REDUCING EXCESSIVE MICROGLIA ACTIVATION TO SLOW NEURODEGENERATION

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EXPLORING METHODS TO COMBAT ALZHEIMER'S DISEASE

INTRODUCTION

The hallmark symptoms of Alzheimer's disease (AD) are: neuroinflammation, oxidative stress and cholinergic neuron injury, all simultaneously contributing to the neurodegeneration of the brain. Investigating how to minimise excessively activated microglia cells through the epigenetic modification of biochemical and cellular mechanisms, is one of the methods to significantly reduce the progression of the disease, as over-activation of these cells accelerates the rate of degeneration. In focusing on the most heavily researched NF-kB subunit, RelA, I sought to investigate how the overactivation of NF-κB signalling not only promotes tumour cell survival, genomic instability, and metabolic abnormalities, but also reshapes the immune-suppressive microenvironment, promoting immune escape and resistance to immunotherapy.

KEY WORDS AND DEFINITIONS

- **Microglia cell**: neuronal support cell that mediates immune responses by acting as a macrophage, clearing cellular debris and dead neurons from nervous tissue
- **DNA methylation/acetylation**: process of regulating gene expression by recruiting methyl or acetyl groups to selectively inhibit the binding of transcription factors to DNA.
- **HATS/HDACs**: Histone deacetylases are enzymes that remove acetyl groups from histones and non-histone proteins, affecting gene expression.
- **Epigenetics/epigenetic modification**: Epigenetics is the study of how cells control gene activity without changing the DNA sequence. These can be modified within cells to observe how cell activity is affected.
- **NF-kB**: Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a family of transcription factor protein complexes that controls transcription of DNA, cytokine production and cell survival.
- **Histone**: a protein that provides structural support for a chromosome; a highly basic protein abundant in lysine and arginine residue

OBJECTIVES

- As there is a variety of complex methods to reduce excessive neuroinflammation, we focused on one protein and modifying it using epigenetic mutations, such as acetylation and methylation.
- Through this and researching other methods of modification, I hoped to contextualize my lab work and to investigate how we can begin to simplify the complexity of finding a cure for AD; through lifestyle choices, epigenetic modifications and drug development

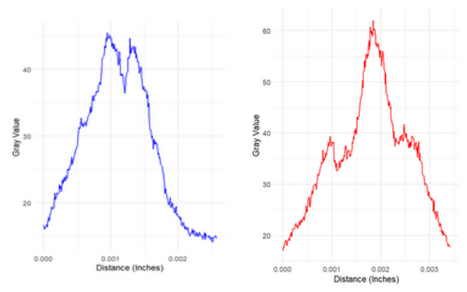
METHODOLOGY

- Utilising a wide range of techniques including cell culturing, fluorescence microscopy, and gene cloning, we transfected BV-2 microglia cells with a plasmid expressing a NF-kB-GFP fusion protein which emits fluorescence. We also transfected cells with mutated versions of this fusion protein through modifying specific lysine residues to see how acetylation affects the localisation of the NF-kB and subsequently, the activation of the microglia cell. Once we transfected the cells with the fusion protein, we activated some of the microglia cells and analysed them under a fluorescence microscope to see if the mutations had affected the localisation of the NF-kB subunit. I informed my research through conducting literature reviews to contextualise what current cures are being investigated, as there are a range of epigenetic modifications being explored.

- After quantifying and plotting the data, I used R studio to measure the total area under the curve then the area under the curve, which I approximated, was the nucleus. As the plot represented the intensity across the whole cell, I needed a method to compare the localisation of the fluorescence, and therefore, the RelA mutants. I substituted what data was necessary to create the plot and calculate the area under the curve (AUC).

- The resulting plot of Gray Values after creating a cross section of the cell images to quantify the fluorescence and to compare how it varies in a cell.

