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THE ROLE OF REELIN AT THE CROSSROADS OF ALZHEIMER'S DISEASE MECHANISMS: TAUOPATHY, AMYLOID TOXICITY AND TRANSMISSIBILITY

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1. Project Summary

Reelin is an extracellular protein that stimulates adult brain plasticity and acts as a homeostatic regulator of correct brain functioning. Moreover, reelin and its signaling pathway are directly involved in the molecular mechanisms of Alzheimer's disease (AD), playing a beneficial role in the context of this pathology through a dual mechanism: on the one hand, reelin positively modulates brain functioning, thus strengthening the system against impairment; on the other, reelin ameliorates disease-linked characteristics at the molecular level. Particularly, reelin activates signaling pathways to inhibit phosphorylation of Tau, a protein directly involved in one of the hallmarks of the disease. Additionally, reelin modulates the other main hallmark of the disease by binding amyloid species to reduce their toxicity, delaying fibril formation and rescuing cognitive deficits associated to amyloidosis in a transgenic mouse model. Together, this wealth of data positions reelin at the crossroads of AD pathologic and protecting processes and strongly encourages considering reelin and its signaling pathway as a therapeutic target to combat AD.

The main objective of the project was to address the precise role of reelin in the molecular mechanisms involved in AD progression, focusing on tauopathy, amyloidogenesis and transmissibility. The project was planned to clarify whether reelin overexpression rescues AD symptoms in Tau models (TgRln/VLW and TgRln/GSK-3β) and whether accelerated AD-progress may occurs in conditional reelin-KO AD-strain (flRln/Cre/J20). Finally, by studying the participation of reelin into the transmissibility of amyloidosis in an AD-mouse model with reelin overexpression (TgRln/J20), the project wanted to evaluate how the interaction of reelin with amyloid species modulates the amyloid propagation and its transmissibility properties, particularly when animal models for the disease were subjected to experimentally-induced amyloidosis.

The most relevant results arising from the project include that reelin is able to reduce the induction of tauopathy indicators in neuronal cultures treated with toxic amyloid species. Additionally, the study of reelin overexpression in tauopathy mouse models evidences that such protection is also occurring in vivo. Thus, reelin reduces phenotypes linked to

tauopathy at the biochemical/histopathological levels and, importantly, also upon electrophysiological and behavioral studies. Within the project, we also studied another manifestation of the disease, amyloidosis, in specific experimental situations that include reelin downregulation and induced amyloidosis. We initiated the evaluation of reelin downregulation in the adult brain through behavioral, histological and electrophysiological characterizations, particularly when crossbreed with the J20 AD-mouse model. Furthermore, reelin's role in transmissibility of amyloidosis was studied mainly in the in vivo model of reelin overexpression subjected to exogenously-induced amyloidosis by intracerebral injections of brain extracts. We find that the increased presence of reelin is able to reduce the propagation of amyloidosis within the brain of experimental animals during early stages after induction.

In summary, data generated within this project show that reelin is beneficial for ameliorating tauopathy as well as induced amyloidosis, thus confirming the hypothesis that reelin protein plays important roles in a diversity of situations linked to the pathological mechanisms of Alzheimer's disease. We conclude that reelin must be further considered as a target for developing new therapies to combat Alzheimer's disease.

2. Results obtained

A first main aim within the project was to study the effect of reelin overexpression in the extent of Tau phosphorylation in two in vivo tauopathy models (VLW and GSK-3β)Reelin overexpression on those models (TgRln/VLW and TgRln/GSK-3β) produced a reduction in Tau phosphorylation, while total levels of Tau protein were maintained. Our data demonstrated a reelin-dependent rescue of the increased phospho-Tau levels, a fact that may modulate the progression of the disease-associated phenotypes in these animals. These results were complemented with a set of in vitro experiments to investigate the participation of reelin in rescuing the Aβ-induced somatodendritic missorting of axonal cytoskeletal proteins such as Tau and neurofilaments. Particularly, reelin was able to modulate the proportion of neurons suffering missorting/translocation of neurofilaments, as well as the fine distribution of Tau protein within translocated dendrites, resulting in the

reduced accumulation of this protein in the proximal part of the dendrites. These findings were reinforced by results from experiments using tauopathy mouse models to explore in vivo somatodendritic missorting of phospho-Tau protein in relevant epitopes linked to AD. Moreover, to further characterize TgRln/VLW mice, we explored their responses at the electrophysiological level. VLW mice presented similar basal synaptic properties and short-term plasticity as compared to wild-types, but showed important deficits in long-term potentiation (LTP) experiments. Interestingly, these LTP deficits were reverted by the overexpression of reelin in TgRln/VLW mice. Similarly, when animals were subjected to behavioral studies, we first found that VLW mice did not present evident motor deficits, but they did show significant deficits in memory retention that were again rescued in the model of reelin overexpression. Together, our findings point to the physiological relevance of the reelin-induced reduction in phosphorylation levels and show that reelin overexpression counteracts tauopathy-related histological, electrophysiological and behavioral abnormalities in animal models.

