Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Molecular insights into the biomechanical deterioration of 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 to the loss of nuclear structural and mechanical properties.
In mammalian cells, the nuclear structural integrity is 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, even though they can directly impact the degree of lamin binding and the consequent nuclear biomechanics. In this project, we aim at carefully designing simplified models with lipid and lamin features characteristic of the NE at different stages of healthy age progression. To do that, membrane models with age-tuned lipid composition will be used, as well as different lamin proteins. Although changes can be accommodated depending on the acquired data, the work should be divided in 3 main sections:

1. Quantification of lamin-membrane interactions: Lamin proteins (fluorescent and non-fluorescent versions) will be expressed in yeast and purified using a protocol already being implemented at the Lab. Association with the membrane will be assessed using fluorescence anisotropy and fluorescence correlation spectroscopy.
2. 2D lamin polymerization: Lamin meshwork formation will be tested and optimized in supported membranes, enabling visualization using confocal microscopy and atomic force microscopy (AFM).
3. Simplified artificial NE: Lamins will be incorporated into micrometer-sized vesicles and polymerization will be attempted in different conditions. Lamin-membrane association will be visualized by confocal microscopy, while the resulting vesicle stiffness will be quantified by AFM-based force spectroscopy. Direct comparison with human primary fibroblasts' nuclei will also be performed.

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

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