Unraveling the role of endocytic pathways of endothelial cells in amyloid-β blood-brain barrier clearance in Alzheimer's disease

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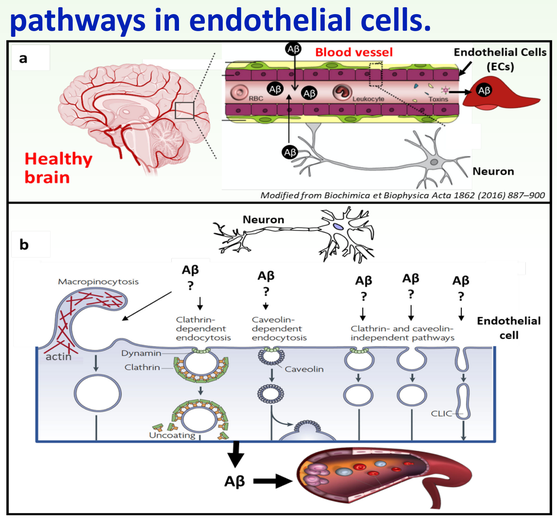
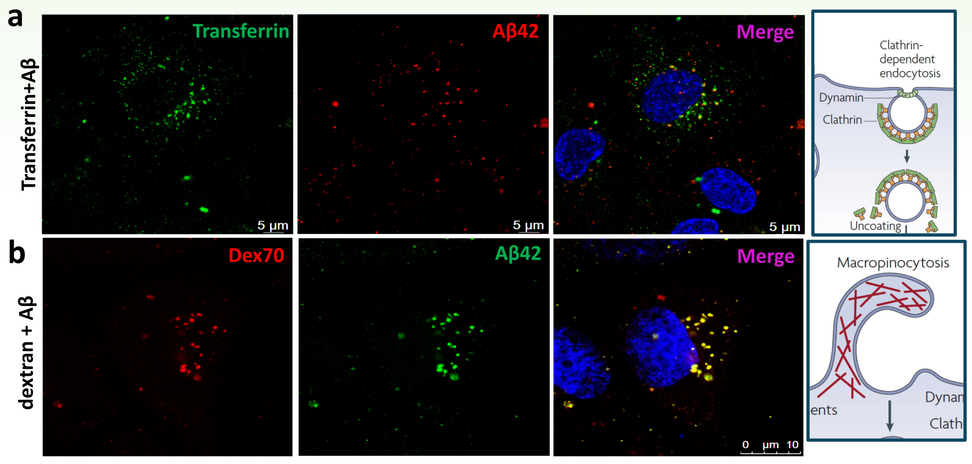
Abstract: Alzheimer's disease (AD) is an age-associated, irreversible neurodegenerative disorder. A fundamental neuropathological hallmark of this disease is the accumulation of the amyloid-beta (Aβ) peptide in the extracellular space and its aggregation in the brain. Interestingly, critical role for a healthy brain is played by clearance mechanisms of endothelial cells, which traverse the Aβ peptide through the Blood Brain Barrier (BBB), a highly selective semipermeable border[1]. Studies in recent years have shown that endothelial cells remove Aβ by endocytosis (Fig. 1), and that impairment of this function is a key contributor to AD progression[2]. However, the exact contribution of the distinct individual endocytic routes (Fig. 1b), as well as the involved molecular mechanisms, are largely unexplored. Here, we used specific chemical inhibitors and siRNAs against regulators of endocytic routes, combined with fluorescence confocal microcopy in brain endothelial cells, to identify the routes of endocytosis of Aβ amyloid. Interestingly, we found that Aβ is taken up by the cells via at least two independent endocytic routes, the pathway of macropinocytosis and the route of clathrin-mediated endocytosis. Ongoing experiments aim to confirm the physiopathological relevance of our findings in a disease relevant in vitro BBB model in transwell inserts, consisting of brain endothelial cells co-cultured with neurons generated from iPSCs from Alzheimer's patients (collaboration with V. Mahairaki, Johns Hopkins), as well as in animal models, mice and C-elegans, in collaboration with I. Charalambopoulos and N. Tavernarakis, respectively. All in all, the results of the present study will shed light in Aβ clearance mechanisms, thus contributing to novel strategies aiming to reduce the load of Aβ peptide in the brain, thereby preventing or delaying the onset of Alzheimer's disease.

