PROJECT 1

DoS: Prof. Carlo Tacchetti

Title: Unraveling the role of Integrated Stress Response signaling in metastatic breast cancer mitochondrial bioenergetics and heterogeneity

Curriculum: BAIO

Link to OSR/UniSR personal page:
https://research.hsr.it/en/centers/experimental-imaging-center.html

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Metastasis is the leading cause of carcinoma-related mortality (1). While the options for metastatic disease treatment remain unsatisfactory, the contribution of non-genetic mechanisms generating phenotypic heterogeneity and refractoriness to conventional therapies is becoming increasingly recognised (2).
Our Studies carried out by Dr. Paola Falletta, revealed that in breast cancer, the deadliest cancer for women worldwide (3), activation of Integrated Stress Response (ISR) increases metastatic potential by reprogramming the transcriptional and translational landscape of the cancer cell. The highest levels of ISR are found in triple-negative breast cancer (TNBC), the most aggressive molecular subtype, and predict poor patient survival.
Showing that ISR-driven translation reprogramming, a mechanism that allows a rapid and reversible response to acute microenvironmental stress, is sufficient to trigger cancer invasiveness and rewire metabolism, leads us to suggest translational regulation as an emerging hallmark of cancer.
Furthermore, identification of the downstream targets of ISR in the metastatic setting, and the dissection of the mechanisms by which ISR regulates them, could allow the discovery of new potential therapeutic targets for this yet untreatable disease.
Based on our previous discoveries (4-6) and our striking laboratory data, we plan to address the hypothesis-driven theory by which the ISR signalling cascade, pivoted by the peIF2α/ATF4 axis, determines cancer aggressiveness by modulating the breast cancer cells mitochondria bioenergetics and heterogeneity.

In this Study, carried out with Prof. Tacchetti as Director of Studies and with the support of Dr. Paola Falletta, we will utilise multiple approaches to dissect the ISR-driven mechanisms taking place in TNBC cell reprogramming. To expose new targetable vulnerabilities, the PhD Student will answer the following key questions:

1) What are the key events triggered by ISR-induced transcription and translation reprogramming that lead to altered bioenergetics and mitochondria signalling implicated in metastatic potential?

The PhD student will create a CRISPR/Cas9 ATF4 knock-out model which will allow to uncouple the transcriptional from the translational role of ISR activation in regulating the mitochondrial bioenergetics. S/He will produce Next Generation Sequencing data and utilise imaging, biochemical and molecular biology tools to characterise the effect of bioenergetics alterations on the TNBC metastatic phenotype.

2) How does the ISR activation drive phenotypic and metabolic heterogeneity in TNBC cells?

The PhD student will create and utilise 2D and 3D models to characterise and mechanistically dissect the ISR-driven signalling pathways driving to heterogeneity.
S/He will study the mechanisms by which the ISR confers innate and acquired TNBC cells resistance to chemo-, radio- and targeted- therapies, by means of biochemical and imaging tools.

Our project aims to address these questions to propose a new therapeutic window for metastatic cancer, identifying and validating new targets, and to develop or reposition drugs for metastatic disease treatment.

Skills to be acquired by the student (*Number of characters*, *including spaces*: max 600):

The Student will acquire:

1- Technical skills

- 3D culture set-up, validation, screening
- Biochemistry and molecular biology basic and advanced techniques
- CRISPR/Cas9 based editing, transfection, retroviral and lentiviral infection
- Basic and advanced imaging techniques

- NGS sample preparation, analysis and interpretation

2- Critical analysis of literature, data interpretation, short- and long-term experimental planning

3- Abstracts, manuscripts, dissertation scientific writing

4- Independent thinking as well as teamwork approach

References (max. 15)

1- Guan, X. Cancer metastases: Challenges and opportunities. *Acta Pharmaceutica Sinica B* vol. 5 402–418 (2015).

2- Licari, E., Sánchez-del-Campo, L. & Falletta, P. The two faces of the Integrated Stress Response in cancer progression and therapeutic strategies. *Int. J. Biochem. Cell Biol.* **139**, (2021).

3- Siegel, R., Miller, K. & Jemal, A. Cancer statistics, 2020. *CA Cancer J Clin* **70**, 7–30 (2020).

4- Falletta, P. *et al.* Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes Dev.* **31**, 18–33 (2017).

5- Louphrasitthiphol, P. *et al.* MITF controls the TCA cycle to modulate the melanoma hypoxia response. *Pigment Cell Melanoma Res.* **32**, 792–808 (2019).

6- Vivas-García, Y. *et al.* Lineage-Restricted Regulation of SCD and Fatty Acid Saturation by MITF Controls Melanoma Phenotypic Plasticity. *Mol. Cell* **77**, 120-137.e9 (2020).