Gene expression and biological pathways associated with differential responsiveness to anti-PD1 immunotherapy in preclinical HPV+ tumors

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Background
- Human papillomavirus induced (HPV+) oropharyngeal cancer is quickly rising in the United States, accounting for as much as 70% of oropharyngeal cancers.
- Understanding the genetic determinants in the tumor microenvironment (TME) is important for rational design of therapeutic strategies.

Hypothesis
- Differences in the expression of genes important for cytotoxic function of innate and adaptive antitumor effector cells (CD8 T cells and NK cells) are associated with response to immunotherapy.
- There is higher expression of cytokines, signaling molecules that regulate and mediate immunity, in the sample of mEER tumors implanted in tongue.

Methods

RNA Extraction and Sequencing
- RNA was extracted from preclinical mEER tumor samples, alongside with immune cell infiltrates, for both the tongue and flank models.
- RNA data was then subjected to quality control testing, leaving 3 samples of the tongue tumors and 2 samples of tumor for further sequencing.

Identification of Differential Gene Expression
- DESeq2 and V-core were performed in the software and employed to evaluate differential gene expression between the groups.
- The gene expression data is calculated based on the ratio of CPM (counts per million) values, normalized between samples, and principal component analysis (PCA) was performed to ensure that tumor location difference accounts for the majority of variance between gene expression data (Fig. 1).
- A sample of the flank implanted mEER tumor group is removed due to an abundance of checkpoint molecules identified for targeting.

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Results (Continued)

Biological Pathway Analysis via IPA
- This set of significantly differentially expressed genes were further analyzed using Ingenuity Pathway Analysis (IPA) to obtain insights on potential pathways associated with treatment responsiveness.
- The p-value for canonical pathways were calculated using Fisher’s A test, and the cutoff threshold was set to log2(p-value) ≥ 1.5.

Results

Identification of Differential Gene Expression
- 485 differentially expressed genes altered by >2 log2 fold change in tongue-implanted mEER tumors relative to flank tumors were identified.
- We observe significant upregulation in the expression of immune function related genes encoding such as IFNγ, Cxcl9, Chil1, and Gzm b in mEER oral tumors (Fig. 2).

Results (Continued)

Natural Killer Activators and Inhibitors
- Upon further examination of natural killer activator and inhibitory molecules and NKG2D, we identified overexpression in C9D9 and PDCD1 (PD1) in mEER tongue tumors, as potential targets for immunotherapy using anti-C9D9 and anti-PD1 checkpoint antibodies (Fig. 2, Table 2).
- Importantly, these gene expression analyses data show validated expression of inhibitory tumor implanted tumors showed better response to anti-C9D9 (unpublished) and anti-PD1 immunotherapies.

Conclusion
- Cytokines and chemokines crucial to immune response is observed to be overexpressed in the tongue implanted mEER tumor samples, which are molecular signatures indicative of an immunologically 'hot' tumor relative to the flank.
- The lack of significant differential expression for most natural killer cell activators and inhibitors such as TIGIT, Lag3, and Tim3 alongside the abundance of cytokines overexpression and evidence of activated interferon signaling in the tongue suggests that while the immune system may recognize the presence of the tongue tumor, there is blockade in effective attack against the tumor itself whereas the immune system may not be recognizing the tumor in the flank implanted tumor sample.
- The overexpression of C9D9 and PD1, despite their role as natural killer cell inhibitors, in the tongue implanted tumor suggests that immunotherapy efficacy is higher for the tongue implanted tumors due to an abundance of checkpoint molecules identified for targeting.
- Further studies should be conducted to determine the exact effects of the overexpressed chemokines in the tongue implanted model.

References

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