Title
Identification and Characterization of an Intra-Tumoral Microbiome in Soft Tissue Sarcomas

Authors
Basmaci, Ugur N.
Jones, Louis B.
Chen, Shuai
et al.

Publication Date
2020

Data Availability
The data associated with this publication are not available for this reason: N/A

Identification and Characterization of an Intra-Tumoral Microbiome in Soft Tissue Sarcomas

Ugur N. Basmaci[1], Louis B. Jones[2], Shuai Chen[3], Cordelia Dunai[4], Alicia A. Gingrich[5], Sean J. Judge[5], Lauren M. Perry[5], Arta M. Monjazeb[6], Robert J. Canter[7]

[1]UC Davis School of Medicine, Sacramento, CA, USA; [2]Department of Orthopedic Surgery; [3]Division of Biostatistics, Department of Public Health Sciences; [4]Department of Dermatology; [5]Department of Surgery; [6]Department of Radiation Oncology; [7]Division of Surgical Oncology, Department of Surgery. UC Davis Medical Center, Sacramento, CA, USA.

UC DAVIS HEALTH | SCHOOL OF MEDICINE

Background

- ✓ Immunotherapy is the "fourth pillar" of cancer treatment (Nobel Prize in 2018 by Drs. Allison and Honjo).
- ✓ The gut microbiome has been shown to impact immunotherapy outcomes in cancer. [1]
- ✓ In human-to-mouse experiments involving fecal transplantation (FMT), the effect of the gut microbiome on immunotherapy outcomes was observed to be transferable.[1]
- ✓ Cancer patients who receive antibiotics prior to initiation of immunotherapy may have worse outcomes, which is hypothesized to be due to the negative impact of antibiotics on the gut microbiome.[2]
- ✓ Microbiota have also been identified in solid tumors of the pancreas and breast—organs that communicate with the outside world.[3,4]
- ✓ In a murine model of pancreatic cancer, the intra-tumoral microbiome was shown to promote inhibitory immune pathways, and antibiotics reversed this effect.[4]

1. V. Gopalakrishnan et al. Science 2018;359:97-103
2. D. Pinato et al. JAMA Oncol. 2019;5(12):1774-1778
3. C. Urbaniak et al. Appl. Environ. Microbiol. 2016;82(16):5039-5048
4. S. Pushalkar et al. Cancer Discov. 2018;8:403-416

Research Questions

1) Is there a soft tissue sarcoma (STS) microbiome?
2) If so, is it clinically relevant?
   - ➢ Impact on immune phenotype (stimulatory vs. inhibitory)?
   - ➢ Correlation with the gut microbiome?
   - ➢ Response to cancer therapy?
   - ➢ Prognosis?

Methods

- ➢ 16S rRNA sequencing was employed to identify microbiome-specific genetic signatures in eight treated (post-radiotherapy) archived STS specimens (**Table 1**), obtained from the UC Davis Comprehensive Cancer Center Biorepository.
- ➢ Abundance and diversity of microbial organisms were examined against a background of reagent-only negative controls.

Results

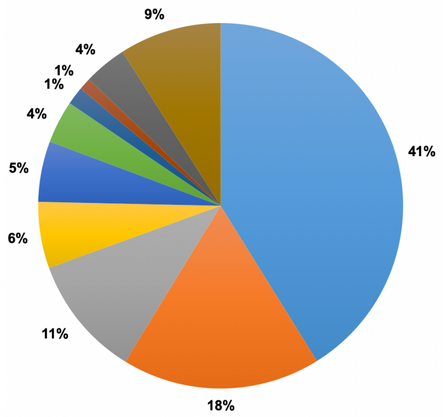

Figure 1. Overview of banked patient sarcoma tissue at the UC Davis Comprehensive Cancer Center Biorepository, classified by sarcoma sub-type (n = 186).

Table 1. Characteristics of chosen STS post-RT specimens for 16S rRNA microbiome analysis (n = 8). UPS = undifferentiated pleomorphic sarcoma. Mean age = 69.5 years, 50% male.

Specimen ID	Diagnosis	Sex	Age	Race
SA0536	Liposarcoma	M	67	White
SA0553	UPS	M	65	White
SA0648	UPS	F	49	White
SA0737	UPS	F	86	White
SA0738	UPS	F	86	White
SA0970	UPS	M	86	White
SA1210	Liposarcoma	F	54	White
SA1216	UPS	M	63	White

Table 2. Relative abundances of the six most abundant families of bacteria in each patient STS sample, expressed as percentages of the total 16S reads per sample.

FAMILY	SA0536 (% of 33,614)	SA0553 (% of 46,143)	SA0648 (% of 42,483)	SA0737 (% of 42,292)	SA0738 (% of 71,789)	SA0970 (% of 51,659)	SA1210 (% of 43,179)	SA1216 (% of 57,148)
Bacillaceae	65.5%	35.8%	1.0%	63.3%	58.3%	47.2%	61.6%	54.7%
Enterobacteriaceae	0.0%	38.0%	0.4%	6.9%	0.0%	11.4%	0.0%	0.0%
Enterococcaceae	0.0%	3.5%	37.3%	0.2%	0.0%	1.1%	0.0%	0.0%
Tannerellaceae	0.0%	3.0%	23.7%	2.1%	0.0%	4.3%	0.0%	0.0%
Lachnospiraceae	0.0%	5.0%	19.0%	1.2%	0.0%	7.5%	0.0%	0.0%
Burkholderiaceae	29.2%	1.9%	0.1%	7.8%	27.3%	5.4%	28.9%	33.6%
Staphylococcaceae	2.4%	2.9%	1.2%	3.0%	9.9%	1.8%	5.1%	3.4%
Other	2.9%	9.8%	17.3%	15.6%	4.5%	21.4%	4.4%	8.4%
TOTAL	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Table 3. Relative abundances of the seven most abundant genera of bacteria in each patient STS sample, expressed as percentages of the total 16S reads per sample.

