The Impact of the Tumor Microbiome on Anti-tumor Immunity in Mucosal Melanoma

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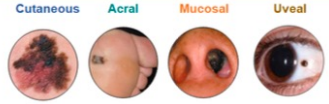
Introduction

Mucosal melanoma is a rare, but lethal, form of melanoma



	Cutaneous	Acral	Mucosal	Uveal
% of US Cases[1]:	87%	3%	1%	5%
5-year survival rate[1]:	81%	80%	25%	75%

Figure 1. Cutaneous melanoma and different rare, non-sun related melanoma subtypes. Mucosal melanomas are typically not detected until late stage, are challenging to surgically resect, and respond poorly to standard melanoma treatments *including immune checkpoint blockade (ICB) therapy.*[2-4]

Mucosal melanoma responds poorly to immune checkpoint blockade (ICB) compared to cutaneous melanoma

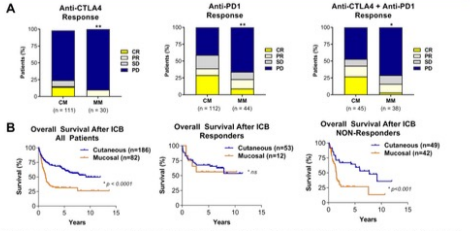


Figure 2. Immune checkpoint blockade therapies are less effective in mucosal melanoma. (A) Percent of CU melanoma clinic patients with complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). *p<0.01, **p<0.001. **(B)** Overall survival from the date of first ICB treatment for patient cohort indicated.

Hypothesis

Given the role of the microbiome in tumor immunity and ICB response, we *hypothesize* distinct mucosal tissue microbiomes contribute to poor anti-tumor immunity and ICB resistance in mucosal melanoma.

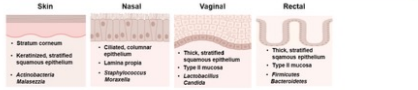
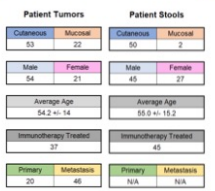


Figure 4. Structural and microbial differences in skin *versus* mucosal tissues.

Methods

Patient gDNA specimens



16S rRNA bacterial sequencing

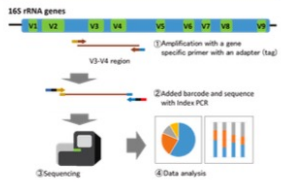


Figure 5. 16S rRNA sequencing of melanoma patient specimens. (A) Specimens were collected by the CU Melanoma Biorepository under COMIRB #05-0309. Genomic DNA was purified using the Qiagen DNeasy UltraClean Microbial Kit (tumors) or Dneasy PowerSoil Pro Kit (stool). **(B)** Diagram of 16S rRNA sequencing process.

Results

~50% of tumors are "positive" for bacteria

Primary mucosal melanoma tumors have higher bacterial load *vs* cutaneous melanomas

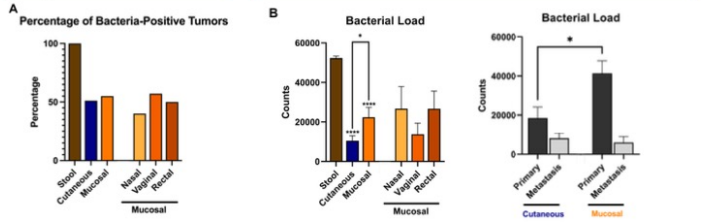


Figure 5. Bacteria positivity and total bacterial load in patient specimens. (A) Percentage of tumors identified as "positive" for the presence of bacteria. **(B)** Bacterial load in specimens shown by subtype/anatomic site and by primary or metastatic tumor status.

