

Uncovering Anatomical Subsite Specific Transcriptional Networks in Oral Cavity Squamous Cell Carcinoma

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Results

Abstract

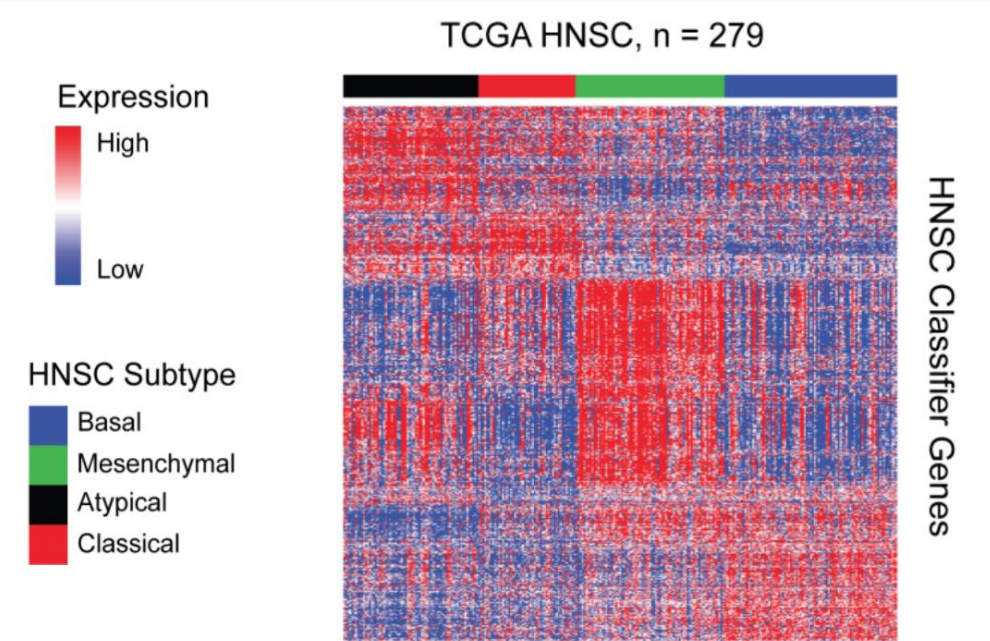
Head and Neck Squamous Cell Carcinoma (HNSCC), in particular in oral cavity cancer, continues to be a challenge in achieving local regional control and improved survival. This is due, in part, to a lack of understanding of the biology of the disease. In this study, we focus on cancers of the oral cavity, which have unpredictable biological behavior. While depth of invasion and T stage has been the focus of clinical treatment, we present data that suggests tumor behavior based on subsite location within the oral cavity is of clinical importance. We hypothesized that there exist key oncogenic transcriptional networks that are associated with unique anatomical subsites and may be correlated with tumor stage and outcome.

To decipher the transcriptomic networks defining the heterogeneity within oral cavity, we examined the gene expression levels of the Cancer Genome Atlas (TCGA) Oral Squamous Cell Carcinoma (OSCC) RNA-Seq dataset. Our comprehensive analysis of the OSCC differential gene-expression profiles has revealed commonalities and dynamic differences among the various anatomical subsites (i.e., Tongue, Floor of Mouth, Alveolar Ridge and the Buccal Mucosa). We demonstrated that differential gene-expression patterns can segregate tumors based on genes uniquely enriched within each anatomical subsite. Interestingly, we found that gene-expression patterns across all subsites defined two main clusters, one containing tumors of higher clinical stage and tumor grade and the other primarily containing tumors of low tumor grade and clinical stage.

Our studies highlight both genes that are systematically over-expressed in each subsite as well as uniquely enriched genes for each oral cavity subsite. These gene-sets have uncovered core gene signatures that highlight crucial aspects of OSCC biology. For example, we observed oncogenic processes such as: HIF1 signaling, angiogenesis and adherens junction signaling for buccal mucosa as well as upregulation of metastasis and cell proliferation genes for oral tongue. Our systematic approach to define and characterize tumor variance as a function of anatomy has thus revealed critical and specific pathways, which can explain the difference in clinical outcomes observed for each subsite and potentially be leveraged for targeted clinical trials.

