FULL PROJECT 3: A MULTI-OMIC APPROACH TO EVALUATE THE ROLE OF THE GUT MICROBIOTA IN MODULATING THE GLIOBLASTOMA RESPONSE TO VIROIMMUNOTHERAPY (2022-2024) Co-leaders: Drs. Candelaria Gomez (MDACC) and Filipa Godoy (UPR)

The overall goal of the project is to assess the role of microbiota and its metabolites in the response of glioblastoma to viroimmunotherapy, which results might constitute the basis for the identification of prognostic biomarkers and discovery of new adjuvant therapies

Inputs

Leaders

Students

- Natalie Melendez (PHD student UPR)
- Miguel Jimenez (Undergraduate, UPR Mayaguez/Godoy Lab
- Andres Lopez-Rivas (MD-PhD student MDAC)

Collaborator Scientists

- Dr Natalyia Chrona (UPR - PR-INBRE metabolomics core)
- Dr Juan Fueyo (MDACC)

Resources

- UPRCCC
- The Center for Tropical and Emerging Infectious Diseases Laboratories
- UPR/MADCC Cores
- IAC
- PSC

Technology

- Multi-Omic integration GC-MS, Next-generation sequencing, shotgun metagenomics and 16S rDNA gene sequencing

Specific Aims & planned initiatives

Aims

1. Determine the role of gut microbial communities in modulating the tumor microenvironment and the efficacy of viroimmunotherapy against glioblastoma.
2. Examine gut microbiota-produced metabolites associated with the response of GBM to viroimmunotherapy

Activities

Aim 1

- Analyze the microbiome (16S and Shotgun) in GSC-005-bearing C57BL/6 mice treated intratumorally with Delta-24-RGDOX oncolytic adenovirus
- Analyze the gut microbial diversity and composition associated with the efficacy of viroimmunotherapy
- Assess the role of gut microbiota signatures as viroimmunotherapy modifiers
- Ascertain the extent to which modifications in the gut microbiota dictates the immune microenvironment of untreated and treated tumors.
- Perform correlative studies analyzing multiple parameters of the tumor microenvironment (flow cytometry, RNA seq, and functional immunological studies). These parameters will be analyzed in glioma-bearing mice that have been supplemented with *Bifidobacterium* or treated with FMT, before and after viroimmunotherapy.

Aim 2

- Examine the shifting of microbiota-derived metabolites during the dysbiosis induced by viroimmunotherapy; we will evaluate untargeted metabolomic changes associated to viroimmunotherapy success.
- Determine key species contributors and their metabolic activity associated to viroimmunotherapy
- Delineate the production of specific anti-cancer metabolites during viroimmunotherapy
- Detect Short Chain Fatty Acids (SCFA) that have related to the response to viroimmunotherapy.
- Train students in microbiome and metabolomics analyses
- Integrate microbiome and metabolomics targeted and untargeted
- Develop lab and bioinformatic SOPs that could be applied to other projects
- Facilitate access to information by following QIITA Metadata resources and giving access to data publicly.
- Work with media to bring knowledge on the microbiome to the community

Outputs

Aim 1

- # students trained in oncolytic therapy and immunology
- # students trained in multi-omics integration
- Validate coculture-based functional immune assays and *in vivo* methods

Aim 2

- Train students in Genomic DNA extractions and Bioinformatics as well as in the use of GC-MS and untargeted metabolomics
- Train students in microbiome, alpha and beta diversity analyses, and statistical tests for microbiota research
- Train students in multi-omic integration using public tools
- Joint publication highlighting oncolytic virotherapy efficacy and gut microbiota signatures of responders including Bifidobacterium supplementation
- AACR Joint Poster presentations (2022, 2023, 2024)

Standardized outputs (overall totals since 09-2022)

- 2 manuscripts in development
- 1 joint publication
- 4 oral presentations
- 5 poster presentations
- 3 collaborations
- 5 students trained in the lab
- 2 grants obtained UPR (R21ES034191); NSF 2243580
- 2 grants obtained MDACC (Alliance for Cancer Gene Therapy; Cure Starts Now)

Outcomes -- Impact

Short-term/ Intermediate

- First-experiment from both labs, to use multi-omics methods to characterize the gut modulation of mice bearing glioma after virotherapy
- Probiotic supplementation could reveal new opportunities for promoting gut homeostasis and the immune response
- Characterize microbial communities and metabolites (alpha, beta diversity, taxonomic profiles and biomarkers and identify possible products and metabolic pathways responsible for improved responses to oncolytic virotherapy (conventionally-raised; germ-free).
- Use of microbiota (fecal matter transplants, FMT) as modulators of viroimmunotherapy.

Long-term

- This project should yield new information about the potential use of probiotic taxa or metabolites as a therapy to improve intestinal microbiota as a potential treatment modifier.
- Bacterial biomarkers associated to success of oncolytic adenoviral therapy.
- Metabolic biomarkers associated to improved responses to therapy.
- An MD-PHD student, connected to this U54, trained at MDACC and exposed to these high-level multi-omic analyses, establishing a bridge between both sites
- Empower the next generation of MD/Microbial ecologists to help in translational medicine.
- Graduation of PhD in May 2024 by Miss Natalie Melendez where the project was instrumental to her scientific formation.

Conditions

- Health
- Human Ecology
- Cancer

Process Evaluation | **Outcome Evaluation**