The Mucosal Melanoma Tumor Microbiome is Distinct from Cutaneous with Unfavorable Proportions of Immune-associated Species:

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Immune checkpoint blockade (ICB) is effective in cutaneous melanoma (CM), but mucosal melanoma (MM) has poor ICB response and low anti-tumor immunity. Tumor microbiome diversity and specific species have been linked to cancer development, immunity, and ICB response. Since the microbial composition of mucosa tissues is unique, we hypothesized the MM tumor microbiome contributes to immune evasion. We performed 16S rRNA sequencing to analyze bacterial in 75 tumors and 72 stool samples from patients with CM or MM. Overall, 50% of tumors were "positive" for bacteria and total bacterial load was higher in primary tumors compared to metastases for CM ($p=0.12$) and MM ($p<0.001$). Tumor bacterial load and species count were higher in MM than CM ($p<0.05$ and $p<0.0001$, respectively). Strikingly, microbiomes from MM tumors (primary and metastasis) originating from various anatomic sites (nasal, vaginal, anorectal) more closely resembled stool than CM tumor microbiomes. The ratio of *Proteobacteria* to *Firmicutes* (P:F) was low in MM tumors (0.03) and stool (0.07) compared to CM (P:F=1.1). As expected, *Staphylococcus* was higher in primary CM tumors compared to MM ($p<0.05$) and metastatic CM ($p<0.005$). Interestingly, MM tumors and stool had high levels of *Clostridia*, an immunosuppressive bacterium, which was not elevated in CM gastrointestinal metastases. While similar, key differences in MM tumor microbiomes from stool included elevated *Prevotella* ($p<0.4x10-5$, prevalent colonizer of mucosal sites), *Actinobacteria* ($p<0.009$), and *Fusobacteria* ($p<0.002$), and a lower proportion of *Akkermansia* ($p<0.1$) which enhances anti-tumor and ICB response. In summary, there are significant differences between CM and MM tumor microbiomes, with MM tumor microbiomes closely resembling the gut microbiome and having unfavorable proportions of immune-related species.