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SEARCHING NEW BIOMARKERS AND THERAPEUTIC TARGETS RELATED TO COGNITIVE DEFICITS IN EARLY STAGES OF ALZHEIMER'S DISEASE: ROLE OF AKAP79/150, CPT1C AND SSAO/VAP-1 IN Aß-MEDIATED AMPAR DYSFUNCTION

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1. Summary

The plasticity of glutamatergic synapses, which involves changes in glutamate receptors' dynamics, has emerged as a core cellular mechanism involved in the processing of various cognitive functions, and it is becoming apparent that alterations in these synapses could be related to early learning and memory deficits observed in mild cognitive impairment (MCI) and early stages of Alzheimer's disease (AD). Our proposal is to elucidate the underlying molecular mechanisms related to regulation of glutamate AMPA receptors (AMPAR) that are involved in early learning and memory dysfunction associated to AD and to identify novel therapeutic targets and biomarkers for promoting AD prevention and diagnosis at MCI and presymptomatic stages of the disease. Building upon preliminary data obtained from our laboratory in transgenic AD mice, we believe that oAβ-mediated synaptic dysfunction associated to early learning and memory deficits is due to an alteration in the presence of functional GluA1-AMPARs in the synaptic membrane as a consequence of a combination of different processes: 1) a down-regulation of AKAP79/150 and/or carnitine palmitoyltransferase I (CPT1C); 2) a decrease in synaptic synthesis of these receptors by altered miRNAs regulation and 3) the overexpression of SSAO/VAP-1 affecting the release of angioneurins needed for proper synaptic AMPAR function. By using different in vitro and in vivo experimental models of AD together with human samples from control and patients with MCI or initial AD, our proposal intends to identify novel therapeutic targets and biomarkers for promoting prevention, diagnosis and future recovery therapies at early stages of the disease.

2. Results

The regulation of abundance of AMPA-type receptors (AMPARs) in the postsynaptic membrane is an important mechanism involved in learning and memory formation and several reports have suggested that an alteration in synaptic AMPA receptors is involved in early cognitive deficits observed in AD. As indicated above, the aim of the project was to test whether AKAP79/150, CPT1C, altered miRNAs regulation and/or disruption of blood-brain barrier contribute to AMPA receptor dysfunction in experimental models of AD.

Excitatory synaptic transmission is tightly regulated by total number and activation of AMPA receptors (AMPAR) present at the synapse. Current evidences suggests that AMPARs are inserted into the postsynaptic membrane during long-term potentiation (LTP) and could be removed from the membrane during long-term depression (LTD). Moreover, oAβ has been shown to disrupt synaptic structure and function, inhibiting LTP and facilitating LTD processes. Dephosphorylation of GluA1 at Ser845 and enhanced endocytosis are critical events in the modulation of LTD. Moreover, changes in scaffold proteins from the postsynaptic density (PSD) are also related to AMPAR regulation in oAβ-mediated synaptopathology. In this study we have analysed the effect of chemical LTD (cLTD) or oAβ on **AKAP150**, a synaptic protein that has been proposed to function as a signalling scaffold that regulates phosphorylation, channel activity, and endosomal trafficking of AMPAR, and AMPARs levels in cultured neurons. oAβ and NMDA-mediated cLTD induces a degradation in AKAP150 protein levels that is dependent on proteosome activation but not on calcineurin (CaN) activity. The reduction in AKAP150 parallels oAβ (and cLTD)-mediated GluA1 AMPARs endocytosis and dephosphorylation of GluA1 Ser-845. A causative relationship between the decrease in AKAP150 levels and the endocytosis of AMPARs is also supported by the dephosphorylation of GluA1 Ser-845 and the endocytosis of GluA1 AMPARs when AKAP150 expression was silenced whereas overexpression of AKAP150, restoring AKAP150 levels, blocked oAβ-mediated AMPARs endocytosis and dephosphorylation of GluA1. Since AKAP79/150 is a synaptic protein that has been proposed to function as a signalling scaffold that regulates AMPAR phosphorylation, channel activity, and endosomal trafficking associated to synaptic plasticity, the oAβ-mediated changes in AKAP 79/150 levels could be related to a deregulation of synaptic AMPA receptors in early stages of AD.

