Alteration of the Intracellular Trafficking in Alzheimer's Disease by Apolipoprotein E

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Abstract

Carrying apolipoprotein E (ApoE) ε4 allele is currently the highest known risk factor for Alzheimer's disease. ApoE has 3 alleles, ε2, ε3, and ε4, which increases Alzheimer's disease risk. ApoE forms lipoproteins that transports cholesterol and other lipids through the bloodstream and in the extracellular space. ATP Binding Cassette Subfamily A Member 1 (ABCA1) facilitates lipidation of ApoE. It is known that APOE4 clumps up more than APOE3 and APOE2. We hypothesize that this APOE4 clumping will affect the intracellular transport of ABCA1. The goal of this study is to differentiate induced pluripotent stem cells into astrocytes and to analyze how the different ApoE genotypes affect intracellular transport of ABCA1 inside of the astrocytes. Our analysis showed that in human APOE4 astrocytes, recycling of ABCA1 to the cell membrane was decreased and, ABCA1 accumulated within late endosomes and lysosomes. This study demonstrated that iPSCs could be differentiated to astrocytes; and image analysis was a feasible technique to analyse intracellular trafficking of ABCA1.

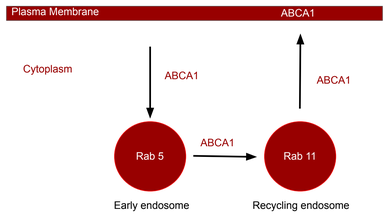
Introduction

Alzheimer's disease, the most common cause of dementia being 60 to 70 percent of cases, currently affects 6.7 million Americans alone over the age of 65. Alzheimer's disease is characterised by its amyloid plaques that are caused by abnormal clumps of β-amyloid, neurofibrillary tangles from abnormal aggregations of a protein called tau in neurons, and chronic inflammation caused by built up in glial cells.

The highest known risk factor for Alzheimer's disease is carrying the ε4 allele of apolipoprotein E (ApoE). ApoE has three alleles ε2, ε3, and ε4. ε3, the most common allele, has less risk of Alzheimer's disease compared to ε4. Whereas the ε2 allele decreases the risk of Alzheimer's disease. ApoE is a protein that forms lipoproteins that transports cholesterol and other lipids through the bloodstream and in the extracellular space. The major cell type that produces ApoE in the brain is astrocytes. Astrocytes are cells found within the nervous system that are a type of glial cell. They create the environment for other cells by holding nerve cells in place for them to develop and work correctly. ATP Binding Cassette Subfamily A Member 1 (ABCA1) is produced in astrocytes. ABCA1 facilitates the shuttling of ApoE into early endosomes which leads to lipidation, loading of lipids and cholesterol to the ApoE forming lipoprotein particles.

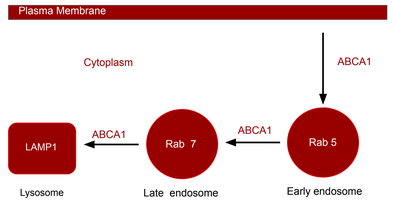
The goal of this study is to analyze how the different ApoE genotypes affect intracellular transport of ABCA1 inside of human astrocytes. Under normal conditions ABCA1 is transported from an early endosome to a recycling endosome and finally gets back to plasma membrane as seen in figure 1.

Figure 1: Transportation of ABCA1 inside an astrocyte with ApoE3



We hypothesize that ABCA1 is more likely to get clogged in ε4 carriers. Due to the clogging, ABCA1 will be transported from an early endosome to a late endosome and then to a lysosome as seen in figure 2.

Figure 2: Transportation of ABCA1 inside an astrocyte with ApoE4



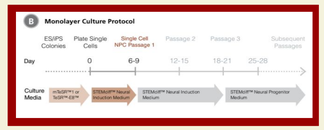
We were able to study human astrocytes by differentiating induced pluripotent stem cells (iPSCs) into astrocytes. iPSCs are cells reprogrammed from skin cells that becomes pluripotent stem cells which can the be differentiated as seen in this experiment. This allowed us to analyze the process of the transportation of ABCA1 between intracellular compartments.

