ORT.15 - Metastasis of HPV-negative oral cavity tumors: an *in silico* study

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Introduction: Oral cavity tumors are the 5th more frequent neoplasms among men according to INCA's last estimative. The occurrence of metastatic processes during tumor development increases the chances of relapse and hamper the treatment. That said, studying the mechanisms that favour metastasis may help identifying biomarkers associated with this process and ultimately improve treatment.

Objective: To analyze *in silico* the HPV-negative tumors from oral cavity comparing those which and without linfonodal metastasis.

Methodology: 20 samples from a collaborator's cohort (AC Camargo - ACC) and 22 samples from The Cancer Genome Atlas (TCGA) were analyzed. The raw data from ACC was filtered and quality checked using FastQC and Trimmomatic, respectively. The ACC reads were then aligned against the human genome GRCh38 using star method and genes were counted with RSEM package. TCGA cohort already possess pre-processed data. Differentially expressed genes were evaluated by DESeq2, the enriched pathways by Webgestalt and tumor microenvironment by xCell. Exclusively for the TCGA available data, the HLA-I alleles were identified by Optitype and subsequent neoantigen prediction was accomplished by netMHCpan. T and B-cell receptors (TCR and BCR) repertoire were identified and analyzed by MiXCR.

Results: We identified 186 DEG for the TCGA cohort, 127 for the ACC and 3 DEG shared genes, from which two of them up-regulated in the same condition: PIWIL2 (up-regulated in the non metastatic group) and ADH1B (up-regulated in the metastatic group). The immune population with the highest correlation coefficient was the memory CD4 T-cell (Pearson: -0,78 in ACC and -0,71 in TCGA) which signature is enriched in the non metastatic group. A prolymphocyte B signature had an elevated correlation coefficient (Pearson: 0,77 in TCGA cohort) with the metastatic outcome. A higher clone number of TCR alpha ($p<0,01$) and beta ($p<0,001$) chains was identified in the non metastatic group. There was no difference between mutation and neoantigen load between groups. Moreover, the sample with higher mutation burden was also the solely bearing a damaging mutation in the antigen processing and presentation pathway. In total, 4 samples had mutations in DNA repair pathways, representing the 4 with higher neoantigen burden. Pathway enrichment analysis as well as correlations between immune populations are currently underway.

Conclusion: The results suggest an association between memory CD4 T-cells and metastasis-free disease. The individual analysis of each sample indicates putative mechanisms of immune escape, for example, the mutated protein in the antigen processing and presentation pathway.

Keywords: Oral cavity; Metastasis; Immune evasion