

 <p>UniSR Università Vita-Salute San Raffaele</p>	<p>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</p>	<p>MO 20-5 ed. 01 del 21/02/2025 PO 20 Page 4 of 10</p>
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PROJECT

Supervisor: Rosanna Mezzapelle

Title: “Unraveling cell interactions in the mesothelioma microenvironment to improve antitumor responses elicited by HMGB1 inhibition”

Curriculum: *Cell and Molecular Biology*

Link to the personal page of the University or relevant hospital site website:

<https://research.hsr.it/en/divisions/genetics-and-cell-biology.html>

Description of the Project (max 3,000 characters including spaces)

Back ground/gap of knowledge

It is now well accepted that both tumor associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) shape the tumor microenvironment and determine its aggressiveness, immunogenicity and sensitivity to treatment. However, how tumor cells, TAMs and CAFs interact within tumor microenvironment is still not well understood. We study malignant mesothelioma (MM), a rare but incurable cancer that arises from mesothelial cells lining the body cavities, mostly of the pleura, after a long period of chronic inflammation. A key determinant in MM onset is High Mobility Group Box 1 protein (HMGB1), a DNA chaperone that, when released outside the cell, works as a signal of danger (1). HMGB1 drives persistent inflammation by acting at least at two levels: 1) it recruits and activates macrophages (2, 3) and 2) it switches resting fibrocytes to CAFs (4); moreover, CAFs secrete HMGB1 that in turn stimulates metastatic spreading of cancer cells (5).

We found that BoxA, an HMGB1 competitor, shows anti-tumor activity by switching macrophage phenotype and activity to boost antitumor immune responses (6). We have preliminary data showing that some mesothelioma cells show a transcriptional profile typical of CAFs. The ability to differentiate into CAFs may be a trait of MM cells that they inherit from mesothelial cells, and therefore be specific to MM; alternatively, cancer cell differentiation into fibroblasts might be a common endpoint of the process of epithelial-to-mesenchymal transition (EMT) that cancer cells undergo in many different tumors.



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MO 20-5

ed. 01 del 21/02/2025

PO 20

Page 5 of 10

Rationale and hypothesis

Our hypothesis is that TAMs and CAFs exert a check and balance role in both driving tumor progression and at the same time flagging tumor antigens to the adaptive immune system. Both activities involve HMGB1; targeting HMGB1 enhances the immunostimulatory activity of TAMs and can mute the immunosuppressive activity of CAFs.

Objectives and specific aims

To test our hypotheses, we will organize the project in two specific aims:

AIM. 1 Reconstruct HMGB1-driven interactions among cancer cells, TAMs and CAFs in a simplified TME.

Using an *in vitro* 3D system of mesosphere in co-culture with macrophages and fibroblasts we will mimic a simplified TME; in this system we will investigate how the different cell populations change in terms of phenotype and activity, and we will dissect the molecules involved in the cross talk. Focusing on HMGB1, we will test if HMGB1 inhibition switches CAFs and TAMs towards an antitumor phenotype.

AIM. 2 Understand and predict cell-cell interactions by spatial transcriptomics of human samples.

Since studying cell heterogeneity is fundamental to understand cancer biology and to find new therapeutic strategies, we will untangle the contribution and the function of CAFs in MM TME by performing spatial transcriptomics in samples from MM patients.

Expected outcomes

The knowledge generated in our project has the potential to lay the foundations for future strategies of intervention in cancer, since it will contribute to define the molecular bases that regulate the interplay between mesothelioma cells, fibroblasts, and macrophages and how they influence each other's fate.

Skills that the student should acquire (max. 600 characters including spaces):

The student will learn how to organize his work and his time, to think critically, to plan his/her experiments and solve and overcome problems.

He/she will work with cancer models developed in-house, including mice and 3D *in vitro* models; learn about immune responses to cancer and how to measure them using multiple assays, primarily flow cytometry and cell imaging.



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MO 20-5

ed. 01 del 21/02/2025

PO 20

Page 6 of 10

He/she will work with a multi-disciplinary team involving bioinformaticians, pathologists and thoracic surgeons.

References (max. 15)

1. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*. 2002;418:191–5. doi: 10.1038/nature00858
- 2 Suarez, J. S. et al. HMGB1 released by mesothelial cells drives the development of asbestos-induced mesothelioma. *Proceedings of the National Academy of Sciences* 120, (2023).
- 3 Yang, H. et al. Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. *Proc Natl Acad Sci U S A* 107, 12611–12616 (2010).
- 4 Borchert, S. et al. Cancer-Associated Fibroblasts Influence Survival in Pleural Mesothelioma: Digital Gene Expression Analysis and Supervised Machine Learning Model. *Int J Mol Sci* 24, 12426 (2023).
- 5 Ren, Y. et al. Autophagic secretion of HMGB1 from cancer-associated fibroblasts promotes metastatic potential of non-small cell lung cancer cells via NFκB signaling. *Cell Death Dis* 12, 858 (2021).
- 6 Mezzapelle R, De Marchis F, Passera C, Leo M, Brambilla F, Colombo F, et al. CXCR4 engagement triggers CD47 internalization and antitumor immunization in a mouse model of mesothelioma. *EMBO Mol Med*.2021;13:1–17. doi: 10.15252/emmm.202012344
- 7 Mayer, S. et al. The tumor microenvironment shows a hierarchy of cell-cell interactions dominated by fibroblasts. *Nat Commun* 14, 5810 (2023).