HNSCC is a Heterogeneous Disease

Integrative Genomic Clustering Analysis, uncovered four distinct molecular classes. Each intrinsic subtype is represented by a unique underlying molecular biology that governs the tumor phenotype.



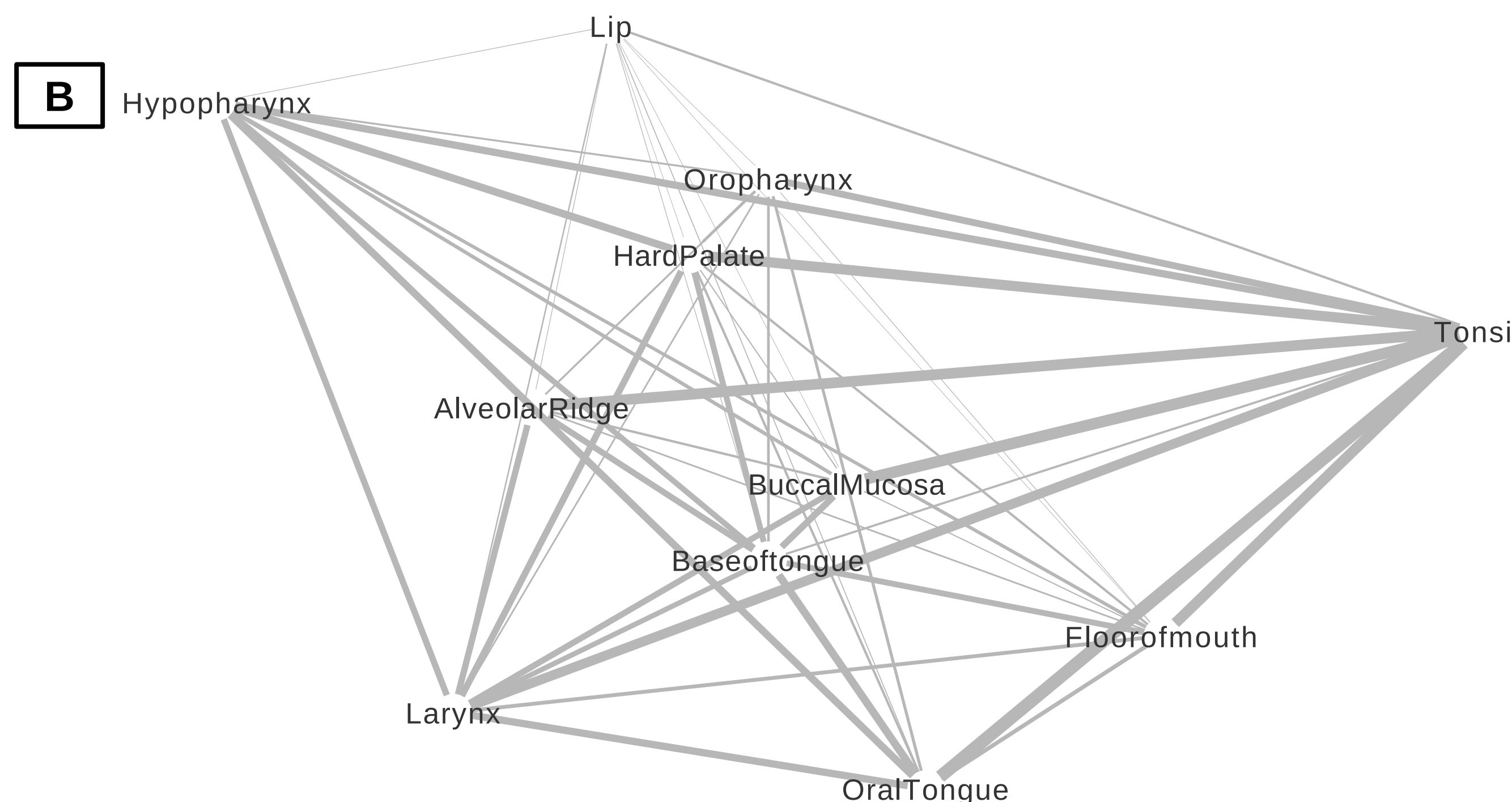
Identifying the Network of Uniquely Expressed Genes across the Landscape of OSCC

Identifying Differentially Expressed Genes Across the TCGA Database

Methodology → Pairwise comparisons between each anatomical subunit, reveals a network of gene programs unique to each comparison.

