Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Studying the nuclear envelope during human healthy aging

Synopsis:

Aging acts as a major risk factor for many human pathologies, including cancer, neurodegenerative disorders, diabetes and cardiovascular diseases. Nonetheless, information on the determinants of biological deterioration is still required if we aim at providing a better quality of life for aging individuals. In our Lab, we are focused on understanding why human cell nuclei tend to lose structural integrity as age progresses, ultimately leading to functional impairment. In other words, we aim at explaining why aged individuals are more prone to certain diseases and how does it relate with the loss of nuclear structural and mechanical properties.

In mammalian cells, the nuclear structural integrity is thought to be safeguarded by the nuclear envelope (NE), in particular the nuclear lamina (NL). The NL is mainly composed of lamin proteins that assemble into filaments and bind both heterochromatin and the inner nuclear membrane. However, the role of specific NE lipids and lipid-lamin interactions in the nuclear compartment has been mainly disregarded, mostly due to the complexity of the double lipid bilayer. In this project, we aim at directly circumventing these issues by carefully designing simplified model systems with lipid features characteristic of the NE at different stages of healthy age progression.

We have started by identifying the age-related lipid composition of the nuclear envelope by mass spectrometry (lipidomics). Our results suggest that age progression leads to a decrease in NE plasmalogens, which are natural antioxidants. Now, the next step is to **design and characterize membrane models with different plasmalogen content to mimic the age-tuned lipid composition of the NE**. Moreover, interaction with peptides derived from lamin proteins will also be addressed, to understand if different lipid compositions result in a different membrane association of lamin proteins (virtually making the nuclei more rigid).

Briefly, small, large and giant unilamellar vesicles will be prepared with age-specific lipid compositions matching the lipidomics results. The biophysical characterization of the models will then be accomplished by a multitude of techniques, including fluorescence spectroscopy and microscopy, dynamic light scattering (DLS) and atomic force microscopy (AFM)-based approaches. Direct comparison with human primary fibroblasts' nuclei will also be performed. Upon characterization, the interaction of lamin-derived peptides with these membranes will be assessed by fluorescence correlation spectroscopy (FCS).

Ultimately, this project will bring us a step forward in the implementation of a free-standing nucleus-sized vesicle system with age-tuned lipid composition, thus helping to create a **novel platform to study nuclear aging *in vitro***.

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