**PNRR: CN-mRNA - Targeting metastases from solid tumors and primary extranodal lymphomas by novel RNA therapies**

**Title**

*Study of the NRF2 role in the Unfolded Protein Response “UPR” in osteosarcoma*

**Project**

The unfolded protein response is the mechanism by which cells control endoplasmic reticulum (ER) protein homeostasis. Under certain stresses, such as hypoxia or altered glycosylation, cells can activate the UPR pathway in response to the accumulation of unfolded proteins [1].

One of the key element in the UPR response is the by the bifunctional IRE1α protein. In fact, IRE1α is a type I transmembrane protein with both serine/threonine kinase and endoribonuclease domains. Recently, activation of UPR has been suggested to play a cytoprotective role in a wide range of human malignancies. However, the molecular definition of the UPR mechanism and the definition of all the players that take part to this pathway are mostly unknown. Recent scientific evidences suggest that the UPR mechanism may also integrate lipid stress by limiting the amount of lipids that accumulate intracellularly. In fact, recent data have shown that osteosarcoma cells in acidic microenvironment activate the UPR to avoid cell death and that those cell lines that under acidosis increase lipid accumulation specifically activate the IRE1α branch, which is responsible for modulating lipid metabolism. Moreover, the acidic environment triggers an intracellular ROS production that in turn lead to the activation of the NRF2 Transcription Factor (TF) mainly involved in the intracellular antioxidant response[1,2].

The main focus of this project is to study the functional axis “UPR-NRF2” in osteosarcoma tumour.

Specifically, the candidate will have to address three main scientific AIMS:

1) The candidate will generate at least three engineered osteosarcoma cell lines for the conditional Knock-Down (Doxycycline inducible sh-RNA) of the NRF2 gene expression. Next, he will evaluate the UPR response on those engineered cells (conditional NRF2-KD) in response to several stimuli such as intracellular lipid accumulation.

2) The candidate will perform transcriptomic analyses in the engineered osteosarcoma cell lines to define new UPR downstream targets directly or in-directly regulated by the NRF2 transcription factor. Next, ChIP-qPCR assays will be performed to validate the direct role NRF2 in the regulation of these new UPR related targets.

3) The candidate, by using specific siRNA molecules, will validate the biological role of at least five new direct UPR-NRF2 target genes involved in the UPR pathway activated by the lipid accumulation.

References

1) The Unfolded Protein Response: An Overview; *Adam Read and Martin Schroder*,

Biology 2021, 10(5), 384; <https://doi.org/10.3390/biology10050384>

2) Unfolded protein response (UPR) integrated signaling networks determine cell fate during hypoxia; *Sylwia Bartoszewska and James F. Collawn*, Cellular & Molecular Biology Letters volume 25, Article number: 18 (2020)