O' Neal Comprehensive Cancer Center Core

Microbiome/Gnotobiotic Shared Facility

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Institutional Core Microbiome Center

Services and Operation

In response to the increasing realization of the role that microbial pathogens play in inflammation, metabolism and energetics that link obesity and cancer, which are major health concerns for the entire Southeast, a pilot Microbiome and Gnotobiotic Core was started in February of 2012. Understanding the role of the microbiome in these diseases requires investigation of the interactions among genes of the microbiota and host. Essential to such research is the ability to manipulate and define the microbiota, i.e., develop defined-flora and "humanized" animal models. Gnotobiotic mice transplantation provides a unique advantage to investigators to study disease links and health implications of differences identified from the microbiome (and metagenome) analysis.

The overall goal of the CCC/Institutional Core/Microbiome Center Microbiome/Gnotobiotics Facility is to provide investigators with access to cutting-edge microbiome analysis methodologies and gnotobiotic animal models designed to facilitate and strengthen the quality of microbiome related research.

1. To provide state-of-the-art microbiome analysis services to investigators.

The Microbiome component will provide investigators with the full spectrum of resources necessary to study the microbiota population present in human and animal model research. This support will include assistance with experimental design and planning, sample collection and preparation and sequencing of 16S ribosomal genes.

2. To provide computational bioinformatics expertise to assist investigators in the analysis and understanding of microbiome data.

The Bioinformatics component will provide investigators with all of the resources necessary to fully support the wide range of informatics needs required for conducting state-of-the art microbiome and metagenome research. The Bioinformatics component will work with the Biostatistics to provide investigators with the integrated bioinformatics and biostatistics resources.

3. To provide Gnotobiotic Animal services.

The Gnotobiotic Animal Facility will provide investigators access to a number of existing gnotobiotic mouse models generated by the facility, will assist them in developing new gnotobiotic models, assist in "humanizing" these animals by transplantation of archived microbiomes and will support full microbial and immunological analyses of the transplanted microbiota and "humanized" mice.

Rationale and Value-Added

The Microbiome Analysis/Gnotobiotic Facility provides:

- Services for microbiome and tissue sample collection, processing and archiving
- Bioinformatics for the collection, analysis, integration, and reporting of data using a UAB-developed web application that collects data from microbiome projects
- Access to the data on a protected intranet site.
- Gnotobiotic mice and services for transplantation of archived samples that can be used to evaluate the potential of patient microbiomes to catalyze inflammation and ultimately cancer.
- Transgenic animals that are of interest to particular diseases studied by UAB investigators have been, and will be, re-derived germ-free by the Gnotobiotic Facility to provide unique gnotobiotic strains.

Examples of Programmatic Research Using Microbiome/Gnotbiotics

Black and White Women Display Differences in Abundances of Colorectal Cancer Associated Gut Microbial Taxa

Tiffany L. Carson[1], Bradford E. Jackson[1], Ranjit Kumar[2], Elliot J. Lefkowitz[2, 3], Casey Morrow[3], Monica L. Baskin[1]

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Introduction

- Black women are at 48% greater risk for colorectal cancer (CRC) compared to white women even after controlling for known risk factors.
- Emerging research suggests that the gut microbiota may contribute to the etiology and pathogenesis of CRC.
- Over- or underrepresentation of several microbial taxa has been observed in individuals with CRC (table 1) suggesting an association between microbial dysbiosis and CRC.

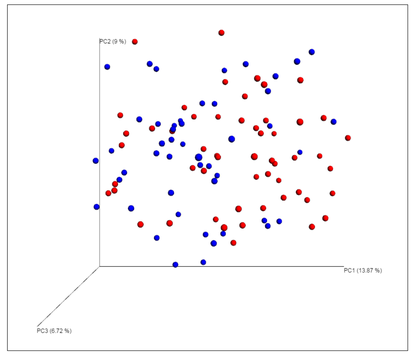


Figure 1. Display of racial differences in composition using Principle Coordinate Analysis

Conclusions

- While black and white women display similar diversity in the gut microbiota, the overall composition of their microbiotas differ by race.
- Many factors (e.g., stress, diet, environment, heredity) influence the gut microbiota which may contribute to the observed differences in microbes.
- Our findings show a relationship between perceived psychological stress and various gut microbes.
- Future research should include examining the relationship between physiological markers of stress (e.g., cortisol) and microbial abundances.
- Future research should also explore the interplay of multiple contributors such as diet, weight, and environment.

