S100A8/A9-associated down-regulation of EGFR could explain better survival of patients with S100A8/A9-high tumors.

**COMPOSITION OF IMMUNE CELL AGGREGATES AT THE INVASION FRONTS OF ORAL CANCER ASSOCIATED WITH CLINICAL OUTCOMES.** C. POH, M. LOPES, K. LIU, Y. LIU, C. NELSON. UNIVERSITY OF VICTORIA, VICTORIA; UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER; FEDERAL UNIVERSITY OF RIO GRANDE DO NORTE, NATAL; AND UNIVERSITY OF ELECTRONIC SCIENCE AND TECHNOLOGY, CHINA.

Objectives: Evading immune destruction from tumor microenvironment has been proposed as one of the hallmarks of cancer. However, little is known in oral cancer (OC). The objective is to investigate the immune cellular composition of invasion fronts (IFs) of OC and associated outcome.

Methods: As an exploratory study, two 5-μm serial sections from the cross-section of 12 highly annotated OC samples were used for 2 sets of multi-color immunostaining with antibodies against: CD8, CD163, FoxP3, CD25; and PD-L1, PD-1, CD3, CD3D, CD20 (Deely Research Lab, Victoria, BC)

Results: PD-L1 tumor-cell expression was heterogeneous in 9 OCs, especially at the IFs. PD-L1- tumors had a better outcome with no nodal disease. In addition, PD-L1+ immune cells with dendritic morphology were seen in all cases. In areas with interconnecting clusters of PD-L1+ immune cells we observed different proportions of CD25+ immune cells and CD8+cells co-localizing within T-cell clusters. We did not observe a relationship between the number of CD8+cells and the outcome; however, clusters of PD-1+ immune cells were commonly seen in patients with poor outcomes. Aggregates containing both expanded B-(CD20) and T-(CD3) cells at the IFs seemed to have a better outcome. CD163 was not exclusively expressed in macrophages. Various patterns of CD25 expression were also found at the IFs of the tumor cells of 11 OCs, including membranous, cytoplasmic, and dot-like. The latter was more evident in poorly differentiated tumors, while well-differentiated tumor nests expressed little to none CD25.

Conclusion: Immune cellular composition at TME, either pro-inflammatory or immune suppressive, are associated with clinical consequences. Further investigation is warranted to shed light of the underlying biology.

**DOWNREGULATION OF DMBT1 PROMOTES INVASION IN HEAD AND NECK CANCER.** S. PIAO, S. PIAO, R. BANERJEE, M. LIU, R.C. INGLEHART, N. D’SILVA. UNIVERSITY OF MICHIGAN, ANN ARBOR, MI.

Deleted in malignant brain tumors 1 (DMBT1) is a tumor suppressor that is downregulated in multiple cancers.

Objective: In the present study, we investigated the expression, function and mechanism of regulation of DMBT1 in human head and neck cancer.

Methods: Using laser capture microdissection we isolated epithelium from head and neck cancer and normal tissue and used quantitative RT-PCR to quantify DMBT1 transcripts. The regulation of DMBT1 was investigated by genetic and biochemical approaches in human head and neck cancer cell lines. The function of DMBT1 was investigated using in vitro and in vivo approaches.

Results: DMBT1 is downregulated in head and neck cancer compared with normal epithelium. DMBT1 expression is inversely correlated with EZH2, an oncogene that promotes invasion in head and neck cancer. Furthermore, EZH2 silences DMBT1 via histone and promoter methylation. In head and neck cancer cell lines with stable downregulation of EZH2, suppression of DMBT1 rescues the invasive phenotype.

Conclusions: DMBT1 is a tumor suppressor that inhibits invasion and is silenced in HNC. (This work was supported by NIDCR grants DE022567 and DE019513).


Odontogenic tumors are a heterogeneous group of lesions that arise from the tissues derived from the tooth forming apparatus that display a range of microscopic patterns and clinical behavior. The most common is the ameloblastoma, a benign, locally infiltrative odontogenic tumor that on rare occasions may undergo malignant transformation. Surprisingly little is known about the molecular biology of most odontogenic tumors but the best known aberration is the presence of a BRAF V600E mutation in 40-60% of mandibular ameloblastomas. The goal of this project is to identify the gene expression profile of ameloblastoma and other common odontogenic neoplasms using laser microdissection and RNA sequencing, with the goal of finding novel markers for ameloblastoma and other odontogenic tumors. We analyzed gene-expression patterns of 18 odontogenic tumors. The epithelial component and the stromal component are obtained using laser capture microdissection, resulting in a total of 42 samples. Smart-3SEQ, a newly designed RNA sequencing method, is then used to prepare the cDNA libraries. 42 libraries pool are then sequenced using SMART-3SEQ. A total of 196.6 million reads are obtained, with an average of 4.68 million reads per sample. Genes with less than 20 reads are excluded from our study. Significant clusterings of several candidate genes are seen in ameloblastoma. We then performed differential expression analysis of the RNA-seq data using DEseq2. Based on the adjusted p-value (<0.05), >500 statistically significant candidate genes are found. We have identified a list of candidate genes that are preferentially expressed in ameloblastoma. These results may provide a framework to identify useful markers for diagnosis of odontogenic tumors.

**A CLINICOPATHOLOGIC AND SURVIVAL ANALYSIS OF HEAD AND NECK SOFT TISSUE SARCOMAS (HNSTS) WITH KNOWN GENETIC ABNORMALITIES.** A. OWOSHO, L. ZHANG, C. ESTILO, J. HURYN, C. ANTONESCU. MEMORIAL SLOAN KETTERING CANCER CENTER, NEW YORK.

Background: HNSTS are rare and diagnostically challenging without the advent of ancillary testing to exclude more common diagnoses such as melanoma and carcinoma. An increasing number of STS are now classified based on their defining molecular genetic abnormalities and thus the criteria for diagnosing HNSTS have evolved from morphologic and immuno histochemical tools alone.