GENUS	SA0536 (% of 33,614)	SA0553 (% of 46,143)	SA0648 (% of 42,483)	SA0737 (% of 42,292)	SA0738 (% of 71,789)	SA0970 (% of 51,659)	SA1210 (% of 43,179)	SA1216 (% of 57,148)
Bacillus	31.5%	21.4%	0.5%	31.9%	27.5%	27.3%	33.3%	25.4%
Anaerobacillus	34.1%	14.4%	0.5%	31.4%	30.9%	19.8%	28.3%	29.3%
Escherichia/Shigella	0.0%	38.0%	0.4%	6.9%	0.0%	11.4%	0.0%	0.0%
Enterococcus	0.0%	3.5%	37.3%	0.2%	0.0%	1.1%	0.0%	0.0%
Parabacteroides	0.0%	3.0%	23.7%	2.1%	0.0%	4.3%	0.0%	0.0%
Blautia	0.0%	1.6%	12.3%	0.8%	0.0%	2.5%	0.0%	0.0%
Ralstonia	29.2%	1.9%	0.1%	7.8%	27.3%	5.4%	28.9%	35.6%
Staphylococcus	2.4%	2.8%	1.2%	3.0%	9.9%	1.8%	5.1%	3.4%
Other	2.8%	13.3%	24.1%	15.9%	4.5%	26.3%	4.4%	6.4%
TOTAL	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

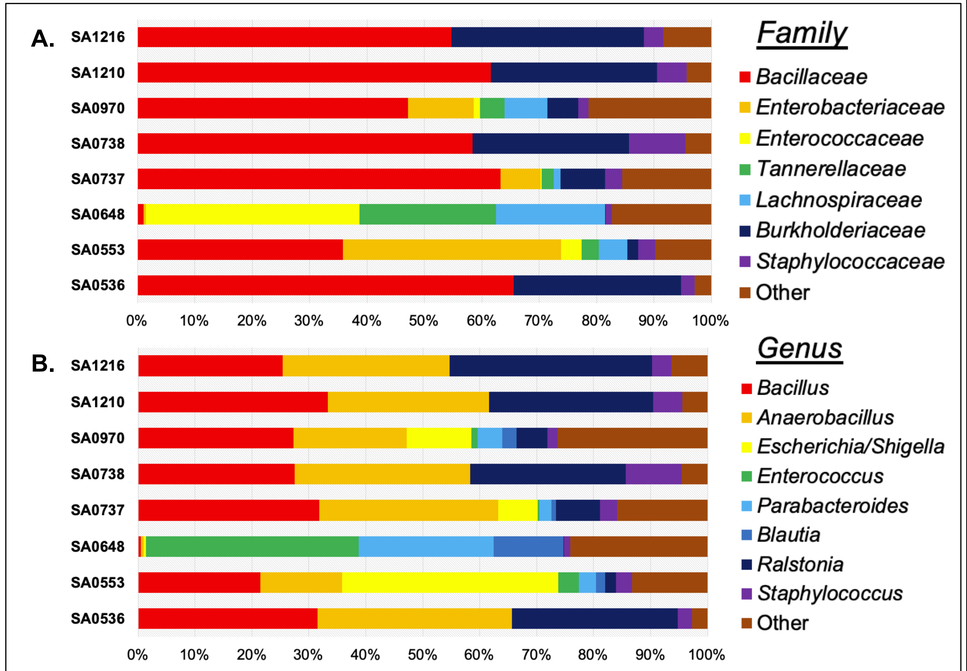

Figure 2 (A-B). Stacked bar plots at the family (A) and genus (B) level showing the relative abundance of each family and genus per patient STS sample, respectively.

Conclusion & Future Directions

- ➢ We report exciting preliminary data demonstrating, for the first time to our knowledge, the existence of an intra-tumoral microbiome in STS.
- ➢ Validation is ongoing, including a prospective collection and assessment of tumor tissue alongside key clinical correlates (**Figure 4**).
- ➢ If our hypotheses are correct, it would be an exciting opportunity for innovation in the treatment of this cancer that poses a formidable challenge in disseminated disease.

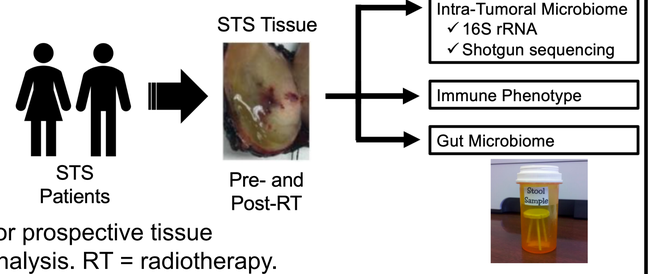

Figure 3. Study schematic for prospective tissue collection and microbiome analysis. RT = radiotherapy.

Acknowledgements: We would like to extend a special thank you to Matthew Rolston and the UC Davis Microbiome Core for their assistance in this project, and the UC Davis Comprehensive Cancer Center Biorepository for providing the patient tissue samples.