PROGETTO

Supervisore::	Mario Leonardo Squadrito
Titolo/*Title*:	***In vivo* genetic engineering of liver metastases to modulate immune circuits**
Curriculum:	Gene and Cell Therapy
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/targeted-cancer-gene-therapy.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Liver metastases are often fatal for cancer patients due to their resistance to conventional therapies such as surgery, chemotherapy, immunotherapy, and targeted therapy. Therefore, unlike primary tumors, which can often be surgically removed and in most cases respond to pharmacological treatments, liver metastases pose a significant challenge due to their invasive nature and resistance to treatment. Understanding the factors contributing to metastatic dissemination in the liver and developing effective therapeutic strategies are critical to improving patient outcomes.

Rationale and hypothesis

The project aims to utilize lentiviral vector (LV)-based gene transfer technologies to deliver molecules capable of reprogramming the tumor microenvironment in metastatic liver cancer mouse models. Our previous studies have demonstrated the potential of LVs to deliver therapeutic payloads, such as interferon-alpha (IFNa), to tumors and enhance the efficacy of cancer immunotherapy. [1-4] Building upon this, the project will explore the use of a new LV platform to deliver engineered proteins that can modulate the immunosuppressive tumor microenvironment and promote anti-tumor immune responses.

Objectives and specific aims

1. Develop and optimize a LV-based liver targeting platform.
2. Use CRISPR/Cas9 technology to improve existing liver metastasis mouse models, including engineering CRC cell lines to reduce immunogenicity and increase their ability to originate spontaneous liver metastases.
3. Investigate the therapeutic efficacy of the LV platform for *in vivo* gene engineering of liver metastases.

4. Employ single-cell RNA sequencing and other omics techniques to study the tumor microenvironment following LV treatment, with a focus on elucidating the molecular mechanisms underlying the efficacy of gene therapy strategies and overcoming mechanisms of resistance to therapy.

Expected outcomes

The projects will enable the optimization of an LV-based platform to effectively deliver immune-activating molecules to LM. Moreover, state-of-the-art technologies will be exploited to characterize the immune landscape of the liver metastases upon treatment. Mechanism of immunosuppression will be studied and then reverted by incorporating additional payloads to the LV design.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

Cell Culture of cell lines and primary cells; mouse handling; lentiviral vector design, production and validation; CRISPR/CAS9-based techniques; gene expression analysis by RNA sequencing, Taqman and ddPCR; molecular biology techniques: PCR, DNA/RNA extraction, electrophoresis; copy number analysis by digital droplet PCR (ddPCR); multicolor flow cytometry; confocal microscopy; experimental design and data analysis; presentation of results, discussion and manuscript writing.

Bibliografia

Escobar, G., Moi, D., Ranghetti, A., Ozkal-Baydin, P., Squadrito, M.L., Kajaste-Rudnitski, A., Bondanza, A., Gentner, B., De Palma, M., Mazzieri, R., and Naldini, L. (2014). Genetic engineering of hematopoiesis for targeted IFN-alpha delivery inhibits breast cancer progression. **Science translational medicine** 6, 217ra213. 10.1126/scitranslmed.3006353.

2. Birocchi, F., Cusimano, M., Rossari, F., Beretta, S., Rancoita, P.M.V., Ranghetti, A., Colombo, S., Costa, B., Angel, P., Sanvito, F., et al. (2022). Targeted inducible delivery of immunoactivating cytokines reprograms glioblastoma microenvironment and inhibits growth in mouse models. **Science translational medicine** 14, eabl4106. 10.1126/scitranslmed.abl4106.

3. Kerzel, T., Giacca, G., Beretta, S., Bresesti, C., Notaro, M., Scotti, G.M., Balestrieri, C., Canu, T., Redegalli, M., Pedica, F., et al. (2023). In vivo macrophage engineering reshapes the tumor microenvironment leading to eradication of liver metastases. **Cancer cell** 41, 1892-1910 e1810. 10.1016/j.ccell.2023.09.014.

4. Giacca, G., Naldini, L., and Squadrito, M.L. (2024). Harnessing lentiviral vectors for in vivo gene therapy of liver metastases. **Clin Transl Med** 14, e1542. 10.1002/ctm2.1542.