The second main aim of the project was related to the study of amyloidosis in a diversity of experimental models subjected to alterations in reelin levels. For this purpose, we used the J20 strain, which expresses a mutated form of the human amyloid precursor protein, to reproduce the amyloidosis phenotype of AD, thus leading to the accumulation of senile plaques in the animals. By crossbreeding these animals with the conditional knock-out of reelin (flRln/Cre) we obtained flRln/Cre/J20 animals. Firstly, we explored the accumulation of deposits at early ages to analyze a potential accelerated appearance of amyloid plaques as compared with J20 mice. In parallel, the study of the electrophysiological implications of reelin depletion were carried out on flRln/Cre animals by analyzing basal characteristics of hippocampal CA3-CA1 synapses, as well as their functional responses following experimentally induced LTP. Our findings indicate that depletion of reelin in postnatal stages leads to a complex phenotype at the electrophysiological and histological levels, in which aberrant accumulation of amyloid species do not occur massively upon short times of reelin depletion. Collected data allowed the design of experiments with longer stages post reelin depletion to better study not only the onset of amyloid deposits, but their rate of propagation throughout the brain, thus linking with the last main goal of the project related to amyloid transmissibility.

Lastly, the project aimed to unravel the possible participation of reelin in propagation of amyloid deposits throughout the brain during the evolution of AD. To study this process, we modeled experimentally the induction of amyloidosis in our animal models. We started by performing a series of preliminary experiments that include the electrophysiological and behavioral characterization of the TgRln/J20 model, the in vitro modeling of induced-aggregation of amyloids, and the optimization of experimentally-induced amyloidosis in vivo in the J20 AD-mouse model. Efficient conditions for implementing experimentally-induced amyloidosis were settled in 4- to 6-month-old mice with incubation periods of 8 weeks post-induction as well as in 2 month-old mice with longer incubation periods over 12 weeks, also observing amyloidosis from 16 weeks onwards in distant areas from the injection site (i.e. entorhinal cortex and contralateral hippocampus). Extensive studies modeling induced amyloidosis in a context of reelin overexpression using the TgRln/J20 model indicate that reelin is able to reduce transmissibility both locally and far from the site of injection for a certain time-window during the initial accumulation of amyloid deposits in each of the areas analyzed.

3. Relevance with the possible future implications

Taken together, our results complement the previously reported beneficial effect of reelin in AD pathology and confer the reelin pathway a pivotal role as a negative regulator of AD progression, by antagonizing both Aβ and Tau pathologies. On the basis of our findings, we propose that the activation of the reelin pathway provides an efficient therapeutic approach to ameliorate several of the most important pathological mechanisms commonly associated with tauopathy and amyloidosis, for AD, and possibly other proteinopathies. Considering the data generated in the present project we conclude that the extracellular protein reelin positively targets several amyloid-related pathological processes (propagation of amyloid aggregates and Aβ-induced Tau translocation), as well as several tauopathy-associated processes (aberrant distribution of phospho-Tau, synaptic deficits, and long-term memory loss). Therefore, the role of reelin as central modulator of both amyloid- and Tau-associated pathological mechanisms for neurodegenerative diseases such as AD deserves further attention for the development of novel therapies.

4. Generated Literature

Rossi D., Gruart A., Contrerars-Murillo G., Ávila J., **Delgado-Garcia J.M**., **Pujadas L**[#]. and Soriano E[#]. "*Reelin reverts biochemical, physiological and cognitive alterations in mouse models of Tauopathy*" (under resubmission, *Progress in Neurobiology*)

Rossi D., Pardo M., Jordan K., Tarutani A., Hasegawa M.S., Soriano E[#]. and **Pujadas L**[#]. "*Reelin decreases prion-like induced-proteinopathy for amyloid and α-synuclein species in murine models*" (in preparation for *Nature Medicine*)

Gruart A., Rossi D., Soriano E., **Pujadas L.** and **Delgado-García J.M** "*Electrophysiological and functional characterization of Reelin -overexpression in the context of Alzheimer's Disease amyloidosis*" (under development, *undetermined journal*)

Manso Y., Vilchez A., Rossi D., Gruart A., **Delgado-García J.M.**, **Pujadas L.** and Soriano E. "*Structural and functional characterization of conditional depletion of reelin in the context of Alzheimer's Disease*" (under development, *undetermined journal*)

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