Recent data suggest that one of the constituents of the AMPAR complex is **carnitine palmitoyltransferase 1 C (CPT1C**), a brain-specific isoform located in the endoplasmic reticulum of neurons. Previous results demonstrated that CPT1C deficiency disrupted spine maturation in hippocampal neurons and impaired spatial learning, but the role of CPT1C in AMPAR physiology remained unknown. In this project we have shown that CPT1C binds GluA1 and GluA2, and that the three proteins have the same expression profile during neuronal maturation. Moreover, in hippocampal neurons of CPT1C knockout (KO) mice, AMPAR-mediated miniature excitatory postsynaptic currents and synaptic levels of AMPAR subunits GluA1 and GluA2 are

greatly reduced. We prove that AMPAR expression is dependent on CPT1C levels because total GluA1 and GluA2 protein levels are decreased in the hippocampus and in hippocampal cultures of CPT1C KO mice and GluA1 and GluA2 levels could be rescued in CPT1C overexpressing neurons, while other synaptic proteins remain unaltered. Notably, mRNA levels of AMPARs remained unchanged in those cultures, indicating that CPT1C is post-transcriptionally involved. We demonstrate that CPT1C is directly involved in the de novo synthesis of GluA1 and not in its degradation. Moreover, in CPT1C KO cultured neurons, GluA1 synthesis after chemical long-term depression was clearly diminished. These data identify CPT1C as a new regulator of AMPAR translation efficiency, and hence of synaptic function in the hippocampus. However, although we observed a decrease in CPT1C protein levels in the hippocampus of AD patients from Braak III/IV, we were unable to observe significant changes in CPT1C protein levels in wild type HPC cultures after oAβ treatment or in APPSw,Ind mice cultures compared to control cells. Thus, no relationship seems to exist between CRT1C levels and the decrease in GluA1 observed in AD experimental models.

Some evidence indicates that cognitive impairment observed in early stages of AD could be explained by alterations in synaptic function that would precede neurodegeneration. Furthermore, some reports have shown that deregulation of synaptic protein levels could be related to early cognitive dysfunction in experimental models of AD. Thus, changes in the regulatory mechanisms involved in the expression of synaptic proteins could be valuable for assessing prognosis and the rate of cognitive decline in AD. Since the levels of synaptic proteins are modulated by **miRNAs** and miRNAs could be used as biomarkers, in this project we analysed the changes in miRNAs profiling in control and AD patients at different stages in different brain areas. From the results obtained, we have examined plasma levels of a number of miRNAs associated with synaptic proteins, and have explored the utility of a subset of these miRNAs as a potential selective biomarker for AD. For these exploratory studies, we initially secured plasma samples from a Spanish cohort composed of control subjects, and those diagnosed with mild cognitive impairment (MCI) or AD. We were greatly encouraged to find a significant upregulation in the expression level of synaptic-related miRNAs, miR-92a-3p, miR-181c-5p and miR-210-3p in both MCI and AD subjects. Furthermore, we found that the expression level of these miRNAs was a good indicator of whether the symptoms of MCI patients progress to AD: the MCI patients that eventually developed AD had higher plasma levels of these miRNAs. We also found that

the expression levels of these miRNas could be reliably used as a potential biomarker for AD, as no changes in their expression levels were observed in plasma obtained from a cohort of frontotemporal dementia patients (FTD). Based on our very encouraging preliminary findings, we propose that, collectively, the synaptic proteins-related miRNAs, miR-92a-3p, miR-181c-5p and miR210-3p, likely constitute a specific molecular signature that can be a potentially developed as a sensitive and selective biomarker for detection of early stage AD and a good predictor of whether MCI is likely to progress to AD.

