Finding Biomarkers for Early Alzheimer's Detection

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is always fatal. AD is pathologically defined by the presence of amyloid plaques composed of the Ab peptide, and neurofibrillary tangles composed primarily of the microtubule-associated protein tau. There are no current treatments or cure for AD and no new therapies have been approved in 20 years. Indeed, clinical trials during this time have all failed. One hypothesis for these failures is that the experimental treatment does not begin until late in the disease and tangles/plaques are already formed. Thus, a major objective is to identify people earlier in the disease progression so that experimental drugs can be tested earlier, during the preclinical stages. The purpose of the current research is to identify a biomarker from human cerebral spinal fluid that would be used for early detection of AD. To attain this goal, we have used phage-peptide display to screen for the presence of abnormal forms of tau protein. Because tau is so closely linked to AD progression, we hypothesize that post-translational changes to tau are occurring through the course of the disease. ELISA screening is ongoing to determine the specificity and sensitivity of candidate phage using the approach.

BACKGROUND

Alzheimer's disease (AD) affects nearly 5.4 million Americans and as the population gets older, this number is expected to double by 2050[1].

In the present study, the focus is on assessing and measuring post-translational modifications to tau that are thought to be linked to disease progression (See Table 1).

Tau has been selected as the biomarker of focus in this study for several reasons, including:

- Tau changes, like amyloid, are pre-clinical and appear to remain present in confirmed Alzheimer's patients
- There is significant research suggesting AD-mediated changes to tau exist such as phosphorylation, oxidation, proteolysis and others[2] as seen in **Table 1**.

Post-Translational Modifications
Glycosylation
Acetylation
Phosphorylation
Nitration
Oxidation
Proteolysis

Table 1. List of potential tau post-translational modifications. Tau can be modified post-translationally by a number of different pathways.

AIM

This project aims to develop a selective and specific tau-based screening profile to aid in the diagnosis of Alzheimer's Disease. We propose an innovative study to use phage display libraries for the identification of phage that can specifically and selectively bind to disease-relevant forms of tau.

This study will include:

- Selection and characterization of phage, testing and optimization of quantitative approaches to maximize assay speed and sensitivity, and testing of selected phage to human CSF samples.

METHOD

This project will focus on utilizing a sandwich ELISA method to identify novel biomarkers of tau. This process is shown in **Figure 1**[3] and outlined below:

Tau protein is isolated from human CSF, and then screened using our phage-display library. The phage are incubated and washed. We then elute this and save for amplification.

This process is repeated two times after each round of amplification. The amplified phage is then sequenced for identification. This process is repeated using the amplified phage two more times to obtain the most specific phage from the original library. The amplified phage is then sequenced for identification so that we may know which phage have high affinity for the relevant tau protein.

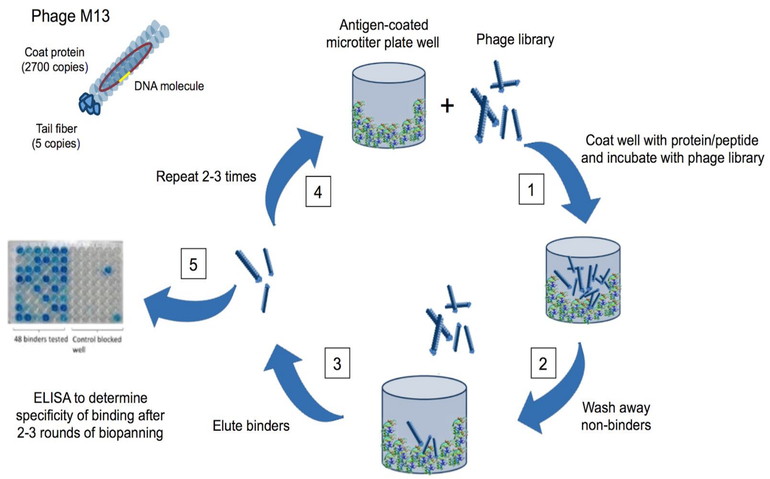

Figure 1: General ELISA methods used to isolate high affinity binders to tau protein found in Alzheimer's Disease

CONCLUSIONS

At the conclusion of this study, we expect that using a phage-display approach will lead to a low-cost, rapid, array-based test targeting multiple disease-relevant PTMs of tau. This test will expand on current biomarker strategies, so clinicians can identify at-risk populations for AD.

A variety of phages have been isolated that appear to bind preferentially to either AD or control CSF, and blood samples are currently being tested.

While we will be testing this array in a clinically diagnosed AD population, future studies to refine this work my screening patients with dementia and following them to see if the biomarker is linked.

Future studies will also focus on screening blood from AD patients against CSF to compare for changes between these biofluids or to use blood in place of CSF.

Lastly, it may be discovered that tau markers established here are insufficient to discriminate AD from other dementias and that other proteins may be better suited.

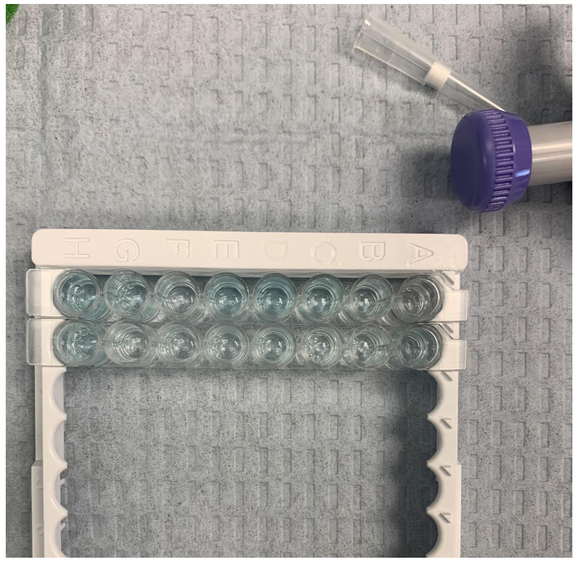

Figure 2. A colorimetric assay is formed with AD samples and control.

ACKNOWLEDGEMENTS

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