Materials and Methods

Induced Pluripotent Stem Cells (iPSC)

- NPCs were generated from isogenic human iPSC lines obtained from the Jackson Laboratory. The cell lines carry the exact genetic material other than one has the ε3 allele and the other was modified to have the ε4 allele.

Neural Progenitor Cells (NPC) and Astrocytes



- In the monolayer culture protocol, single cell hPSCs are resuspended in STEMdiff Neural Induction Medium and cultured as a monolayer. Following neural induction, NPCs are expanded using STEMdiffTM Neural Progenitor Medium.
- To generate astrocytes NPC were plated on matrigel-coated plates and were grown in astrocyte medium (ScienCell) for 40 days.

Induced Pluripotent Stem Cells (iPSC)

Colocalization:

- Using Magic wand select red channel
- Select all red outside of the nucleus changing tolerance until the late endosomes or lysosomes are outlined
- Repeat on all quantifiable cells
- Save ROI
- Run Coloc 2
- Save wanted data
- Run T-Test

Signal Intensity:

- Outline high intensity areas indicating recycling endosomes
- Select ROI of the same size to normalize ABCA1 intensity for each cell.
- Quantify ABCA1 signal intensities in both ROIs
- Run T-Test

Results

Differentiation

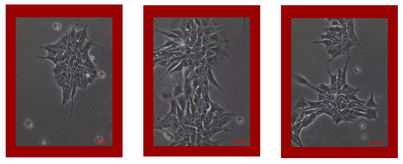


Figure 3: Induced pluripotent stem cells photo taking at 10x on day 0 of their differentiation.

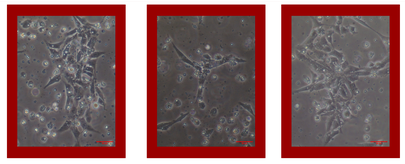
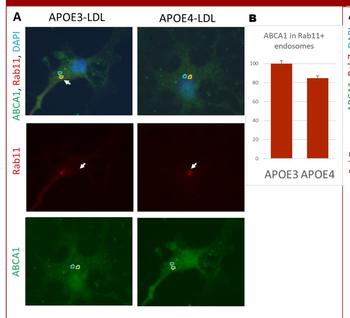
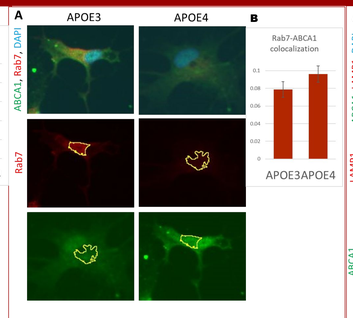


Figure 4: Differentiating IPSCs photo taking at 10x on day 1 of their differentiation.

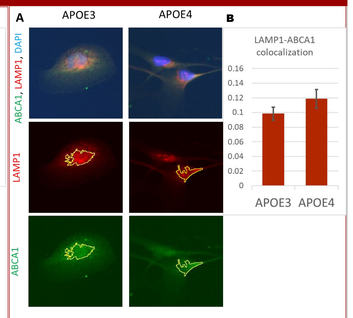
Image Analysis



A) Astrocytes were stained for ABCA1 (green) and Rab11 (red). Rab11$^+$ recycling endosomes (arrow; yellow outline).B) Standardized ABCA1 for each cell was plotted. *APOE3*-LDL n=125, *APOE4*-LDL n=134; 4 cell lines (2 *APOE3* and 2 *APOE4* lines). T-Test: $p < 0.05$



