UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.11

Proponent: Prof. Luisa Fiandra

Project Title: RNA nanodelivery to pancreatic cancer microenvironment for the stimulation of tumor immune response

Scientific background and 'open issues'

Pancreatic Ductal Adenocarcinoma (PDAC) is a common type of pancreatic cancer with dismal mortality rates. To date, complete surgical resection is the one and only cure for advanced PDAC. However, less than 20% of all PDAC patients are eligible for surgery.

Peculiar features of the tumor microenvironment (TME), such as immunosuppressive leukocytes, cancer-associated fibroblasts, and a highly fibrotic stroma causing tumor hypo-vascularization and vessel abnormalities, have undermined previous attempts at developing effective therapies. In addition, the complexity of TME makes studying PDAC cells in isolation of limited translational relevance. Hence, finding more effective treatment options that target the TME in advanced PDAC is an unmet medical need that must be urgently addressed.

Targeting the TME with selective nanocarriers is now considered a hot topic in cancer research. The delivery of nucleic acids is attracting increasing interest with the aim to activate antitumor action, including TME remodulation, stimulation of the immune response and activation of specific apoptotic processes. Yet, a major hurdle in studying novel PDAC therapeutic strategies resides in the lack of reliable in vitro systems recapitulating the complexity of the TME.

Objectives

Objective of project is the validation of advanced 3D cell culture models as tools to study the interplay between tumor cells and fibroblasts, with the aim to detect the molecular actors involved in this interaction and detect possible therapeutic targets for the tumor treatment. In particular, the project will be focused on aggressive metastatic cancers, as the TNBC and the PDAC, which are characterized by a dense and desmoplastic matrix and a large amount of CAFs into the TME, which highly contribute to the aggressiveness and poor prognosis of these tumors.

Once identified the target/s, appropriate nanoparticles will be formulated to deliver genetic materials (mRNA or siRNA), with the aim to produce proteins able to inhibit specific pathways or directly hinder the production of molecular actors involved in the TME-tumor cells cross-talk and his pro-tumoral effect.

Methodologies

According to the experimental design, the PhD students will be involved in:

1) designing and characterizing lipid nanoparticles and innovative polymer nanoparticles suitable for stable RNA incorporation and delivery
2) determining the most efficient RNA nanocomplex in terms of loading, stability, and delivery
3) developing an *in vitro* 3D vascularized PDAC model to assess the transcytosis of the nanocarriers across the tumor endothelial barrier and its release into the TME
4) assessing the anti-cancer and endothelium-normalizing effects of mRNA-nanocarriers in the 3D *in vitro* T cell-endowed vascularized PDAC model
5) evaluating the ability of mRNA-nanocarriers to reach tumor in mouse models of PDAC
6) studying the potential synergistic effect of mRNA-nanocarriers combined with immune-checkpoint inhibitors in hampering PDAC growth in vivo.

Collaboration / Co-tutoring opportunities

The project will be carry out in collaboration with the "Tumor Microenvironment Laboratory at Candiolo Cancer Institute" (https://www.irccs.org/tumor-microenvironment), where are present competences and equipment for the study of the mRNA-nanocarriers in mouse PDAC models.

Project's Sustainability & Mobility

The project will mainly take place in the two laboratories of "Advanced Cells Culture" of BtBs, directed by Prof. Luisa Fiandra, and of "Cell pathobiology and molecular treatment", directed by Dr. Metello Innocenti, where are present all competences and equipment necessary to 1) reproduce the tumor 3D models, 2) study their biological activity, and 3) validate the activity of nanoformulations' on these systems.
This project will be also performed in collaboration with the NanoBioLab, laboratory of nanobiotechnology and nanomedicine of BtBs, directed by Prof. Prosperi, whose main activity has been recently devoted to the production of lipidic and protein-based nanosystems for the delivery of genetic material, and where RNA-nanocarriers will be produced and characterized.

Pertinent research articles authored by the proponent research team:

1) Del Nero, M., ….. **Innocenti, M**., **Prosperi D.**, Colombo M., **Fiandra L.**. (2023). Advanced Cell Culture Models Illuminate the Interplay between Mammary Tumor Cells and Activated Fibroblasts. CANCERS, 15(9)
2) Zhao, Y., ….. **Innocenti M**; Kong B. (2021). Complex to Promote Kras G12D-Induced Acinar-to-Ductal Metaplasia and Early Pancreatic Carcinogenesis. GASTROENTEROLOGY, 160(5), 1755-1770.
3) Salvioni, L., … **Prosperi D**, Riccardo Vago, Miriam Colombo. (2020). Nanoparticle-Mediated Suicide Gene Therapy for Triple Negative Breast Cancer Treatment. ADVANCED THERAPEUTICS, 3(8)

The mobility to foreign institutions could include: 1) the laboratory of Dr. Bo Kong of Ulm University Hospital (Germany), that is devoted to the study of the molecular pathways involved in pancreatic carcinogenesis using patient-derived pancreatic cancer models; 2)

the laboratory of gene delivery of Prof. Dan Peer at Tel Aviv University allows student exchange during the PhD internship.

References

(1) Bejarano L, et al. Cancer Discov. 2021; 11, 933–959.
(2) Manji GA, et al. Clin Cancer Res. 2017 Apr 1;23(7):1670-1678.
(3) Tian L, et al. Nature 2017; 544:250-254.
(4) Mastrantonio R, et al. Theranostics 2021; 11:3262-3277.
(5) Das R, et al. Bioconjug Chem, 2022. 33:1996–2007.
(6) Hou X, et al. Nat Rev Mater, 2021. 6:1078–1094.
(7) Gioielli N. et al. Sci Transl Med. 2018 May 23;10(442).