IBEC- VHIR INTERNATIONAL PhD PROGRAMME

Position

1. Project Title/ Job Position title:
   Effects of cytosolic crowding by age-dependent accumulation of neuromelanin on neuronal mechanics
2. Research project/Research Group description

Humans accumulate with age the dark-brown pigment neuromelanin (NM) inside specific neuronal groups. Neurons with the highest NM levels, i.e. dopaminergic (DA) neurons of the substantia nigra pars compacta (SNpc) and noradrenergic neurons of the locus coeruleus (LC), are preferentially susceptible to degeneration in Parkinson disease (PD), resulting in characteristic motor and non-motor symptoms. While the loss of pigmented nigral neurons in PD was first established a century ago and constitutes the cardinal pathologic diagnostic criteria for the disease, *the potential contribution of NM to PD pathogenesis has remained largely unknown* because NM does not appear spontaneously in most animal species, including rodents, i.e. the abundant NM levels in the brainstem are unique to humans. ***Remarkably, humans are the only species that naturally develops PD***.

This PhD project will be developed in the group of Neurodegenerative Diseases of Vall D'Hebron Research Institute and the group of Nanoscale Bioelectrical Characterization of IBEC. The first group developed the first experimental *in vivo* rodent models exhibiting age-dependent production and accumulation of human-like NM within PD-vulnerable neurons, at levels up to those reached in elderly humans (Carballo-Carbajal et al., Nat. Commun. 2019; Laguna et al., Nat. Commun. 2024). Using these animal models, they found that progressive intracellular build-up of NM with age compromised neuronal function when accumulating above a specific threshold, eventually triggering main pathological features of PD. Importantly, the lowering of intracellular NM to levels below this pathogenic threshold resulted in a major attenuation of the PD phenotype, both at the behavioral and neuropathological level. The second group is leader in atomic force microscopy (AFM)-based mechanical characterization of cells and biological tissues for disease detection and drug screening at high throughput (Calò et al., Sci Rep. 2020; Tello et al., Immunity 2021).

3. Job position description

Despite NM accumulation has a primary role in pathology and neurodegeneration (PD, Lewy-body, LB), ***the molecular mechanisms driving NM-linked neuronal pathology in both PD and***

brain aging remain uncertain, thereby precluding the development of potential therapeutic strategies targeting NM-related pathogenic mechanisms within the aged or diseased brain. Intracellular NM progressively accumulates over a lifetime inside undegraded autophagic structures until occupying most of the neuronal cytoplasm. This process is associated with an increase in cell size/volume and a general failure of cellular proteostasis, leading to LB formation and neurodegeneration. It is expected that the continuous cytoplasmic accumulation of NM with age **also alters other biophysical properties** beyond neurons' size and volume, *like cell stiffness, (visco)elasticity, adhesion and plasma membrane tension*, all of which will ultimately breakup the cell. ***The aim of this project will be to analyze all these parameters in an age-dependent manner through direct interrogation using the AFM.***

Throughout the proposal, different experimental models will be used, i.e. neurons from human brains and NM-producing rodents. Post-mortem human SN will be used, including: (i) age-matched PD patients and non-PD controls (N≥13 for each sex); (ii) control brains at different ages, from 5-10 to 71-90 years old (N≥3 for each sex). Furthermore, either AAV-Tyr-injected rats/mice or TgNM mice will be used, where PD-like neuronal dysfunction/degeneration progress in a protracted manner in parallel to age-dependent intracellular NM buildup. Experimental analyses will be performed at various ages/disease stages, from pre-symptomatic PD to early and full PD. Six to eight animals of both sexes will be used. Control groups will include either wt littermates (when using TgNM) or rats/mice injected with an empty viral vector (when using AAV-Tyr-injected animals); non-injected contralateral brain hemisphere of AAV-Tyr-injected animals will serve as additional internal control.

Group Leader IBEC

1. Title: Associate Professor, Senior Researcher
2. Full name: Annalisa Calò
3. Email: acalo@ibecbarcelona.eu
4. Research Group: Nanoscale Bioelectrical Characterization

Collaborator in the other institution

1. Title: ICREA Professor, Group Leader
2. Full name: Miquel Vila
3. Email: miquel.vila@vhir.org
4. Institute: Vall d'Hebron Research Institute
5. Research group: Neurodegenerative Diseases