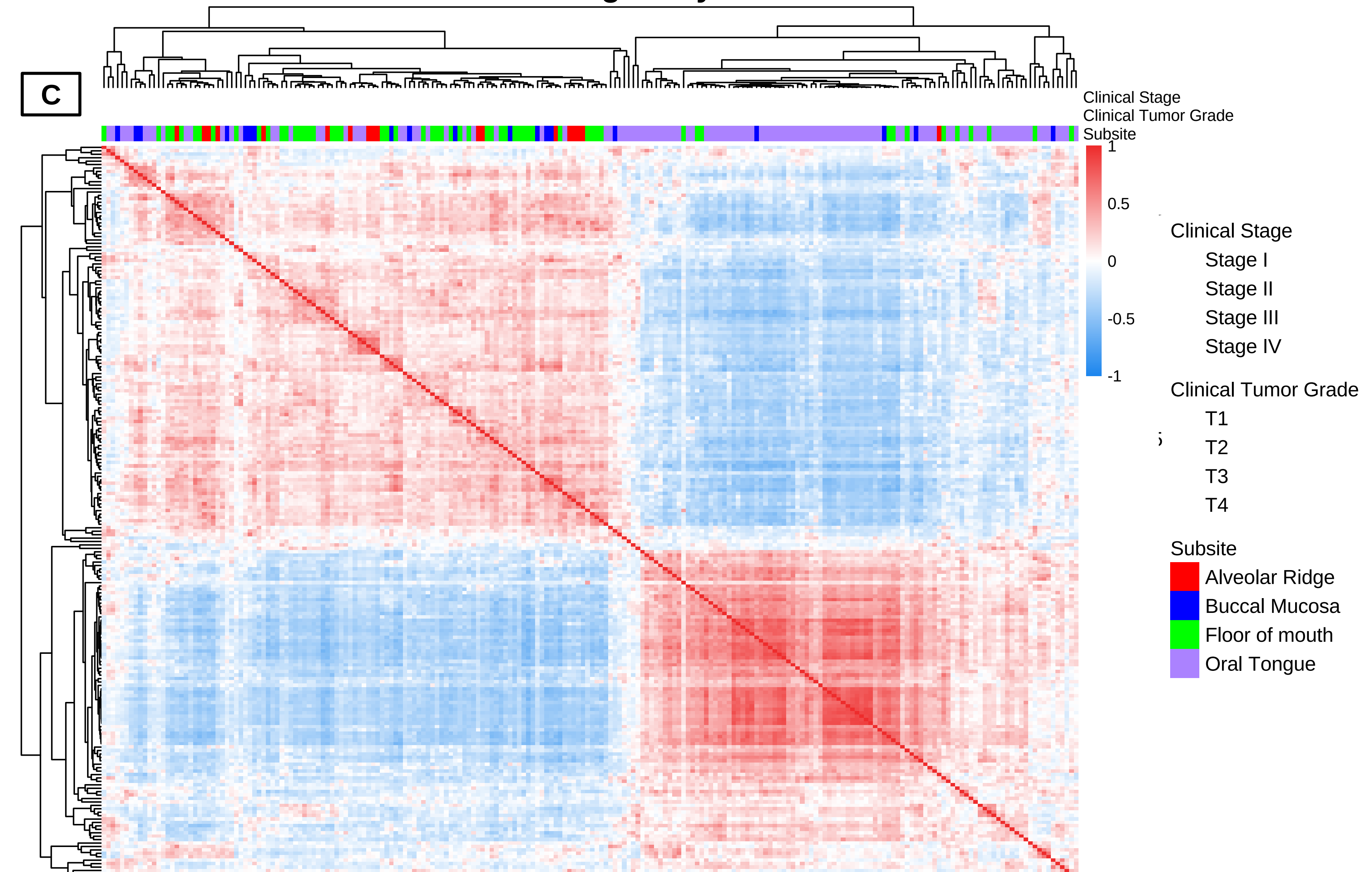
Summary_All_DESeq2_Combinations

Comparison	t.summary_results_all
OralTongue_vs_Larynx	4154
OralTongue_vs_Floorofmouth	2287
OralTongue_vs_OralCavity	1574
OralTongue_vs_Baseoftongue	4438
OralTongue_vs_BuccalMucosa	1456
OralTongue_vs_AlveolarRidge	2429
OralTongue_vs_Tonsil	7236
OralTongue_vs_HardPalate	1320
OralTongue_vs_Oropharynx	1668
OralTongue_vs_Hypopharynx	4056
OralTongue_vs_Lip	603
Larynx_vs_Floorofmouth	2132
Larynx_vs_OralCavity	4153
Larynx_vs_Baseoftongue	2940
Larynx_vs_BuccalMucosa	3443
Larynx_vs_AlveolarRidge	3543
Larynx_vs_Tonsil	5539
Larynx_vs_HardPalate	3716
Larynx_vs_Oropharynx	966
Larynx_vs_Hypopharynx	3603
Larynx_vs_Lip	784
Floorofmouth_vs_OralCavity	1805
Floorofmouth_vs_Baseoftongue	3345
Floorofmouth_vs_BuccalMucosa	715
Floorofmouth_vs_AlveolarRidge	924
Floorofmouth_vs_Tonsil	6052
Floorofmouth_vs_HardPalate	1337
Floorofmouth_vs_Oropharynx	463
Floorofmouth_vs_Hypopharynx	1997



- A) Table highlighting the pairwise gene expression analyses performed and the summary of the cohorts of genes specifically differentially expressed between tissue pairs.
- B) Network analysis highlighting the magnitude of genes unique to each tissue per comparison performed via the DESeq2 analysis of the TCGA dataset. The thickness of the lines correlates to the number of genes differentially regulated between the tissue nodes.
- C) Heatmap displaying the hierarchical clustering of the unique gene signatures for each oral cavity subsite. The oral cavity tumors naturally delineated the tongue SCCs from the rest of the oral cavity subsites. Interestingly, this analysis also highlights that the non-tongue SCCs tend to be of higher clinical stage.

Identifying the Correlation between Gene Expression Programs and Clinical Data relative the OSCC Heterogeneity