1. Clearance of amyloid-β peptides by the endocytic pathways in endothelial cells.



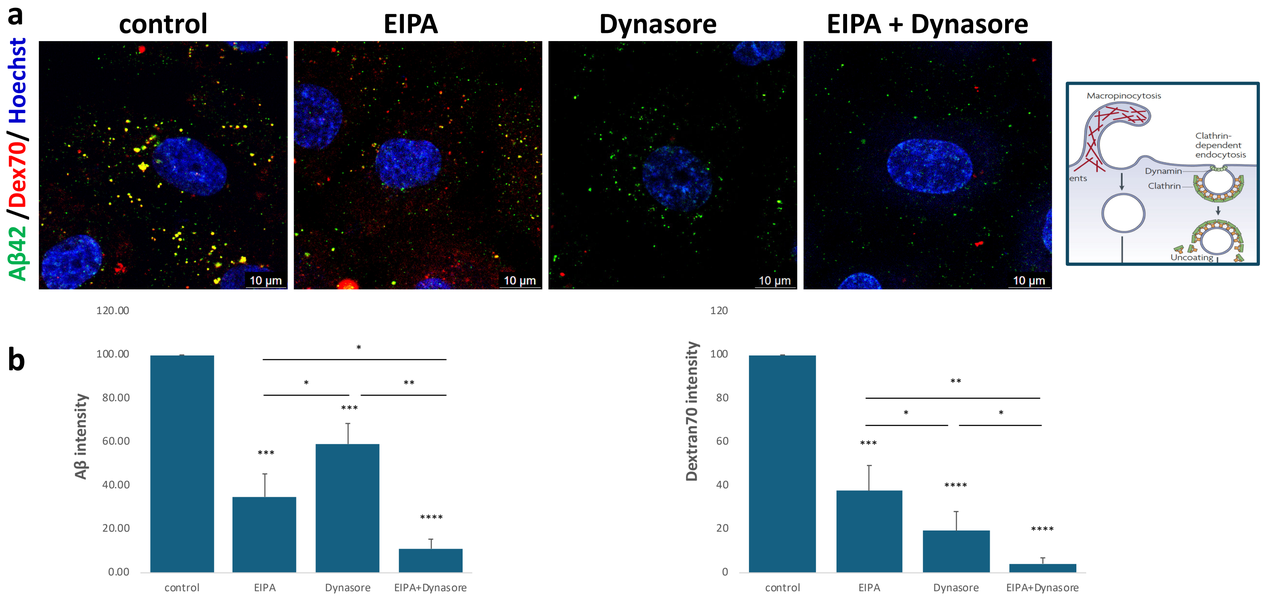
a. Transport of Aβ peptide through the brain endothelial cells, towards the blood circulation, is a physiological clearing process in the BBB.
b. Overview of known endocytic pathways, whose role in Aβ clearance remains to be addressed.

2. Internalized Aβ42 is colocalized with transferrin and dextran, markers of clathrin-mediated endocytosis and macropinocytosis, respectively.