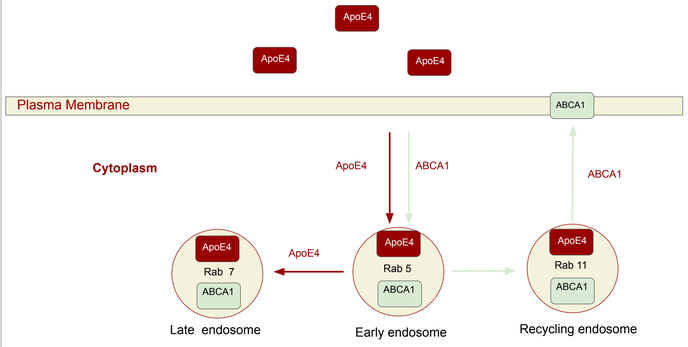
A) Astrocytes were stained for ABCA1 (green) and Rab7 (red). A Rab7$^+$ recycling endosomes rich area (yellow outline) B) Li's ICQ value between ABCA1 and Rab7 was calculated. *APOE3*-LDL n=70, *APOE4*-LDL n=66; 4 cell lines (2 *APOE3* and 2 *APOE4* lines). T-Test: $p = 0.093$



A) Astrocytes were stained for ABCA1 (green) and LAMP1 (red). A LAMP1$^+$ lysosome rich area (yellow outline). B) Li's ICQ value between ABCA1 and LAMP1 was calculated. *APOE3*-LDL n=26, *APOE4*-LDL n=64; 2 cell lines (1 *APOE3* and 1 *APOE4* lines). T-Test: $p = 0.051$

Conclusion

- In conclusion this study demonstrated that:
  - iPSCs could be differentiated to NPCs and NPCs to astrocytes;
  - iPSCs allowed studying human neurological diseases in a dish; and
  - Image analysis was a feasible technique to analyse intracellular trafficking of ABCA1.
- Our analysis showed that in human APOE4 astrocytes,
  - recycling of ABCA1 to the cell membrane was decreased,
  - ABCA1 accumulated within late endosomes and lysosomes
- In combination with previous data, these results suggested that ABCA1 was more likely to get stuck in the intracellular compartments in ε4 homozygote astrocytes than the ε3 homozygote astrocytes, resulting in degradation of ABCA1.



References

- *Astrocytes. National Cancer Institute*,www.cancer.gov/publications/dictionaries/cancer-terms/def/astrocyte. Accessed 19 July 2023.
- Bhuin, Tanmay, and Jagat Kumar Roy. "Rab11 in Disease Progression." IJMCM, winter 2015. National Library of Medicine National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/pmc/articles/PMC4359700/. Accessed 19 July 2023.
- "Alzheimer's Disease Facts and Figures." *Alzheimer's Association*. *Alzheimer's Association*,www.alz.org/alzheimers-dementia/facts-figures#:~:text=More%20than%206%20million%20Americans%20of%20all%20ages%20have%20Alzheimer's,older%20(10.7%25)%20has%20Alzheimer's. Accessed 19 July 2023.
- Nagata, K. O., Nakada, C., Kasai, R. S., Kusumi, A., & Ueda, K. (2013). ABCA1 dimer–monomer interconversion during HDL generation revealed by single-molecule imaging. *Proceedings of the National Academy of Sciences*, *110*(13), 5034-5039.
- Bardy, C., Van Den Hurk, M., Eames, T., Marchand, C., Hernandez, R. V., Kellogg, M., ... & Gage, F. H. (2015). Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. *Proceedings of the National Academy of Sciences*, *112*(20), E2725-E2734.
- Julia, T. C. W., Wang, M., Pimenova, A. A., Bowles, K. R., Hartley, B. J., Lacin, E., ... & Brennand, K. J. (2017). An efficient platform for astrocyte differentiation from human induced pluripotent stem cells. *Stem cell reports*, *9*(2), 600-614.
- Rawat, V., Wang, S., Sima, J., Bar, R., Liraz, O., Gundimeda, U., ... & Yassine, H. N. (2019). ApoE4 alters ABCA1 membrane trafficking in astrocytes. *Journal of Neuroscience*, *39*(48), 9611-9622.

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