 2 infectious particles per cell or plain media (mock) at equal volumes. These were incubated for one hour with rocking to allow viral binding and entry. Following the hour incubation, the inoculum was replaced with fresh media. Untreated had no media change. Foscarnet, an inhibitor of viral DNA replication, was added to some of the plates with fresh media. Plates were incubated at 37˚C until harvested with scraping in western lysis buffer at indicated times and then frozen at -80˚C until use. I conducted western blots with equal volumes of sample and loaded them on 10% SDS-PAGE gels and ran with a PageRuler Plus protein ladder until the dye front fell off to separate the proteins. Proteins were transferred to nitrocellulose membranes before blocking and probing with the antibodies indicated in the results. All blots were probed for mouse anti-α-tubulin (~50 kDa) as a loading control to compare relative number of cells loaded. Various additional antibodies were used to probe for viral and cellular proteins.

Current Conclusions

- Unlike previous experiments, hyperphosphorylation of tau post infection was not seen in these samples.
- Foscarnet did not cause clear changes in tau after CMV-infection in rat neuroblastoma cells.

What next?

We would like to investigate a wider range of time points of MCMV infection in B35s to further understand at which point post infection phosphorylation and tau modifications occur.

Cellular localization of tau throughout these time points may also provide insight as to functions of intracellular tau as well as how the cell reacts to MCMV infection the possible role this plays in tau hyperphosphorylation.

If we don't see the decreased phosphorylation under early conditions in MCMV-infected B35s like we did in fibroblast cells, there may be differences in what mediates decreased phosphorylation of tau in different cell types.

Acknowledgements

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References

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(2) Fowler, K., Mucha, J., Neumann, M. *et al.* A systematic literature review of the global seroprevalence of cytomegalovirus: possible implications for treatment, screening, and vaccine development. *BMC Public Health* 22, 1659 (2022).