ROLE OF THE CELLULAR PRION PROTEIN AS "CROSS-TALK" PROTEIN BETWEEN A-SYN/ LRRK2 AND P-TAU IN SPORADIC AND FAMILIAL PARKINSON'S DISEASE

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1. Project Summary

The general objectives of the project are the following:

i) We would like to determine whether PrP^c is involved in the cell spreading of α-syn and if this spreading also triggers phosphorylation of Tau in a *PRNP* dependent manner as determined for Alzheimer's disease. This will be performed in vivo as well as in vitro.

ii) To discriminate whether the presence of p-Tau in PDD + AD is mediated by α-syn or ADDLs.

iii) Due to that, it seems that dementia is not fully associated with LRRK2 mutations. We would like to explore whether LRRK2 play a role in parallel to PrP^c in controlling p-Tau and microtubule dynamics. Thus, we will use using cellular models (i.e., modulating PrP^c expression in LRRK2 expressing cells lines or developing similar experiments in M17 cell overexpressing α-syn) will be analysed.

iv) To better understand these roles in the closest "patient" that we would like to develop, a time-course cellular and biochemical characterization of p-Tau regulation and accumulation was derived in differentiated neurons (dopaminergic as well as "non-dopaminergic" neurons) derived from IPS^c of PD patients (familial and idiopathic) and controls. In this PD-derived IPSc with increased α-syn, the gene dosage of *PRNP* will also be modulated genetically.

v) All these data will be correlated with the biochemical and histological analysis of PrPc, p-Tau, α-syn, LRRK2 in different regions (i.e., substantia nigra, cortex, striatum, amygdala) in PD, PDD and DLB (20 cases/group) and LRRK2 (G2019S) patients.

The specific objectives of the project are the following:

Objective 1. Role of PrP^c in neural spreading of α-syn *in vitro* and *in vivo*.
Objective 2. p-Tau in PDD + AD is generated directly by α-syn or indirectly by ADDLs?
Objective 3. To explore whether LRRK2 play a role in parallel to PrPc in controlling p-Tau and microtubule dynamics in alpha-synucleinopathies.
Objective 4. Analysis of the role of Prnp dosage in p-Tau phosphorylation and α-syn

generation in IPSc-derived from PD (sporadic and familial) and LRRK2 (G2019S) patients.

Objective 5. Biochemical and histological studies in human samples.

2. Results

Objective 1. We have determined that PrP^C is a new receptor for α-synuclein (Urrea et al., 2018a, 2018b, del Rio et al., 2018). This is developed in vitro and in vivo using two different mica strains. We have determined that the region of the PrP^C responsible for binding a-synuclein protofibrils (PFFs) is the charged region of the molecule. This is relevant since this region also shares its binding with Aβ.

Objective 2. We determined that a-synuclein (PFFs) treatment induces p-Tau in primary cortical neurons. This treatment involves Fyn activation in contrast to other peptides showing β-rich structures such us $PrP_{106-126}$. Since $PrP_{106-126}$ binds to aa 113-133 (HR) of PrP^C in contrast to a-synuclein and β-amyloid, the integrity of the HR domain is mandatory to induce the aggregation of PrP^C in membrane and to enhance p-Tau. On the other hand, overexpression of α-synuclein enhances the generation of p-Tau after ADDLs treatment.

Although p-Tau generation is very low after α-synuclein fibril treatment, we generated a double mutant mice overexpressing three different mutations of Tau (VLW mice) in absence of PrP^C (ZH3 mice). Current data suggests that changes observed in both PrP^C and Tau are related to an epigenetic control of Tau on the *PRNP* promoter and changes in microRNA (mi132p). In fact p-Tau is able to induce the activation of the promoter. In fact only the PPFs of p-Tau are able to be internalized by cultured neurons. Their action on the promoter is modulated by Sp1 and AP1 transcription factors.

Objective 3. Although LRRK2 has been implicated in mictotubule binding, unfortunately, LRRK2 do not bind to PrP^C. However, it competes for the regulation of Thr188 phosphorylation by GSK3β. We have confirmed this data during this year using Biacore and CO-IP without positive results.

Objective 4. We started to differentiate the IPSc using two different protocols, SNM formation or direct differentiation. To date we are not able to reproduce the transport of α-synuclein using IPSc in microfluidic devices due to the low number of TH+ neurons generated from our IPSC. As an alternative we are developing new cultures using direct populations of TH+ neurons selected by FACS and overexpressing mutated forms of synuclein in these cells to check whether there transport of the pathogenic forms takes place in these cells. These experiments are in process as a direct continuation of the funded project.

Objective 5. Biochemical and histological studies in human samples. During the project we collected samples of PD and controls from four different biobanks (Hospital Clinic, Navarra, HUB-Bellvitge and DZNE-Germany). We started to determine p-Tau levels in these samples. We used two different epitopes AT8 and PFH1. Samples indicated that p-Tau is increased in neocortex of PD patients. We included PDD (Parkinson disease samples in the study). Preliminary results demonstrated that molecules modulating Tau phosphorylation (i.e., reelin) are not modified in PD areas in contrast to AD. Similar data also affects PrP^C. Data analysed is included in a preliminary version of an article. A total of > 200 patient samples were employed including CSF samples from Germany, Navarra, CIMA, Hospital Clinic and Mutua de Terrassa.

3. Relevance and potential future implications

In this project we have been able to describe for the first time that the cellular prion protein is a receptor for α-synuclein. In addition, we have been able to determine the region of binding between both proteins. Thus, we believe that new therapeutic pathways are opened to try to block this union and the dissemination of synucleinopathy in the brain of the patients. In this regard, it is relevant to mention that the region or docking area between a-synuclein and PrP^C is the same as for ADDLs in Alzheimer's disease. This opens, even more if possible, the putative therapeutic strategies centred in the CC (binding region) region of the PrP^C for neurodegeneration. These strategies are actually focused in immunotherapy and site directed pharmacology targeting.

4. Publications

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