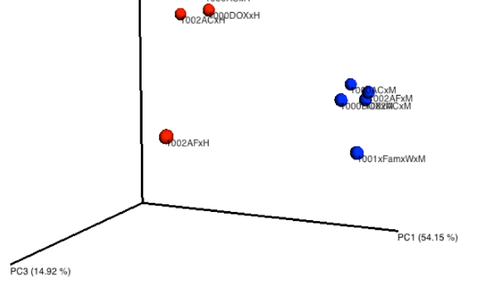
Mechanisms Linking Doxorubicin, Polyphenols and Microbiome in Breast Cancer

Stephen Barnes, Christine Skibola, Trygve Tollefsbol.

In this CCC Pilot Program Project Grant project the principal goal is to determine whether breast cancer patients who are undergoing treatment with doxorubicin and turn to complementary and alternative medicine (CAM) to relieve side effects receive benefit from the CAM modality. The focus will be on the use of CAM herbals or dietary modification/supplements that involve polyphenols. *A central hypothesis will be examined: Are the actions of doxorubicin and the modulation by dietary and CAM polyphenols mediated by effects on the gut microbiome?*

The application consists of project 1 (Christine Skibola) will examine patients on doxorubicin therapy, their intake of polyphenols and their tumor response rates as well as their frequency and severity of side effects and changes on the gut microbiome and project 2 (Trygve Tollefsbol) on the development of fecally humanized mouse models

Semirigid isolators in the gnotobiotic facility



Principle Coordinate Analysis of Human Fecal Samples (red dots) and Gnotobiotic mice transplanted with Human Fecal samples (blue dots).This analysis shows the capacity to transplant mice. Taxa analysis revealed that the transplanted mice had the major taxa found in the human samples.

Current and Projected Use

To enhance the opportunities for Microbiome research, the CCC UAB Insitutional Core and Microbiome Center has established an *innovative* program that provides support for microbiome analysis (by the purchase of reagents necessary for NextGen sequencing on the Illumina MiSeq instrument) and pilot studies in gnotobiotic animals. This *innovative* use of funds has facilitated new studies focused on the role of the microbiome in cancer. As a metric of this added value, since September of 2013 we have analyzed 16,000 microbiome samples for 85 total users. We have also expanded our services to include metagenomic analysis and to date, have performed metagenome analysis on 50 samples. We have established an analytical pipeline from acquisition of samples and multiplex DNA sequencing using the Illumina MiSeq sequencing platform (in collaboration with the Genomic Analysis Facility (Michael Crowley).

Operating Costs and Budget

There is minimal cost to investigators who want 16S microbiome analysis to generate preliminary results for grants. We request that CCC investigators budget a cost of $50 per sample in grants to cover costs which represents the reagent cost for the analysis. Our costs represent a considerable saving to since obtaining the service from outside vendor's costs can be $100 per sample. In addition, the Microbiome/Gnotobiotic facility provides personal interaction to trouble shoot or provide answers for questions. For metagenomic analysis, we anticipate a cost of $700 per sample, which covers the cost of reagents since for our protocol only 10 samples can be analyzed per MiSeq DNA chip.

The Microbiome/Gnotobiotics Facility will provide all mice at a cost of $50 each. The fee to utilize a facility isolator is $200/month, which includes maintenance, food and microbiological monitoring (including Microbiome analysis). Derivations to generate new gnotobiotic strains can be performed for $1,000 per attempt. The facility will maintain the new strains in the isolators at a reduced rate. The fees can also be negotiated for new investigators and those wishing to obtain preliminary results for grants.

Future Plans

- A metagenomic analysis pipeline to provide investigators with the capacity to identify microbes from complex communities at the *species* level. In addition, using single nucleotide polymorphisms (SNP) we can analyze individual microbe populations. to "track" the impact of cancer and cancer treatments on specific microbes in an individual's community. We can also use bioinformatics to investigate the impact on metabolic pathways (e.g. KEGG pathways). Ultimately, we will use this technology to help guide our efforts to reconstitute microbe populations that might be damaged as a result of disease or treatments (e.g. chemotherapy).

- To re-derive germ-free mice for investigators. There is a growing awareness as to a potential role of the gut microbiome in cancer and other diseases. Transgenic mice are of great use for studying these diseases. For example, *Apc-Min* mice, and p53 and p27 knockout mice have shown great utility to study the generation of cancer, as have TRUC mice, which lack an adaptive immune system and the T-bet transcription factor. With seed funds, the core has recently produced a colony TRUC mice into which has been introgressed a colitis susceptibility locus (*Cdcs1*) to facilitate microbiome-driven colonic dysplasia in mice on a pure B6 genetic background.