Tumor Microbiome in Murine 4T1 Triple Negative Breast Cancer
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Abstract
The presence of certain microorganisms has been known to have a strong association with the prognosis and development of human cancers. Recent findings show that the gut microbiome can have a significant role in activating antitumor innate immune responses. In addition, the microbiome in the tumor and its compartment has been found to impact progression and immunotherapy outcomes in patients.

Herein, we utilized 16S deep sequencing platform to profile 4T1 tumor microbiome of mice treated with immune checkpoint therapy (anti-PD-1 + anti-CTLA4) and found that: 1) 4T1 tumors are mostly colonized by aerobic bacteria, 2) tumor progression may be affected by gut microbiota composition, and 3) immune checkpoint therapy may affect microbiome profile and the biodiversity. Furthermore, preliminary qPCR evidence indicates 4T1 tumors may feature a low microbial load, compared with pancreatic, liver, and lung tumors.

Future applications of probiotics as an anti-tumor drug vectors remain open for future exploration.

Key Questions
1. Are 4T1 tumors colonized by bacteria?
2. Does the microbiome affect tumor progression?
3. Does ICT treatment affect the tumor microbiome?

This is important because it can...
- Help understand factors that determine failure or success of treatment.
- Open a new drug delivery route using oral intake of probiotics.

Methods

Results/Observations

Question 1
Are 4T1 Tumors colonized by bacteria?

Does gut microbiome affect tumor microbiome?

1. Bacteria colonizes 4T1
2. Tumor microbiome affected by oral antibiotic treatment

Question 2
Does the 4T1 tumor microbiome affect tumor progression?

1. Untreated mice have lower tumor volume and BLI than Baytril group, indicating relationship between microbiome and tumor progression

Question 3
Does Immune Checkpoint Therapy (ICT) affect the tumor microbiome?

The Simpson's reciprocal index quantifies biodiversity by taking into account species richness and evenness. High species diversity suggests a greater number of successful species and a more stable ecosystem.

Results/Observations

Future Work

1. Identify bacterial candidates
   - Previously known to colonized human tumors
   - Biosafety level 1
   - Commercially available
   - Genome sequenced

2. Culture bacteria – for lab repository and experimental purposes

3. Experimental Opportunities
   - In vivo real time imaging of gut bacteria colonization of tumors – engineer Lux operon into bacteria
   - Bacteria as drug-delivery vector

Candidate Microorganisms

- Pelomonas saccharophila
- ~60% relative abundance in vehicle control mice 1, genome sequenced
- Aerobic, gram negative, Biosafety level 1, commercially available

Lachnospiraceae NK4A126
- ~20% relative abundance in ICT mice 1, genome sequenced
- Anaerobic, gram negative, Biosafety level 1, commercially available

Application

1. Tumor Microbiome Screen (A)
2. Bacteria Culture
3. Bacteria Construction
4. Ideal use-case

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