**DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO**

**39 CYCLE**

**Project proposal for a Sapienza PhD scholarship**

**Other research line**

**Title:Characterization of epigenetic mechanisms involved in the regulation of pancreaticcancer associated fibroblasts upon metabolic alterations**

**Supervisor:** Francesco Spallotta

**e-mail:** francesco.spallotta@uniroma1.it

<https://corsidilaurea.uniroma1.it/it/users/francescospallottauniroma1it>

<https://orcid.org/0000-0002-0466-6286>

**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 projectwill 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 analyseswill be conducted to investigate how dysmetabolism affects the transition ofPSCs into CAFs and the activation of specific transcriptional programsassociated with stromal activation. Attention will be given to the DNAmethylation cycle and its impact on gene expression.

To validatethe findings from in vitro studies, a biobank of freshly isolated CAFs derivedfrom a cohort of PDAC patients with or without a history of metabolic syndromewill be exploited.

Furthermore,the project will explore the potential of pharmacological strategies based onhypoglycemic agents to reverse or slow down PDAC progression and increase theresponse to therapy. Hypoglycemic agents (i.e. Metformin),will be tested to evaluate their impact on the dysmetabolic-mediated transitionof PSCs into CAFs and on human isolated CAFs derived from patients with ahistory of metabolic syndrome. Additionally, the effect of hypoglycemic agentson overcoming therapy refractoriness, particularly in response to gemcitabineand abraxane, will be assessed using 3D co-culture systems of CAFs and tumorcells.

The projectwill take advantage of different molecular biology techniques, including qRT-PCR, chromatinimmunoprecipitation (ChIP), and Western blotting, to study transcriptomic and epigenetic landscape. ELISA assays will be used to quantify specificmetabolites 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 theactivation and function of CAFs in PDAC, with the goal of identifying potentialtherapeutic strategies to target the desmoplastic stroma and improve treatmentoutcomes.

**Pertinent Publications of the proponent (last 5 years)**

1. 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 in press.
2. 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.
3. 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.
4. 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.
5. 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.
6. Azzimato V, Chen P, Barreby E, Morgantini C, Levi L, Vankova A, Jager J, Sulen A, Diotallevi M, Shen JX, Miller A, Ellis E, Rydén M, Näslund E, Thorell A, Lauschke VM, Channon KM, Crabtree MJ, Haschemi A, Craige SM, Mori M, Spallotta F, Aouadi M. Hepatic miR-144 Drives Fumarase Activity Preventing NRF2 Activation During Obesity. *Gastroenterology*2021, Dec;161(6):1982-1997.e11.
7. 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.
8. Salvatori L, Spallotta F, Gaetano C, Illi B. Pillars and Gaps of S-Nitrosylation-Dependent Epigenetic Regulation in Physiology and Cancer. *Life (Basel).* 2021 Dec 17;11(12):1424.
9. Bankov K, Döring C, Ustaszewski A, Giefing M, Herling M, Cencioni C, Spallotta F, Gaetano C, Küppers R, Hansmann ML, Hartmann S. Fibroblasts in Nodular Sclerosing Classical Hodgkin Lymphoma Are Defined by a Specific Phenotype and Protect TumorCells From Brentuximab-Vedotin Induced Injury. *Cancers (Basel).* 2019 Oct 30;11(11):1687.
10. Cencioni C, Gaetano C, Spallotta F. Dissecting Cytosine Methylation Mechanics of Dysmetabolism. *Aging (Albany NY).* 2019 Jan 23;11(3):837-838.
11. Cencioni C, Heid J, Krepelova A, Rasa SMM, Kuenne C, Guenther S, Baumgart M, Cellerino A, Neri F, Spallotta F, Gaetano C. Aging Triggers H3K27 Trimethylation Hoarding in the Chromatin of Nothobranchiusfurzeri Skeletal Muscle. *Cells*. 2019 Sep 28;8(10):1169.
12. Savoia M, Cencioni C, Mori M, Atlante S, Zaccagnini G, Devanna P, Di Marcotullio L, Botta B, Martelli F, Zeiher AM, Pontecorvi A, Farsetti A, Spallotta F, Gaetano C. P300/CBP-associated Factor Regulates Transcription and Function of Isocitrate Dehydrogenase 2 During Muscle Differentiation. *FASEB J.* 2019 Mar;33(3):4107-4123.

**References:**

1. Braun S, Bitton-Worms K, LeRoith D. The link between the metabolic syndrome and cancer. *Int J Biol Sci* 2011; 2011:7(7):1003-15.
2. von Ahrens D, Bhagat TD, Nagrath D, Maitra A, Verma A. The role of stromal cancer-associated fibroblasts in pancreatic cancer. *J Hematol Oncol*. 2017 Mar 28;10(1):76.
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
4. Lu C, Thompson CB. Metabolic regulation of epigenetics. *Cell Metab* 2012;16:9-17
5. Testa R, Bonfigli AR, Prattichizzo F, La Sala L, De Nigris V, Ceriello A.The "Metabolic Memory" Theory and the Early Treatment of Hyperglycemia in Prevention of Diabetic Complications.*Nutrients* 2017 Apr 28;9(5):437.
6. Michelet X, Dyck L, Hogan A, Loftus RM, Duquette D, Wei K, BeyazS, TavakkoliA, Foley C, Donnelly R, O'FarrellyC, RaverdeauM, Vernon M, PetteeW, O'Shea D, NikolajczykBS, Mills KHG, Brenner MB, Finlay D, Lynch L.Metabolic reprogramming of natural killer cells in obesity limits antitumor responses.*Nat Immunol*2018 Dec;19(12):1330-1340.
7. Cencioni C, Atlante S, Savoia M, Martelli F, Farsetti A, Capogrossi MC, Zeiher AM, Gaetano C, Spallotta F. The double life of cardiac mesenchymal cells: Epimetabolic sensors and therapeutic assets for heart regeneration.*PharmacolTher* 2017 Mar;171:43-55.
8. Cascetta P, Cavaliere A, Piro G, Torroni L, Santoro R, Tortora G, Melisi D, Carbone C. Pancreatic Cancer and Obesity: Molecular Mechanisms of Cell Transformation and Chemoresistance. *Int J Mol Sci.* 2018 Oct 25;19(11):3331.
9. Ohlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med* 2017;214:579-96
10. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. *Cancer Discov* 2019Aug;9(8):1102-1123.
11. Moffitt RA,Marayati R,Flate EL,Volmar KE,Herrera Loeza SG, Hoadley KA, Rashid NU,Williams LA, Eaton SC,Chung AH, Smyla JK,AndersonJM,Kim HJ,Bentrem DJ,Talamonti MS,Iacobuzio-Donahue CA, Hollingsworth MA and Yeh JJ. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma.*Nat Genet.* 2015 Oct; 47(10): 1168–1178.