UNIVERSITÀ DEGLI STUDI DI MILANO

D-MEM PhD-UNIMI | Doctorate program Milan EXPERIMENTAL MEDICINE

Tumor-associated microbiota as new player in modulating the immune response in the tumor microenvironment and in promoting immunotherapy resistance

Lucia.sfondrini@unimi.it
Molecular Targeting Unit- Research Department AMADEOLab, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan (Italy); Department of Biomedical Science for Health, University of Milan

Beside the gut, several tissues harbor a unique microbiota. Recent findings revealed specific changes of the microbiome profile in tumoral tissues of various histotypes versus the normal counterparts and their effects on different cancer (Nejman). Interestingly, in lung and pancreatic cancer, tumor associated microbiome has been demonstrated to affect cancer progression through the recruitment of ìmmunosuppressive/ inflammatory cells able to inhibit the anti-tumor immune response (Le Noci; Jin; Pushalkar). In addition, direct effects induced by tumoral tissue-associated microbiota have been revealed in colon and mammary tumors, where the release of bacterial toxins or metabolites able to cause DNA damages and influence tumor growth /migration have been demonstrated (Parida; Sears). These data raise the possibility that the tumor-associated microbiome may be an additional player in the complex tumor ecosystem (Zhao).
We published that lung microbiota manipulation by local delivery of aerosolized antibiotics or probiotics, a feasible and non-invasive strategy, can revert the immunosuppressive status and promote the immune control of experimental metastases induced by iv injection of B16 melanoma cells (Le Noci).
Thus, commensal microorganisms that colonize the respiratory tract might influence clinical outcome in lung cancer patients and represent a therapeutic target to counteract the immunosuppressive status.

This project has two primary **objectives**:
1) to define whether and how tumors with distinct histotypes affect lung microbiota populations and promote immunosuppression and to determine the relationship between lung microbiota populations and tumor growth/progression
2) to establish if a specific microbiota is associated with immunotherapy resistance and the relevance of its targeting in the responses to immunotherapy.

Experimental Plan:
Task 1. Do tumors with distinct histotypes and immunogenicities have differing effects on the local microbiota population? Which bacterial species are associated with specific histotypes, and how do lung bacterial communities influence the immunosuppressive microenvironment?.
We will inject mice with tumor cells of different histotype that may metastatize to the lung. Modifications in the microbiota compositions that are specifically associated with the growth of

different tumors will be assessed by 16S rRNA gene profiling. To determine the immunosuppressive/inflammatory populations activated by the bacterial communities associated to the different tumors and their effects on the activation of anti-tumor T and NK cells, mice will be treated with aerosolized antibiotics and the different subpopulation of lynfoid and myeloid compartments assessed by multiparametric flow cytometry (FC) and immunohystochemistry (IHC). We will also isolate the specific bacterial communities from lungs of tumor-bearing mice by culture techinque and analyze in vitro their immunological effects.

Task 2. Do lung microbiota compositions affect the clinical outcomes of NSCLC patients?

We will analyze the microbiota compositions of a large retrospective NSCLC patients cohort from the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, comparing the taxonomic profiles of lung microbiota isolated from frozen specimens collected from patients with progressive disease profiles who died within the first 2 years after surgery with those of samples from patients (matched by stage, nodes, and histology) who survived, without disease progression, for at least 5 years of follow up. The results of this study will define whether specific microbial communities (or the relative abundance of specific OTUs) are associated with differential prognoses. Moreover, the microbiota profiling results will be correlated with profiles of tumor-infiltrating immune cells, based on immunohistochemistry (IHC) analyses.

Task 3. Does the lung microbiota have an impact on the response to immune checkpoint inhibitors and can modulating the microbial community improve their effects?

It will be evaluated whether aerosolization of antibiotics or probiotics can improve the efficacy of anti-PD1 antibody in two models which respond differently to anti-PD-1 antibody. Mice will be treated intraperitoneally (i.p.) with anti-PD-1 alone or in combination with aerosolized antibiotics or probiotics. The improvement in anti-PD-1 activity will be analyzed based on the number and size of lung metastases and on the local and systemic activation of an anti-tumor immune response.

This project is supported by Associazione Italiana Ricerca sul Cancro (Progetto IG 2020 ID 24718 2021-2026; PI: Dr. Lucia Sfondrini).
For further information please e-mail lucia.sfondrini@unimi.it..

REFERENCES

Nejman et al. Science 2020, 368: 973
Le Noci V et al. Cell Rep. 2018 24: 3528.
Jin C et al. Cell 2019, 176:998.
Pushalkar S et al. Cancer Discov 2018, 8:403
Parida S. et al. Cancer Discov. 11 (2021) 1138-1157.
Sears CL.et al. J Clin Invest. 124 (2014) 4166-4172.
Zhao K. et al.Signal Transduct.Target Ther. 5 (2020) 136.

UNIVERSITÀ DEGLI STUDI DI MILANO

D-MEM PhD-UNIMI

Doctorate program Milan EXPERIMENTAL MEDICINE

Graphical Abstract