REPORT
25th SOCIAL RETURN OF THE RESEARCH
CANCER

ANALYSIS OF MICROBIOTA PATTERNS ASSOCIATED WITH PANCREATIC CANCER AND STUDY OF THEIR ROLE IN INFLAMMATION OF THE HOST, OXIDATIVE STRESS AND IMMUNE STATE

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1. Project summary

This project aimed at analyzing the microbiota patterns linked to pancreatic cancer in order to shed light on their possible role in host inflammation, oxidative stress, and immune status. Indeed, the oral and gut microbiomes may influence cancer risk and progression through systemic inflammation. Bacteria within the pancreas have been described, but their role in local inflammation (i.e. chronic pancreatitis, CP), precursor and benign lesions (e.g. IPMNs and other cysts) and pancreatic ductal adenocarcinoma (PDA) progression is poorly understood. Evidence from animal model systems suggests that endogenous pancreatic bacteria (EPB) promote the progression of PDA through induction of immunosuppression, and enhancement of resistance to the chemotherapeutic drug gemcitabine. The role of EPB on PDA patient outcomes and prognosis is unknown.

Patients were recruited in both centers (ICO-HUB-IDIBELL and IDIBGI). Clinical data were collected from patients with pancreatic cancer and controls from a liver donor program. Two databases were created with the clinical data: a REDCap database and a more limited database for subproject monitoring. Fresh resected pancreas tissue specimens were collected from patients with PDA and controls. Additional formalin-fixed paraffin-embedded (FFPE) samples from short-term (STS) and long-term survivors (LTS) were also collected to increase the sample size.

Due to different reasons (see next section) the interpretation of the results is still ongoing and only preliminary data are currently available. Metagenomics DNA profiles of the microbiomes were characterized using 16S rRNA bacterial gene sequencing for bacterial and viral genome discovery. Bacterial taxa and relative abundance were compared between the different microbiome compartments and will be related with host inflammation/immune status, and finally, prognostic outcomes in PDA patients.

2. Results obtained

First, we would like to emphasize that the COVID-19 pandemic drastically slowed down the subject recruitment and samples collection during the first two years of the project. Furthermore, the process of analyzing the samples for the RNA sequencing has been

prolonged for several months, and the current results described below are still preliminary.

REDCap clinical and covariate database

Study-authorized personnel (i.e., clinicians, documentarians) retrieved limited information provided by study participants on lifestyle (e.g. smoking status, alcohol intake in previous year). Relevant demographic information, family history of cancer, and relevant clinical data were extracted to construct an anonymized database. A REDCap database was created including the clinical data of 129 patients with pancreatic cancer and 32 controls from a liver donor program. Data included variables such as sample ID, scanned signed consent, reason of non-participation, clinical history number, name, surname, sex, ethnicity, recent smoking status and alcohol intake, date of birth and (if deceased) death date, cause of death, diagnosis date, disease stage, resection extent, antibiotic use and type, proton pump inhibitor use and dose, drug consumption, number of biopsies, tumor grade, and number and type of sample. A second more limited database for subproject monitoring has been created taking into consideration just the following strings: sample ID, diagnosis, sex, age rank, stool sample, blood sample, saliva sample, tissue sample, and vital status.

16S ribosomal RNA subunit gene (16S rRNA) analysis on fresh samples

The data of 87 patients (n=55 pancreatic cancer patients and 32 controls) were sent for a marker-based approach using the 16S rRNA to study the bacterial diversity of 127 fresh-frozen tissue samples. This analysis allowed the description and quantification of the microbial alpha and beta diversity as well as to study the taxonomic profiles from phylum to species levels.

The thorough interpretation of the results is still currently ongoing.

(16S rRNA) analysis on FFPE samples

The data of 78 patients with pancreatic cancer were also analyzed with 16S rRNA. A total of 78 samples were analyzed.

As for the data for the analysis with fresh samples, the thorough interpretation of the results is still currently ongoing.

RNA sequencing

We applied for external funding to analyze data from RNA sequencing and to study the association with the metagenome (16S rRNA data).
Data of 89 patients (n=57 pancreatic cancer patients and n=32 controls) were analyzed. The preliminary analysis showed a very good quality of the samples and highlighted differences between normal and tumor samples.
The thorough analysis and interpretation of the results is still currently ongoing.

Bioinformatics and statistical analysis of risks, outcomes, and survival

We have characterized the bacterial diversity (Shannon H diversity index) and classified its abundance and taxonomy in all pancreatic tissue samples. Additionally, we have carried out search algorithms for known animal virus sequences in the shotgun sequence readings.

Statistical analyses of the microbiomes were performed using beta regression models to examine determinants, demographic (e.g. age, sex, BMI) and clinical (e.g. perioperative antibiotic usage, stent usage before surgery, neoadjuvant therapy), of the abundance and microbiome taxonomy. We have also been able to plot bacterial diversity within and across the pancreatic sample types, as well as multivariate statistical comparisons of microbiomes (abundance and taxonomy) between sample types. The multivariate statistical analysis of bacterial abundance and taxonomy (including taxa with CDDL and NupC+ genes or gemcitabine-metabolizer variants) according to prognostic variables in patients with PDA and the survival analyses (Kaplan-Meier and Cox regression) are still ongoing.

3. Relevance to possible future implications

The work will provide the scientific and clinical community with new findings that can be translated into new clinical practice, early diagnostic tools or even a more ambitious definition of microorganisms with probiotic behaviour in pancreatic tissue.
In the long term this project could provide information about the pancreatic microbiome and biomarkers of host response to the microbiome in PDA patients. This information, in combination with other significant discoveries in the field of biomarkers

of cancer early detection, could move the field toward identifying pancreatic cancer in its earlier stages when the tumor can still be resected.

The final translation of the results might include, but are not limited to, the development of new non-invasive tools for the early detection of pancreatic cancer in high-risk individuals. This would provide clinicians with tools and strategies to reduce PDA mortality and, in case of a putative effect of microbiome/inflammation axis on pancreatic cancer development, to open a new avenue to search for treatments to modulate microbiome to prevent it. However, this tool may need further validation in a larger cohort. It could be implemented in the national health system as a pancreatic cancer early detection tool.

4. Scientific bibliography generated

Unfortunately, given that as yet we have only preliminary results of the project, to this date no scientific bibliography could be generated during the implementation of the project.