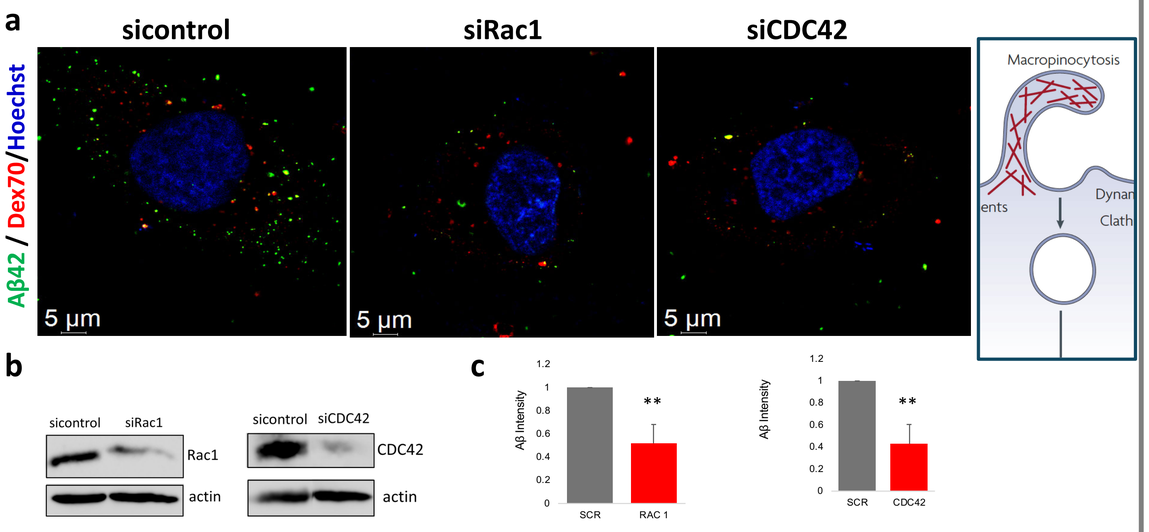
HUVECs were serum-starved and treated with Aβ42 and (a) transferrin or (b) dextran Texas red 70kDa. Then, the cells were fixed and stained with (a) anti-transferrin (green) and anti-Aβ42 (red) and (b) anti-Aβ42 (green) antibodies. Nuclei were stained with Hoechst (blue).

3. Chemical inhibition of macropinocytosis and clathrin mediated endocytosis reduces Aβ42 uptake in Brain Endothelial Cells.



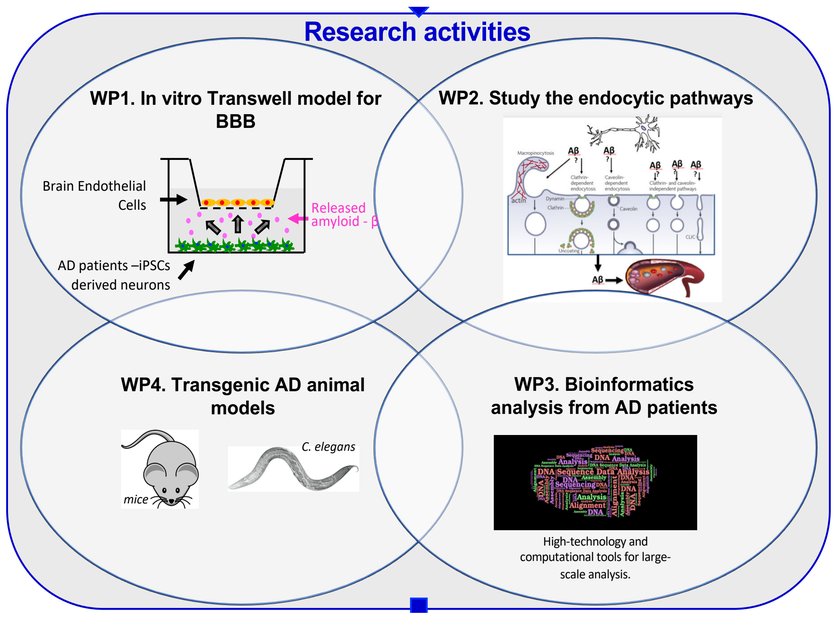
Brain endothelial cells (BECs) were serum-starved and treated with a chemical inhibitor of macropinocytosis (EIPA) and/or Clathrin-mediated endocytosis (dynasore). Afterwards, they were incubated with Aβ42 and dextran. a. Cells were fixed and stained with anti-Aβ42 (green) and Hoechst (blue), representative images. b. Quantification of Aβ and Dextran 70 intensity (N=3).

4. Knock down of Rac1 and CDC42, as key genes in macropinocytosis, blocks endocytosis of Aβ42 in Brain Endothelial cells.



BECs were transfected with control siRNAs or with siRNAs against Rac1 and CDC42 respectively, which are GTPases and modulators of macropinocytosis. Then, cells were serum-starved and treated with Aβ42 and dextran 70. a. Cells were fixed and stained with anti-Aβ42 (green). Nuclei were stained with Hoechst (blue). b. Knockdown of Rac1 and CDC42 confirmed by western blot analysis. c. Quantification of Aβ intensity after knockdown of Rac1 and CDC42 (N=3).