Besides the two main pathological hallmarks of Alzheimer's disease (AD), which include extraneuronal β-amyloid (Aβ) plaques and neurofibrillary tangles, other traits are also present in AD, such as cerebral amyloid angiopathy (CAA). Literature nowadays supports the idea that the cerebrovasculature makes an important contribution to the onset and progression of AD, postulating the existence of a strong link between vascular damage and this pathology. Therefore, **the study of neurovascular crosstalk and its alterations is important to understand the molecular basis of AD**. Here, we have studied the contribution of vascular SSAO/VAP-1 to the BBB dysfunction in AD. Our results show that SSAO/VAP-1 expression is associated to endothelial activation by altering the release of pro-inflammatory and pro-angiogenic angioneurins such as IL-6, IL8 or VEGF. It is also related to a BBB structure alteration, with a decrease of tight-junction proteins such as zona occludens or claudin-5. Moreover, the BBB function revealed increased permeability and leukocyte adhesion in cells expressing SSAO/VAP-1, as well as an enhancement of the vascular Ab deposition induced by mechanisms both dependent and independent of the enzymatic activity of SSAO/VAP-1. Moreover, we have observed a decrease in BDNF release from hSSAO/VAP-1 over-expressing endothelial cells that affect synaptic maturation. In this conditions we have observed a reduction of synaptic AMPA receptors and a significant decrease of PSD95 and AKAP79/150 in the postsynaptic terminal. These results confirm the role of vascular SSAO/VAP-1 in BBB dysfunction related to AD and suggest that by differential release of angioneurins could affect synaptic development and function. Altogether, our results open a new window in the search for alternative therapeutic targets for fighting AD.

3. Future relevance

The results obtained in this project have confirmed that the postsynaptic proteins AKAP79/150 and endothelial SSAO could be new potential targets for future therapeutic approaches. However, we still have to follow up the studies initiated in this project before that work can be undertaken. By contrast, the results that could have a greater impact in less time are those regarding the molecular plasma miRNAs signature. Early diagnosis, and thus early intervention before AD symptoms appear, offers the best chance of slowing or stopping the progression of AD and it is of general interest to identify efficient and cost-effective biomarkers for the benefit of patients and their relatives. Earlier diagnosis of AD will mean: 1) patients can begin health measures to preserve their existing cognitive function for as long as possible (for example by minimisation of vascular risk factors or increasing mental activity); 2) treatment of symptoms with medications or other interventions can start; 3) participation in a clinical trial can be maximised; 4) there will be more time to assemble medical and care-giving teams to provide support and treat medical concerns; 5) individuals and family members can learn what to expect for the future and plan accordingly while the patients are cognitively able to make decisions; 6) accurate diagnosis of non-Alzheimer MCI. In the next few years we plan to validate the molecular signature that we have identified as a universally sensitive and selective biomarker for AD by extending our analysis to MCI and AD cohorts from a number of different countries. With this validation in hand, the second goal would be to use our findings as a platform to explore the development of a plasma-based, affordable diagnostic tool for routine clinical screening. Since, no blood-based biomarker is currently approved or available for in vitro diagnosis of early stages of AD, ours would likely be one of the first, thus representing a significant advance for the field.

4. Patents and Research Papers

Patents

Rodriguez-Alvarez, J, Miñano-Molina, AJ & Siedlecki-Wüllich,D. "Circulating miRNAs as biomarkers for diagnosis of mild cognitive impairment and Alzheimer's disease". Patent EP18382427 (15th June 2018).

Research Papers

Siedlecki-Wullich D, Catala-Solsona J, Fabregas C, Hernandez I, Clarimon J, Lleó A, Boada M, Saura CA, Rodriguez-Alvarez J and Miñano-Molina AJ. Alteration of microRNAs related to synaptic function as potential plasma biomarkers for early stages of Alzheimer disease. *Alzheimer Res & Ther*. (submitted)

Solé M, Esteban M, Taltavull, B, Fabregas C, Rodríguez-Álvarez J, Miñano-Molina AJ and Unzeta M. Blood-brain barrier dysfunction underlying Alzheimer's disease is induced by and SSAO/VAP-1-dependent cerebrovascular activation with enhanced Aβ deposition. *BBA-Molecular Basis of Disease* (2nd revision)

Miñano-Molina A, Cheng W, Siedlecki-Wullich D, Calvet, E., Fadó R., Fabregas, C., Quiroz-Baez R, Casals, N., Aguilera J, Saura C.A & J. Rodríguez-Alvarez. AKAP150 degradation is associated to LTD-mediated endocytosis of synaptic AMPA receptors in cultured neurons. *Science Advances (submitted).*

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Fado R., Soto D., Miñano-Molina A., Pozo M., Carrasco P., Yefimenko N., Rodríguez-Alvarez J & Casals N. Specific regulation of GluA subunit synthesis and AMPA receptor-mediated synaptic function by CPT1C in the hippocampus. *J Biol Chem 290:25548-25560 (2015)*