RESULTS



WITHOUT LPS

MUTANT TYPE	AUC	NUCLEUS AUC
K314R (A)	0.04231	0.01591
K314R (D)	0.02818	0.01546
K314R (F)	0.07552	0.03245

WITH LPS

MUTANT TYPE	AUC	NUCLEUS AUC
K314R (D)	0.02539	0.04307
K314R (E)	0.0673	0.04571
K314R (H)	0.04779	0.03222

DISCUSSION

- When comparing the plots in Figure 4., the fluorescence varies greatly when comparing the cytoplasm and nucleus. In the left plot, there is a visible decline in the Gray Value (fluorescence) at the area that represents the cell nucleus. In comparison to the right plot, there is a sizable increase of fluorescence in the cell nucleus.
- Acetylation on the lysine residues 122 and 123 reduces binding of NF-κB to the DNA κB enhancer and facilitates binding to IκBα and subsequent export from the cytoplasm. Acetylation at lysine residues 314 and 315 do not affect the general transcriptional activity of NF-kB but it modulates the expression of specific sets of genes.
- As can be seen in Figure 2. and Figure 3., the location of the NF-kB-GFP fusion protein changes when comparing cells activated by LPS and the resting cells. As the expected impact of K314 residue mutations is a change in what specific genes are expressed, it is expected that the NF-kB should still move into the nucleus after activation of the cell via a stimulus. After collecting the images of the successful transfected cells, I used ImageJ to quantify how the intensity of the fluorescence, or the Gray Value, differs in different areas of the cell. As I found some difficulty with culturing the cells, the transfection and translocation in some of the mutants was not successful, therefore, I only gained viable results from the K314R mutants. Though this was very much a downside in my project as I lost the ability to compare how different mutants interacted within an activated cell, the experience and results I gained were formative and I believe representative of previous research data.
- Upon analysing the accumulation of this data, it can be concluded that direct acetylation and deacetylation of specific lysine residues in the p50/p65 subunits of NF-kB play an important regulatory role in the functions of the transcription factor. This indicates that the resulting effect of both HAT and HDAC inhibition depends on the selectivity for specific NF-κB acetylation sites. The crucial role of NF-κB acetylation and deacetylation in the regulation of NF-κB-mediated gene expression raises the idea to modulate inflammatory responses by modulating NF-κB acetylation levels with HAT and HDAC inhibitors.
- Figure 5. The table above presents the individual cell images that were measured to quantify the fluorescence. In the first table, the cell mutants that had not been activated by LPS were measured. The total area under the curve (AUC) presents how much of the fluorescence is present in the cell. The higher the number, the higher the average fluorescence across the cell. As we were attempting to discern how the fluorescence, and therefore the mutants, changed after activation of the cell, I also compared the approximated area of the curve that represents the nucleus. When comparing the average AUC of the cell and nucleus between activated and inactivated mutants, on average the fluorescence in the nucleus increases as the K314R mutant does not necessarily impact localisation of the protein, but it impacts what genes are expressed. Subsequently, though the cells still seem to be successfully activated, further research would be required to investigate whether gene expression remains the same.

FUTURE OF THE FIELD

- Recent evidence has suggested that lifestyle has just as much of an impact as genetics. Sleep, diet, and suffering from other cardiovascular diseases has been shown to increase the risk of developing AD. Therefore, it can be argued that a cure is not only rooted in epigenetic modifications and inflammatory suppressants, but also early in our lives where we still have a chance to make a difference. More advanced and closer to the real human environment research methodologies are required, encompassing spatial multi-omics, single-cell sequencing, organoids, genetically engineered animal models, 3D bioprinting, and other cutting-edge techniques.
- Florida based biotechnology company Vaxxinity are planning to begin phase three clinical trials on their most advanced vaccine against AD, UB-311. By 2030, the vaccine could revolutionise how we deal with AD and cognitive decline. In contrast, Massachusetts based biotech company, Biogen, has recently begun developing a new monoclonal antibody alongside Japanese firm, Eisai. The new drug, Lecanemab, can produce significant reductions in cognitive decline.

LIFESTYLE

- It has been increasingly made apparent that our cardiovascular health, and how we maintain it, can influence our chances of developing AD, with various autopsies signifying that 80% of people with AD also suffer from cardiovascular diseases. This includes high blood pressure, diabetes, obesity, high cholesterol, all possible contributors to dementia. Studies have shown that it is the maintenance we do when we are young that can truly impact our health as we age. Not only is it our physical health that can influence this likelihood, but also maintaining our neural plasticity and cognitive reserve.

- Neural plasticity is the ability to constantly remake new synapse connections, through learning new skills and seeking out new experiences. In maintaining this habit, even as you age, it can prove to be a sufficient buffer as ageing depletes your synapses as well as making it more difficult to form new synapses. Cognitive reserve is the amount of excess functional synapses an individual has, which can also act as a buffer for those that develop dementia. Ideally, learning new things and building more cognitive reserve is not done through simply learning and retrieving information; ideally, they should be as deep as possible, engaging your emotions and senses consistently.

CONCLUSION

Summary of possible epigenetic modifications to combat AD:

DNA Methylation:
- Alterations in DNA methylation are linked to AD. Increased methylation is seen in genes such as PSEN1, APOE, and MTHFR.
- DNA methylation is managed by DNA methyltransferase (DNMT) enzymes, but targeting these for treatment is challenging due to the lack of specific inhibitors.

Histone Modifications:
- Histone deacetylase (HDAC) enzymes play a role in AD pathology, with increased expression of HDAC2 and HDAC3 negatively impacting cognitive function.
- HDAC inhibitors have shown promise in improving cognition in AD models, though they do not prevent neuronal degeneration.

Non-coding RNAs (ncRNAs):
- Altered expression of ncRNAs, especially microRNAs, is linked to AD. For example, miR-29 and miR-16 regulate the expression of genes involved in amyloid pathology.
- Targeting ncRNAs offers therapeutic potential, but delivery challenges remain a barrier.

Summary of possible lifestyle and medicinal changes to combat AD:

1. Monoclonal antibodies (Lenecamb)
2. Vaccine (UB-31)
3. Increasing neural plasticity
4. Increasing Cognitive reserve
5. Reducing risk of cardiovascular disease
6. Regular exercise and sleep