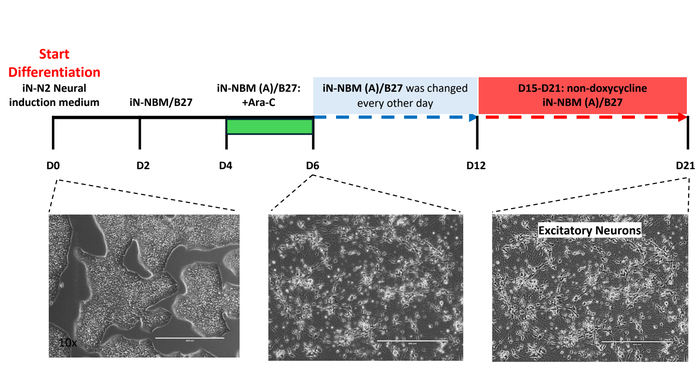
Ongoing experiments (Marie Curie project)



WP1: Establishment of an AD in vitro BBB model, using iPSC-derived brain endothelial cells seeded on the upper surface of permeable supports, while iPSC-derived neurons will be placed at the bottom of the culture well.
WP2: Investigation of distinct endocytic pathways involved in uptake and transport of Aβ across BECs
WP3: Bioinformatic analysis of available genome-wide and transcriptome datasets of the Neurodegenerative Disease BioBanks.
WP4: Validation of the *in vitro* data in transgenic animal models of AD pathology, in particular, C. elegans and mice (collaboration with N. Tavernarakis and I. Charalampopoulos groups, IMBB/FORTH).

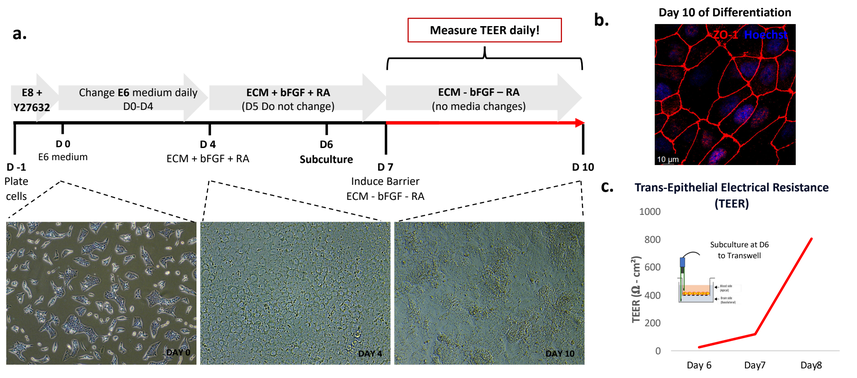
Protocols of differentiation of AD hiPSCs to neurons and BECs

i. Neuronal Differentiation of Ngn2 Transduced iPSCs



Schematic illustration of the followed protocol for the differentiation of AD iPSCs into phenotypically and physiologically mature neurons, following already established protocols[3].

ii. Differentiation of iPSCs to Blood Brain Barrier – Endothelial Cells



a. Schematic illustration of the followed protocol for the differentiation of AD iPSCs into brain endothelial cells[4]. b. At day 10, cells were fixed and stained with anti-ZO1 (red), which is an endothelial marker. Nuclei were stained with Hoechst (blue). c. At day 8 of differentiation, we measured TEER on transwell inserts to evaluate the barrier development.

References:

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2. Zhao, Z., et al., Central role for PICALM in amyloid-beta blood-brain barrier transcytosis and clearance. Nat Neurosci, 2015. 18(7): p. 978-87.
3. Mahairaki V., et alInduced pluripotent stem cells from familial Alzheimer's disease patients differentiate into mature neurons with amyloidogenic properties. Stem Cells 2014 Dev 23: 2996-30102014.
4. Lippmann, E., Wilson, H., & Neal, E. (2020). Protocol for Differentiation of Blood-Brain Barrier endothelial cells from human Pluripotent stem cells V2. BBB Endothelial Cells.

Acknowledgements

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