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Program: PSM Cell and Molecular Biology

Internship Site: Michigan State University

Semester of Completion: Winter 2023

Table of Contents:

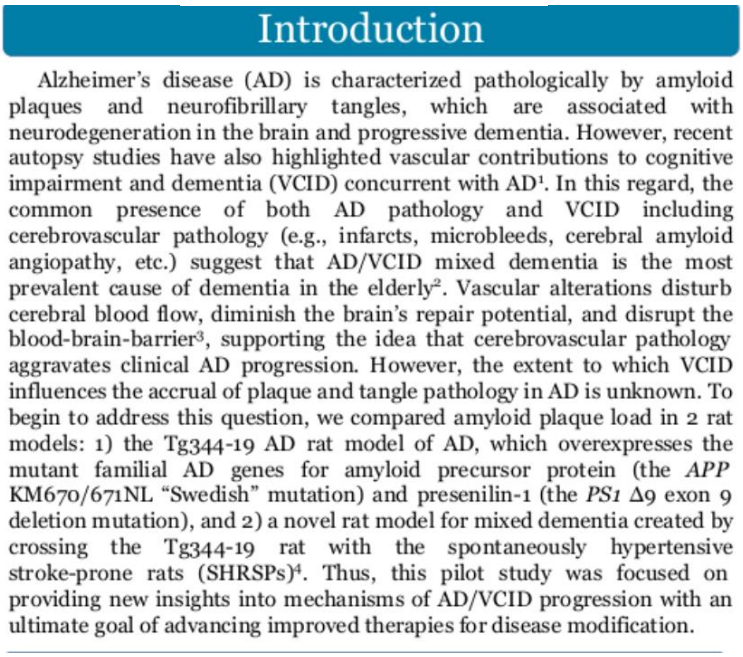
Learning Objective/Internship Objectives

Immunohistochemistry

- AxioScan (Machine Learning)
- HALO (Machine Learning & Data Collection)
- Aseptic Technique
- Good Documentation Practice (GDP)

