PROJECT 1

DoS: **Giovanni Sitia**

Title: **Development of hyaluronan-based (HA) hydrogels for peri-operative immunotherapies**

Curriculum: Basic and Applied Immunology and Oncology

Link to OSR/UniSR personal page:

https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/experimental-hepatology/giovanni-sitia.html

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

Colorectal cancer (CRC) are among the most common and deadly cancers worldwide [1]. Surgical resection of the primary tumor is the mainstay of treatment [2,3]. Unfortunately, despite chemotherapy and targeted adjuvant therapies, up to 50% of these patients often develop life-threatening metastases in subsequent years [2,3]. While the overall benefit of surgery is well established [2,3], there is also evidence that this procedure can promote metastases by increasing the spread of tumor cells in the bloodstream [4,5], causing adhesion of circulating tumor cells to the endothelium [6], or causing transient immunosuppression that awakens dormant micrometastases [7]. Accordingly, there is growing recognition that the use of perioperative immunotherapies in patients undergoing surgical resection of their primary cancer may provide a unique treatment window to prevent metastatic colonization of distal organs and control minimal residual disease [8,9]. In this context, we recently defined the efficacy and mode of action of continuous IFNa therapy using mouse models of liver colorectal cancer metastases (CRC). In particular, we found that: (a) the efficacy of IFNa immunotherapy depends on Ifnar1 expression by liver sinusoidal endothelial cells (LSECS); (b) continuous intraperitoneal release of IFNa by acting on hepatic endothelial cells, shapes a vascular antimetastatic barrier that impedes tumor cells trans-sinusoidal migration promoting protective long-term antitumor immunity. However, we need to develop delivery strategies suitable for clinical translation. To this regard, the use of fully biodegradable FDA-approved scaffolds containing IFNs may represent a convenient way to deliver IFNs and other drugs that promote local expression of type I interferons [5], such as agonists of the stimulator of interferon genes (STING) or Toll-like receptor 7/8 (TLR7/8) to hepatic endothelial cells to shape antimetastatic vascular barriers and transform the properties of a "healthy" metastatic-permissive organ into an anti-metastatic state preventing metastatic colonization and controlling minimal residual disease [5].

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

In collaboration with Prof Filippo Rossi (Politecnico di Milano), test the in vitro degradation kinetics of HA-hydrogels with different molecular weights. Prepare HA-hydrogels containing IFNa and define the in vitro kinetics of IFNa release. Define the in vivo degradation profile of HA-IFNa using ELISA assays on serum from mice implanted with HA-IFNa and other drugs. Maintain tumor cell lines and tumor CRC organoids in culture. Perform in vivo non invasive bioluminescence imaging. Perform flow cytometry analyses of murine PBMCs, liver infiltrating leukocytes or tumor infiltrating leukocytes. Perform total RNA extraction from murine tissues. Perform multiparametric confocal IF. Design, interpret and communicate experimental results.

References (max. 15)

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3. Gennari, A., et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 32, 1475-1495 (2021).
4. Deneve, E., et al. Capture of viable circulating tumor cells in the liver of colorectal cancer patients. Clin Chem 59, 1384-1392 (2013).
5. Park, C.G., et al. Extended release of perioperative immunotherapy prevents tumor recurrence and eliminates metastases. Science translational medicine 10(2018).
6. Chambers, A.F., Groom, A.C. & MacDonald, I.C. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2, 563-572 (2002).
7. Ananth, A.A., et al. Surgical Stress Abrogates Pre-Existing Protective T Cell Mediated Anti-Tumor Immunity Leading to Postoperative Cancer Recurrence. PloS one 11, e0155947 (2016).
8. Horowitz, M., Neeman, E., Sharon, E. & Ben-Eliyahu, S. Exploiting the critical perioperative period to improve long-term cancer outcomes. Nature reviews. Clinical oncology 12, 213-226 (2015).
9. Badia-Ramentol, J., Linares, J., Gomez-Llonin, A. & Calon, A. Minimal Residual Disease, Metastasis and Immunity. Biomolecules 11(2021).
10. Catarinella, M., et al. IFNalpha gene/cell therapy curbs colorectal cancer colonization of the liver by acting on the hepatic microenvironment. EMBO Mol Med 8, 155-170 (2016).