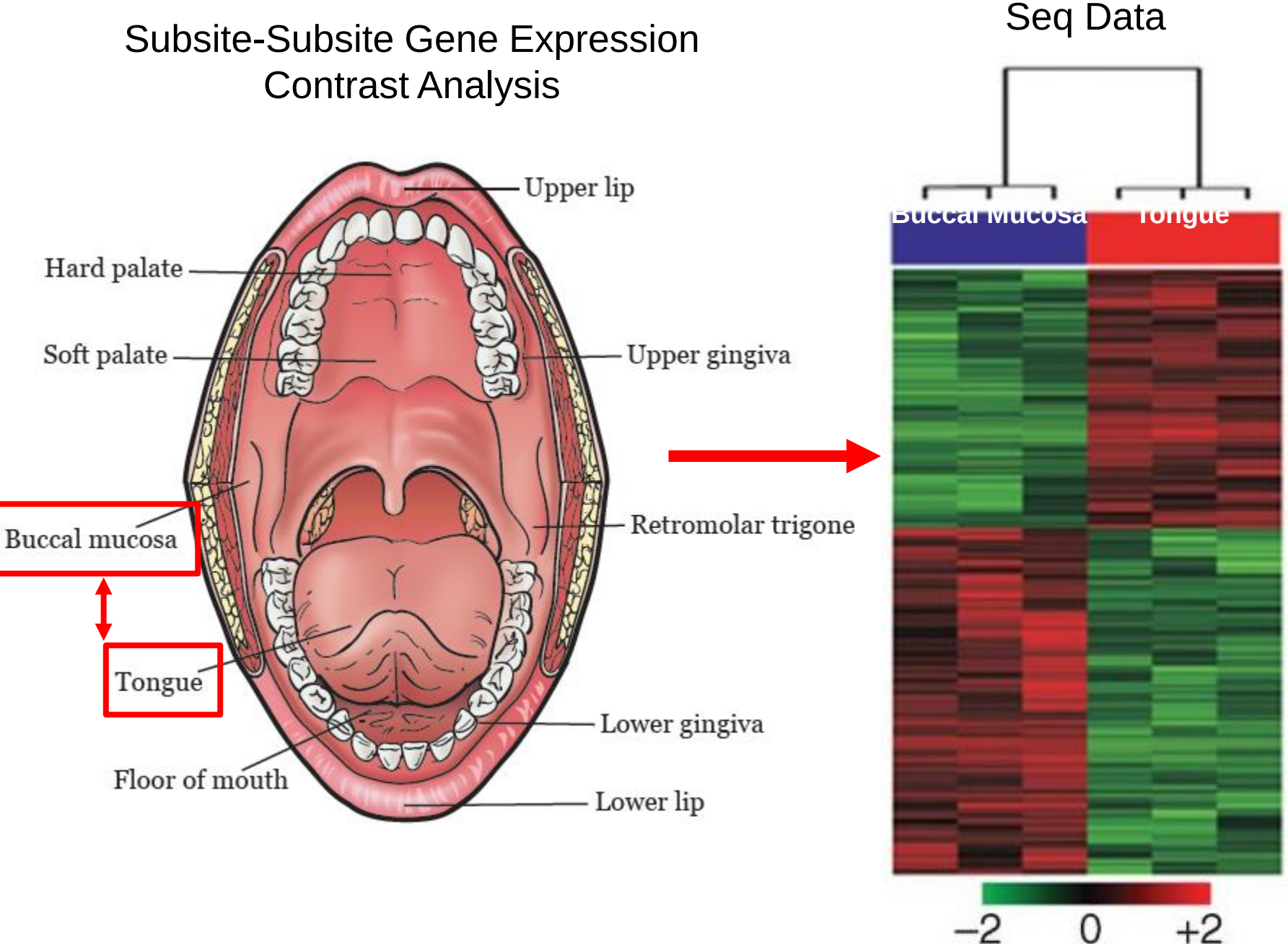


What underlying gene signatures define oral cavity anatomical subsites?

Hypothesis: Unique gene programs, that are specific to each underlying oral cavity anatomical subsite, drive the heterogeneity found in OSCC Tumors.

