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

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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, and that impairment of this function is a key contributor to AD progression (2). However, the exact contribution of the distinct individual endocytic routes, as well as the involved molecular mechanisms, are largely unexplored. To shed light in this issue here we employed primary endothelial cells isolated from umbilical vein, or from human brain, as an *in vitro* model for investigating the endocytic pathways involved in uptake and transport of Aβ across the BBB. Interestingly, treatment of these cells with specific inhibitors of the individual endocytic routes, or with siRNAs against known endocytic modulators, followed by analysis by confocal microscopy between endocytosed Aβ peptide and markers of the endocytic routes, showed that Aβ is taken up by endothelial 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 endothelial cells generated from iPSCs from Alzheimer's patients (collaboration with V. Mahairaki, Johns Hopkins), cultured in transwell inserts, an in vitro model of BBB, as well as in animal models, mice and C-elegans, in collaboration with labs of IMBB-FORTH, the groups of 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.

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References

1. Zlokovic, B.V., *Neurovascular mechanisms of Alzheimer's neurodegeneration.* Trends Neurosci, 2005. **28**(4): p. 202-8.

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.