Mucosal melanoma tumors have more bacteria species *vs* cutaneous melanomas

Tumors clustering with stools are mucosal melanoma tumors

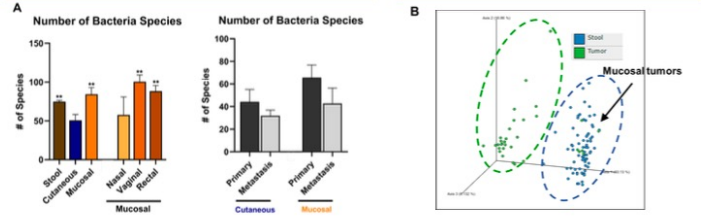


Figure 6. Bacteria species counts and principal component analysis (PCA). (A) Total number of bacteria species shown by subtype/anatomic site and by primary or metastatic tumor status. **(B)** Principal component analysis of data using QIME 2.0 software.

Mucosal melanoma tumor microbiomes more closely resemble stool than cutaneous melanoma tumors

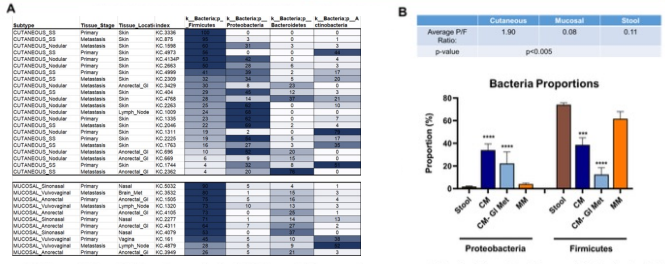


Figure 7. Taxa plot and P:F ratio analysis. (A) Taxa plot for specimens generated with Qime 2 software. **(B)** Analysis of the Proteobacteria to Firmicutes ratio (P:F), which is a common metric used to generally characterize microbiome composition.

Specimens have expected proportions of known skin and gut commensal bacteria

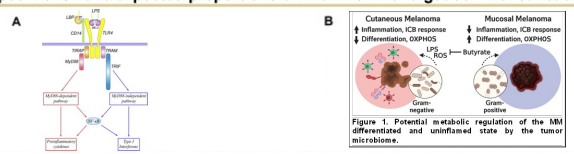


Figure 1. Potential metabolic regulation of the MM differentiated and uninflamed state by the tumor microbiome.

Figure 8. LPS Signaling diagram and effects on immunity. (A) LPS impacts inflammation via two major pathways. **(B)** LPS signaling is postulated to result in decreased ICB response in MM due to these mechanisms.

Results (Cont.)

Mucosal melanoma tumors have proportions of bacteria consistent with poor anti-tumor immunity

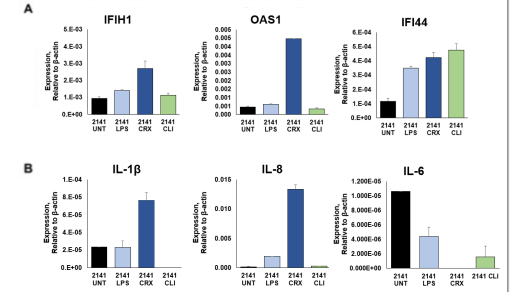


Figure 9. Impacts on Immune Genes in Vitro. (A) Type 1 Interferon Pathway end products show a variable response. **(B)** Inflammatory cytokines resulting from LPS pathway, markedly decreased IL-6 expression.

Conclusions

- Mucosal melanoma is a rare, but particularly lethal, form of melanoma which responds poorly to ICB compared to cutaneous melanomas.
- The mucosal melanoma tumor microbiome is significantly different from cutaneous melanoma, and it more closely resembles the gut microbiome.
- MM tumors have unfavorable proportions of bacteria species controlling tumorigenicity and immunity.

Future Directions

- Multiomics analysis with RNAseq and ATACseq to determine the correlation between the microbiome and epigenetic-mediated immune gene silencing in mucosal melanoma.
- Mucosal melanoma microbiome (gut and tumor) reconstitution in mice to determine effect on tumor epigenetics, immunity, and ICB response.
- ITS sequencing of specimens for fungal species analysis.

References

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Acknowledgements

Special thanks to Morgan Macbeth for research guidance and technical assistance, the melanoma clinic faculty and staff, the patients and their families, and to funding sources including NIH grant 1R25CA240122-01, the American Cancer Society Diversity in Cancer Research Grant, and the Department of Defense CDMRP Melanoma Research Program.