Introduction

[1] Title of internship



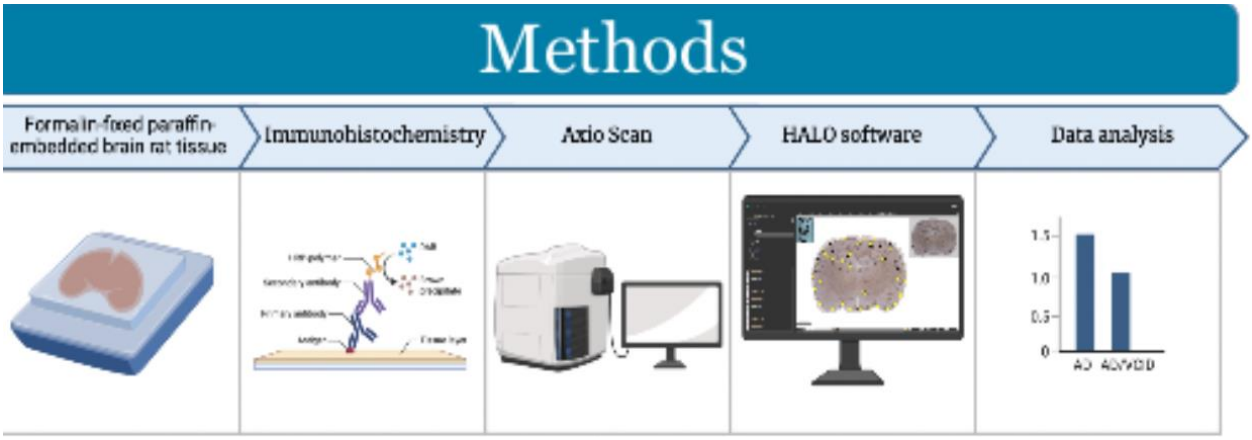
Introduction

Alzheimer's disease (AD) is characterized pathologically by amyloid plaques and neurofibrillary tangles, which are associated with neurodegeneration in the brain and progressive dementia. However, recent autopsy studies have also highlighted vascular contributions to cognitive impairment and dementia (VCID) concurrent with AD[1]. In this regard, the common presence of both AD pathology and VCID including cerebrovascular pathology (e.g., infarcts, microbleeds, cerebral amyloid angiopathy, etc.) suggest that AD/VCID mixed dementia is the most prevalent cause of dementia in the elderly[2]. Vascular alterations disturb cerebral blood flow, diminish the brain's repair potential, and disrupt the blood-brain-barrier[3], supporting the idea that cerebrovascular pathology aggravates clinical AD progression. However, the extent to which VCID influences the accrual of plaque and tangle pathology in AD is unknown. To begin to address this question, we compared amyloid plaque load in 2 rat models: 1) the Tg344-19 AD rat model of AD, which overexpresses the mutant familial AD genes for amyloid precursor protein (the *APP* KM670/671NL "Swedish" mutation) and presenilin-1 (the *PS1* Δ9 exon 9 deletion mutation), and 2) a novel rat model for mixed dementia created by crossing the Tg344-19 rat with the spontaneously hypertensive stroke-prone rats (SHRSPs)[4]. Thus, this pilot study was focused on providing new insights into mechanisms of AD/VCID progression with an ultimate goal of advancing improved therapies for disease modification.

[2] Introduction to internship

The objective of this internship was to continue with the progression of a project that was started in the Summer of 2022. The project compared the development of Alzheimer's disease in 3-month, 6-month, and 12-month mice using 3 distinct models. The three mouse models were labeled FrankenRat (FR) which was the combination of Alzheimer disease positive (AD^+) mice that were bred with vascular disease positive (VD^+) mice to produce the labeled FrankenRat (FR). The FR_AD_TG^+ model contained mice carrying both Alzheimer's disease and vascular disease. The FR_AD^+_TG^- is the second model of mice that contained Alzheimer's disease but did not carry the transgene. Lastly, FR_AD_TG^- model that contained neither Alzheimer's nor vascular disease, this model served as the control for the study. The previous intern focused only on male and female rats within the 9-month model. However, for this project all three models from all three groups were used to rapidly advance the project to near completion.

Description of Work



Following the format of the previous project, all three groups of models had been sent off to a third party sectioning company which sectioned and embedded the brain tissued onto formalin-fixed paraffin slides. All slides where then taken through a 30-step immunohistochemistry procedure. This procedure was crucial in eliminating non-specific binding sites and applying our primary/secondary antibody to observe the potential altercations in brain chemistry from all three models. Specifically, in defining the altercation that the FR_AD_TG^{+} model has in relation to the accrual of plaque and tangle pathology in AD. After ICH was successfully performed, all slides where then placed into AxioScan. AxioScan is a software that carefully observes each tissue to create a precise digital replica. After digitally imaging all slides, images are then uploaded into HALO. HALO is another software that allows the user to edit the digital image and identify regions of interest while further eliminating any background noise that might interfere with the observation of AD pathology. After editing all slides within HALO, an analysis is then run through the software. This analysis will eventually allow the researcher to develop conclusions to compare with the initial hypothesis, as well as create and depict figures using the newly gathered data. The specific statistical analysis that will be used is Two-Way ANOVA.

Internship Discussion

At the conclusion of the internship, we were able to successfully perform IHC on all slides and transition them into AxioScan. After slides were digitally imaged we began editing the images slides with HALO but did not finish. The skills that I learned throughout this experience consisted of a mixture of technical and professional skills. The technical skills learned were centered around immunohistochemistry, dilutions, and machine learning via AxioScan and HALO. The professional skills I took away from this experience included effective communication, teamwork, the ability to work independently, and networking. Before starting the PSM program, I took part in this same internship when it was in it's infancy stage. The PSM program laid a solid foundation of fundamental skills and understanding so that when I returned, it was with a broader skillset and knowledge of the methods and technical skills being used. This allowed me to effectively contribute to the progression of the research. Some of the challenges I faced in the duration of the internship was dealing with some technical skills such as cover slipping and some mathematical formulations. I was able to overcome both through the connections I had built and maintained throughout the experience. I learned new ways to improve my cover slipping techniques and also new perspectives to consider when doing certain formulations of dilutions of components. Overall, this experience not only pushed me to grow in areas where I was weak, but it also equipped me with the tools and mindset needed to thrive within the industry upon graduating.