Intra-tumor microbes correlate with tumor-infiltrating lymphocytes in all cancer biopsies

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Background

- Non-human sequences have been found in many tumors, but their effect on outcomes remains poorly understood.
- Hypothesis:
  - *Intra-tumor microbes affect the recruitment of immune cells through local immuno-stimulatory effects including activation nucleic acid sensing pathways.*

Methods

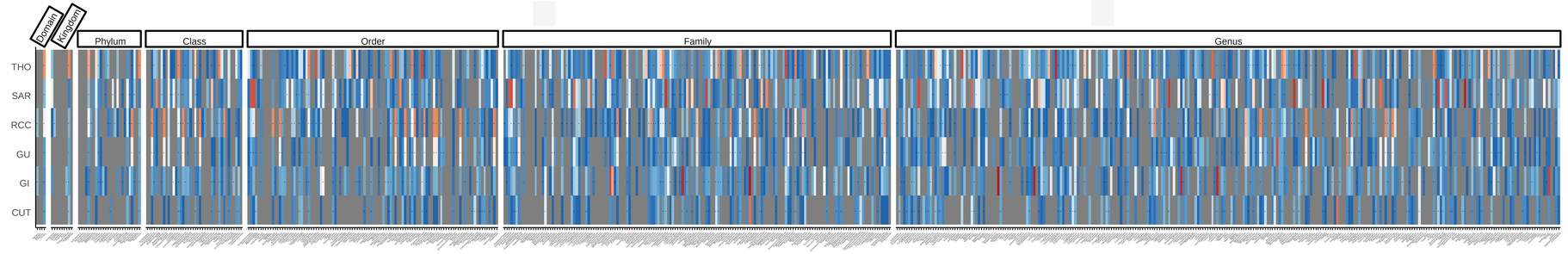
- We obtained RNA-seq data from 480 tumor biopsies
- Patients treated at The Ohio State University Comprehensive Cancer Center as part of the Oncology Research Information Exchange Network (ORIEN)
- Reads aligned to human and exogenous genomes using TopHat2[1] and Kraken2/Bracken[2], respectively
- Human gene expression was deconvolved to absolute abundances of immune cells using CIBERSORT[3].
- CUT = melanoma, GI = colorectal, GU = genitourinary, RCC= renal cell carcinoma, SAR = sarcoma and THO = thoracic, mostly non-small cell lung cancer (NSCLC)
- All analyses performed in R (packages reference [4]).

Table 1. Cohort summary

		CUT	GI	GU	RCC	SAR	THO
Demographic	Age	59 (10.78)	59 (11.72)	61 (12.22)	56 (11.68)	56 (15.99)	63 (10.04)
	BMI	34 (8.72)	29 (6.18)	31 (8.98)	34 (7.68)	29 (6.03)	27 (5.82)
	Perc Male	0.75	0.6	0.8	0.75	0.53	0.53
	Total n	16	104	20	20	118	202
Treatment Type	Chemo	5	91	17	12	90	142
	IO	13	58	12	9	32	54
Stage	0A	0	0	3	0	0	0
	1	0	3	1	2	4	50
	1A	1	0	0	0	4	1
	1B	0	0	0	0	8	3
	2	2	14	1	3	4	13
	2A	0	4	0	0	6	19
	2B	1	1	0	0	9	19
	2C	2	0	0	0	0	0
	3	6	5	7	9	40	16
	3A	1	0	0	0	0	14
	3B	0	20	0	0	0	1
	3C	0	6	0	0	0	0
	4	0	18	3	6	12	42
	4A	0	23	0	0	0	0
	4B	0	5	0	0	2	0
	4C	0	1	0	0	0	0
	Unknown	3	4	5	0	29	24

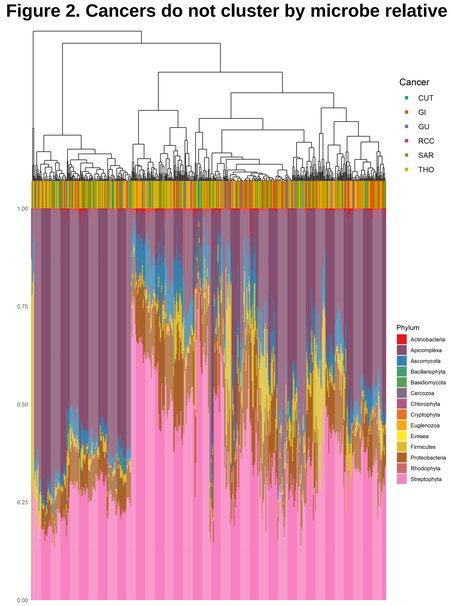
Results

Figure 1.The incidences of microbes in tumor biopsy RNA-seq relates to overall survival