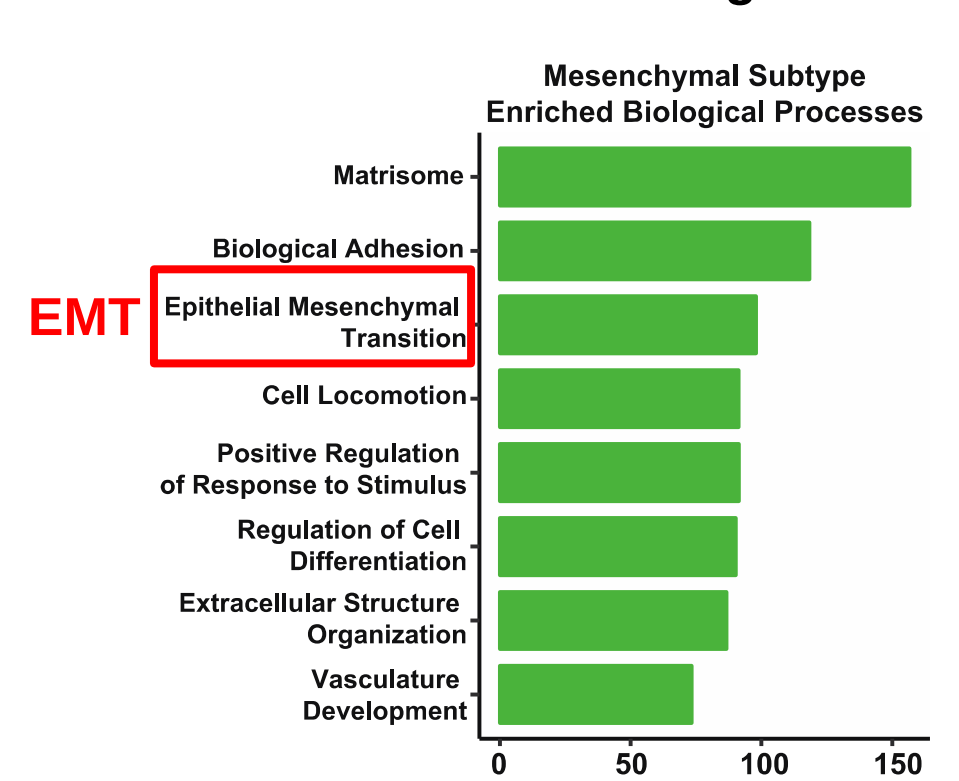
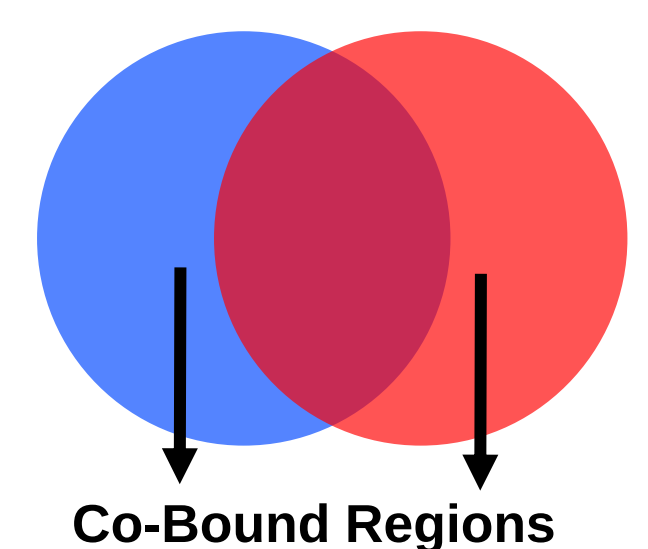
Step 1: Leverage the TCGA RNA-Seq database to perform gene expression analysis to identify **OSCC Subtype Specific Gene Signatures**.

