DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

40th CYCLE
Project proposal for a Sapienza PhD scholarship

Main research line

Title: Characterization of epigenetic mechanisms involved in the regulation of pancreatic cancer associated fibroblasts upon metabolic alterations

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Summary

Epidemiological studies pointed out a correlation between dysmetabolism and pancreatic cancer. Pancreatic ductal adenocarcinoma (PDAC), which comprises 85% of all the pancreatic cancers, is one of the most aggressive and deadly tumor with less than 8% of survival 5 years after diagnosis. PDAC is characterized by a dense fibrotic stroma contributing to the poor clinical outcome and the lack of chemotherapy response.
The present project focuses on investigating the effects of chronic metabolic alterations on the transition of pancreatic stellate cells (PSCs) into pancreatic cancer-associated fibroblasts (CAFs), the most abundant cell type in the pancreatic tumor microenvironment, during the onset and progression of PDAC. Obesity and diabetes will be two dysmetabolic conditions considered in the present study. The goal will be to understand how dysmetabolism influences CAFs in supporting PDAC stroma deposition, ultimately affecting immune response evasion and therapy resistance. CAF differentiation and organization within the tumor microenvironment will be investigated with a particular emphasis on the DNA methylation cycle involvement and sensitivity to metabolic rewiring. Indeed, DNA methylation cycle is a pathway particular sensitive to cancer metabolic rewiring, which modifying the cytosine methylation patterns affects cell transcriptomes and contributes to establish an "epi-metabolic memory."
The project will employ a combination of in vitro and ex vivo approaches. At first, commercially available PSCs will be cultured under dysmetabolic conditions, mimicking high glucose and fatty acid levels. Molecular and cellular analyses will be conducted to investigate how dysmetabolism affects the transition of PSCs into CAFs and the activation of specific transcriptional programs associated with stromal activation. Attention will be given to the DNA methylation cycle and its impact on gene expression. To validate the findings from in vitro studies, a biobank of freshly isolated CAFs derived from a cohort of PDAC patients with or without a history of metabolic syndrome will be exploited.

Furthermore, the project will explore the potential of pharmacological strategies based on hypoglycemic agents to reverse or slow down PDAC progression and increase the response to therapy. Hypoglycemic agents (i.e. Metformin), will be tested to evaluate their impact on the dysmetabolic-mediated transition of PSCs into CAFs and on human isolated CAFs derived from patients with a history of metabolic syndrome. Additionally, the effect of hypoglycemic agents on overcoming therapy refractoriness, particularly in response to gemcitabine and abraxane, will be assessed using 3D co-culture systems of CAFs and tumor cells. The project will take advantage of different molecular biology techniques, including qRT-PCR, chromatin immunoprecipitation (ChIP), and Western blotting, to study transcriptomic and epigenetic landscape. ELISA assays will be used to quantify specific metabolites involved in the DNA methylation enzymatic machinery regulation and determine metabolomic background. Overall, this project aims to shed light on the impact of dysmetabolism on the activation and function of CAFs in PDAC, with the goal of identifying potential therapeutic strategies to target the desmoplastic stroma and improve treatment outcomes.

Pertinent Publications of the proponent

1. Amormino C, Russo E, Tedeschi V, Fiorillo MT, Paiardini A, Spallotta F, Rosanò L, Tuosto L, Kunkl M. Targeting staphylococcal enterotoxin B binding to CD28 as a new strategy for dampening superantigen-mediated intestinal epithelial barrier dysfunctions *Front Immunol.* 2024 Mar 6;15:1365074.
2. Kunkl M, Amormino C, Spallotta F, Caristi S, Fiorillo MT, Paiardini A, Kaempfer R, Tuosto L. Bivalent binding of staphylococcal superantigens to the TCR and CD28 triggers inflammatory signals independently of antigen presenting cells. *Front Immunol.* 2023 May 3;14:1170821. doi: 10.3389/fimmu.2023.
3. Cencioni C, Scagnoli F, Spallotta F, Nasi S, Illi B. The "Superoncogene" Myc at the Crossroad between Metabolism and Gene Expression in Glioblastoma Multiforme. *Int J Mol Sci*. 2023 Feb 20;24(4):4217.
4. De Martino S, Iorio E, Cencioni C, Aiello A, Spallotta F, Chirico M, Pisanu ME, Grassi C, Pontecorvi A, Gaetano C, Nanni S, Farsetti A MALAT1 as a Regulator of the Androgen-Dependent Choline Kinase A Gene in the Metabolic Rewiring of Prostate Cancer. *Cancers (Basel)* 2022 Jun 12;14(12):2902.
5. Cencioni C, Trestini I, Piro G, Bria E, Tortora G, Carbone C, Spallotta F. Gastrointestinal Cancer Patient Nutritional Management: From Specific Needs to Novel Epigenetic Dietary Approaches. *Nutrients* 2022, Apr 8;14(8):1542.
6. Hammer Q, Dunst J, Christ W, Picarazzi F, Wendorff M, Momayyezi P, Huhn O, Netskar HK, Maleki KT, García M, Sekine T, Sohlberg E, Azzimato V, Aouadi M, Karolinska COVID-19 Study Group, Severe COVID-19 GWAS Group, Degenhardt F, Franke A, Spallotta F, Mori M, Michaëlsson J, Björkström NK, Rückert T, Romagnani C, Horowitz A, Klingström J, Ljunggren HG, Malmberg KJ. SARS-CoV-2 Nsp13 encodes for an HLA-E-stabilizing peptide that abrogates inhibition of NKG2A-expressing NK cells. *Cell Rep* 2022, Mar 8;38(10):110503.
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8. Carbone C, Piro G, Agostini A, Delfino P, De Sanctis F, Nasca V, Spallotta F, Sette C, Martini M, Ugel S, Corbo V, Cappello P, Bria E, Scarpa A, Tortora G. Intratumoral injection of TLR9 agonist promotes an immunopermissive microenvironment transition and causes cooperative antitumor activity in combination with anti-PD1 in pancreatic cancer. *J Immunother Cancer.* 2021 Sep;9(9):e002876.

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3. Shan T, Chen S, Chen X, Lin WR, Li W, Ma J, et al. Cancer-associated fibroblasts enhance pancreatic cancer cell invasion by remodeling the metabolic conversion mechanism. Oncol Rep 2017;37:1971-9
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