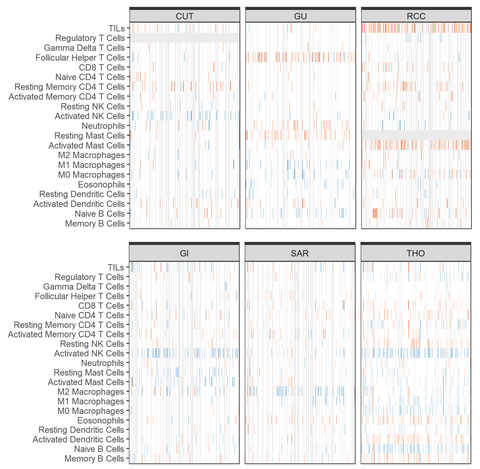
The relationship between the incidence of each microbe, at every taxonomic level and for each cancer, and overall survival is shown. Incidence is defined as any observable counts that pass quality thresholds. Presence versus absence of the microbe is used as a stratifying variable for a Kaplan-Meier survival curve for the time from collection of the biopsy to last follow-up or death. The color of each cell indicates the p-value of the stratification, with dark gray indicating the relationship could not be assessed. Cells containing a "+" indicate the hazard ratio (HR) of the interaction was less than 1 (i.e. overall survival was increased when the microbe was observed); no symbol indicates HR > 1.

Figure 2. Cancers do not cluster by microbe relative abundances



Relative abundances of exogenous sequences found in tumor RNA-seq data, aggregated to the phylum level, are shown. Phyla belonging to bacteria as well as several eukaryotes including fungi, plants and algae were consistently observed (stacked bars). Hierarchical clustering by phylum-level abundances did not separate samples by cancer type (top color row), suggesting similar microbes infiltrate tumors across cancer types.

Figure 3. Microbe relative abundances correlate with estimated immune cell abundances



Estimated absolute abundances of immune cells from deconvolution of the RNA-seq data were related to species-level relative abundances of microbes by Spearman correlation. Significant relationships are colored by correlation coefficient (p-value < 0.05), white are non-significant, and gray are not estimable. In some tumors an immune cell is consistently correlated with many microbes and in the same direction (e.g. activated NK cells in GI and THO cancers). Other relationships are more varied (e.g. Tumor Infiltrating Lymphocytes (TILs, and aggregation of all T cells, B cells, natural killer (NK) cells, macrophages, neutrophils, dendritic cells, mast cells, and eosinophils) in melanoma (CUT) and NSCLC (THO).

Conclusions

Taxon Level	Taxon	Significantly correlates with: cGAS and/or RIG-I expression	Survival	Immune cell relative abundance
family	*Malasseziaceae*	1	1	1
family	*Paenibacillaceae*	1	1	1
genus	*Cicer*	1	1	1
genus	*Leuconostoc*	1	1	1
order	*Glomerellales*	1	1	1
species	*Bacillus megaterium*	1	1	1
species	*Colletotrichum higginsianum*	1	1	1
species	*Theileria annulata*	1	1	1
class	*Alphaproteobacteria*	0	1	1
class	*Bacillariophyceae*	0	1	1
class	*Betaproteobacteria*	0	1	1

- Many microbes are associated with differences in survival, including both reductions and increases
- Non cancer-specific microbial signature was found
- Some tumors show consistent correlations between microbes and immune cells
- Some taxa that were significant across several analyses are not commonly associated with humans, suggesting the need for alternative filtering strategies or thorough validation

Acknowledgements & References

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[3] Newman, A. M. et al. Robust enumeration of cell subsets from tissue expression profiles. Nature Methods 12, 453 (2015).
[4] broom, devEMF, dplyr, flextable, forcats, ggdendro, ggforce, ggplot2, glmnet, glue, Hmisc, officer, RColorBrewer, readxl, survival, survminer, survMisc, tableone, tibble, tidyr

The poster and a full table of correlations between microbes and immune cells available at https://u.osu.edu/spakowiczlab/