DESeq2 Analysis of TCGA RNA-Seq Data



Step 2: Identify uniquely enriched pathways and biomarkers that define each anatomical subunit to infer unique biological and oncological features of each tumor type.

Subsite #1 Subsite #2



(A) Leverage Unique Genes Identified in DESeq2 Pairwise Comparisons between Subsites

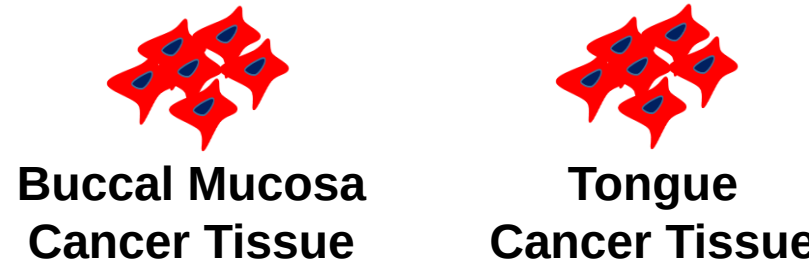
(B) Using IPA and GSEA Analysis, we identify enriched pathways and biological processes that are unique to each subsite-subsite comparison. We then can identify unique gene signatures that define each subsite of OSCC.

OSCC Subsites are Defined by Unique Gene Signatures that Highlight Key Targetable Oncogenic Pathways

