Title:

The mucosal melanoma tumor microbiome is distinct from cutaneous with unfavorable proportions of immune-associated species

Authors:

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Purpose of Study:

Immune checkpoint blockade (ICB) is effective in cutaneous melanoma (CM), but mucosal melanoma (MM) has poor ICB response and low anti-tumor immunity. We discovered the microbial composition of MM tumors is unique, with a higher proportion of Gram-positive bacteria *Firmicutes* compared to CM. *Firmicutes* does not contain lipopolysaccharides (LPS) which are predominantly found within Gram-negative species' cell walls and trigger type I interferon (T1IFN) and inflammatory pathways via Toll-like receptor 4 (TLR4) signaling in both immune and melanoma cells. In addition to lacking LPS, *Firmicutes* produces high levels of the immunosuppressive metabolite butyrate, which inhibits LPS and TLR4 signaling. Based on these data, we hypothesized the *Firmicutes* rich MM tumor microbiome contributes to immune evasion and ICB resistance by suppressing LPS-TLR4 mediated T1IFN signaling and inflammation (Figure 1).

Methods:

We performed 16S rRNA sequencing to analyze bacterial compositions in 75 tumors and 72 stool samples from patients with CM or MM. We then evaluated immune gene expression by qRT-PCR in MM cell lines treated *in vitro* to analyze the impacts of LPS, CD14-independent TLR4 agonist CRX-527, TLR4 inhibitor CLI-095, and butyrate.

Summary of Results:

16S rRNA analyses on tumors showed that the ratio of *Proteobacteria* to *Firmicutes* (P:F), a metric commonly used to compare various tissue types, was low in MM tumors (0.03) and stool (0.07) compared to CM (1.1). Overall, *Firmicutes* was found in greater proportions in MM compared to CM. *In vitro* studies with MM cell lines showed increased expression of T1IFN response genes (IFIH1, OAS1, IFI44) following treatment with LPS or CRX-527, and decreased expression with CLI-095 addition. Inflammatory cytokines (IL-1β and IL-8) were also significantly increased with CRX-527 treatment, with variable response to direct LPS agonist.

Conclusions:

Overall, the tumor microbiomes of CM and MM are significantly different and may be a critical factor underlying the “cold” tumor microenvironment and poor ICB response of MM tumors. Furthermore, these effects may be partially mediated through the interactions of LPS with the TLR4 signaling cascade directly on tumor cells. Unlike LPS, CRX-527 is a synthetic lipid A mimetic that does not require the cofactor CD14 to activate TLR4 signaling, likely explaining its ability to better activate T1IFN and inflammatory signaling compared to standard LPS. CRX-527, used clinically as a vaccine adjuvant, may be an effective treatment modality in combination with ICB to improve anti-tumor immune responses in patients with MM.