Identifying Potential Mesenchymal Tumor Specific Therapies

A

Highlighting the Differences Between Buccal And Tongue Squamous Cell Carcinoma



DESeq2 Mediated Differential Gene Expression Analysis

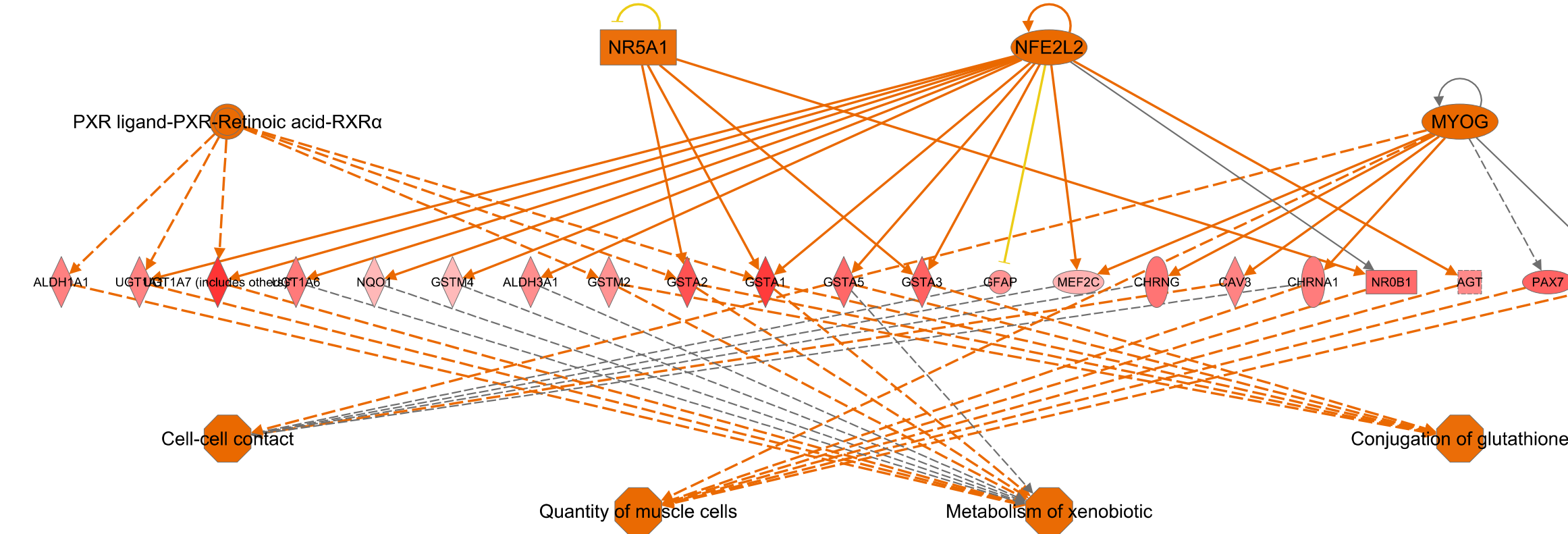
1456 Differentially Expressed Genes

(A) Differences between Buccal SCC vs Tongue SCC analysis were highlighted. 1456 Differentially Expressed Genes were Identified and leveled as part of the larger analysis to define subsite unique gene signatures.

(B) IPA Upstream Analysis was performed on the Tongue Gene Signature to identify potential activated regulators controlling specific pathways and biological processes within the specific tumor subsite.

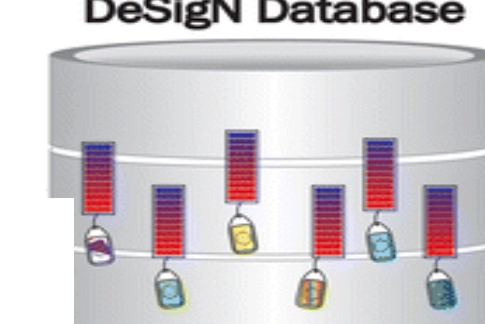
B

Highlighting the Differences Between Buccal And Tongue Squamous Cell Carcinoma



Genomics of Drug Sensitivity Project

DeSigN Database



Oral Tongue Signature

Leverage DeSigN Database to Identify Targeted Therapy Specific to Subsite Gene Signatures

Summary

• **Unique** Gene Signatures were identified for the oral cavity subsites profiled via RNA-Seq analysis in the TCGA Database. These unique expression programs segregated the subsites into unique clusters within a larger expression network and these clusters can be leveraged for targeted therapy.

Future Directions

- Perform validation experiments in RPCI Tissue Bank of OSCC to identify overexpression of markers identified in our TCGA Analysis
- Validate potential therapeutic targets in cell and animal models based on predicted drug sensitivities per our bioinformatic analysis pipeline.

DeSigN Drug Selections
Tongue SCC Gene Signature

Drug	TARGET
Sunitinib	PDGFR, KIT, VEGFR, FLT3, RET, CSF1R
AMG-706	undefined
VX-702	MAPK11, MAPK12, MAPK14
PAC-1	CASP3
AZD6244	undefined
PD-0332991	CDK4, CDK6
ATRA	undefined
GSK-450394	undefined
17-AAG	HSP90AA1
Vorinostat	HDAC1, HDAC10, HDAC11, HDAC2, HDAC3, HDAC5, HDAC6, HDAC8, HDAC9
Obalocicax-Mesylate	undefined
AUY922	undefined
Bosutinib	ABL1, BCR, CAMK1D, CAMK2G, CDK2, FRK, FYN, HCK, LYN, MAP2K1, MAP2K2, MAP3K2, MAP4K5, SRC, STK10, STK24, STK4, TNK2, TKK
Midostaurin	FLT3, PRKCG