Characterization of Oral Cancer Stem-like Cells by Whole Transcriptome Analysis

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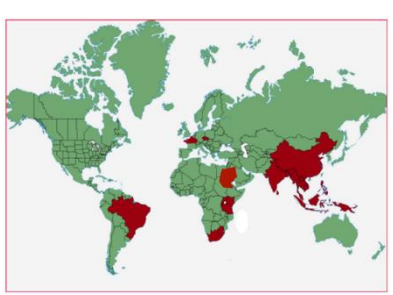
Methods:

Primary tumorsphere formation: Media for the tumorspheres (TS) was made using Mammocult media, methyl cellulose and supplements. Cells were then trypsinized and filtered to ensure single cells and then counted to calculate volume needed for 20,000 cells to be distributed evenly among 4 wells. After 8 days the TS growth was examined and imaged with Celigo in Microscopy Core. Secondary TS were generated by passaging cells from dissociated primary spheres.

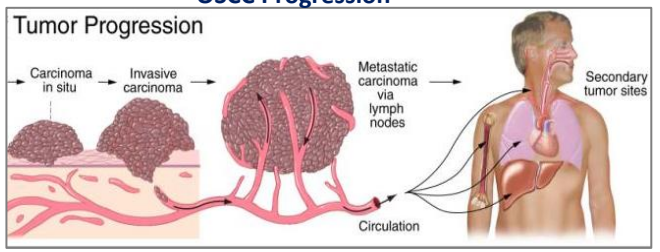
Global multi-omics analyses: TS-derived from CAL27 and HSC-3 OSCC cell lines will be analyzed for gene expression signatures by RNAseq before and after E7386 treatment. The sequencing data will be processed and QC-ed and differential gene expression signatures will be derived for each treatment and annotated by pathway enrichment analysis based on the MSigDB compendia and the Connectivity Map (CMap).

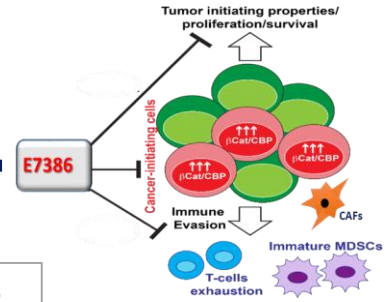
Introduction:

Head and neck cancer, and its major subsite, oral squamous cell carcinoma (OSCC), accounts for 12% of all malignancies worldwide and 3% in the US. Collective evidence indicates that OSCC is driven by a subpopulation of cells known as cancer stem cells (CSCs). These cells can grow in the absence of attachment to the substratum, reflecting their aggressive properties. To date, there is a limited understanding of the molecular features of OSCC CSCs. This project aims to fill this gap in knowledge by generating global transcriptomes of OSCC CSCs and analyzing them in the context of disease progression.



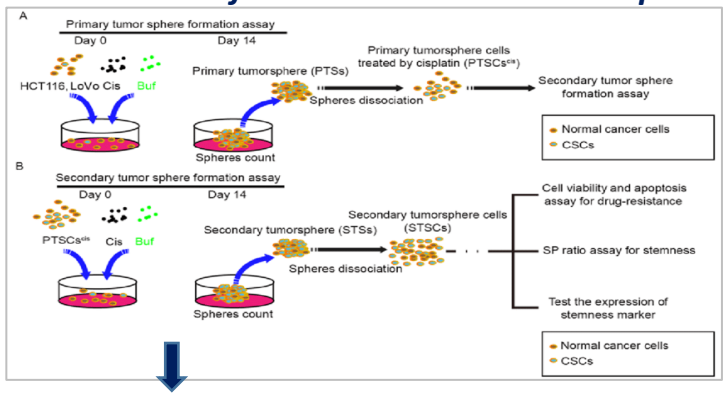
OSCC Progression

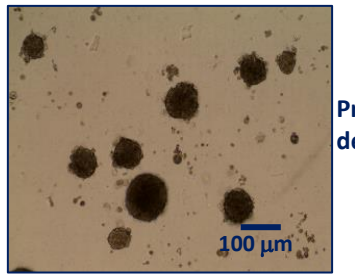




Results: Ongoing and Future Studies

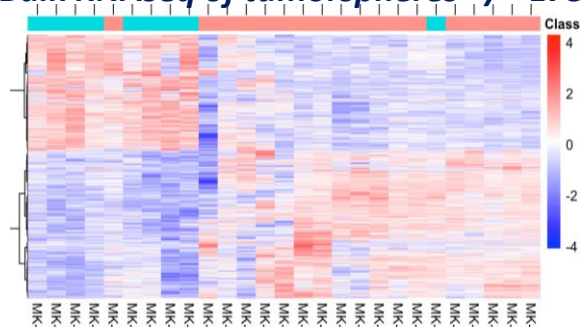
1. *Generation of OSCC cell-derived tumorspheres*





Primary CAL27 cell-derived tumospheres

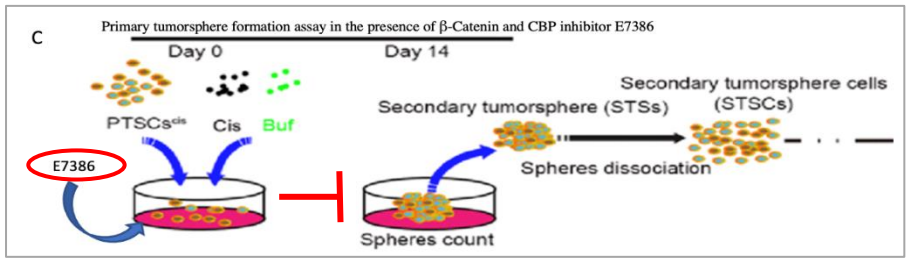
2. *Bulk RNAseq of tumorspheres -/+ E7386*

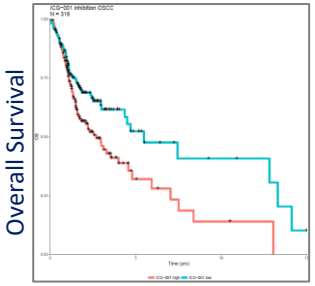


TCGA

Multiomic analysis: projection of gene expression signatures onto the space of OSCC RNAseq in TCGA

3. *Survival Benefit in Human Patients*





Conclusions:

- OSCC cells readily form TS suggesting their aggressive stem cell-like properties
- E7386 is expected to interfere with TS formation
- TS-derived gene signatures will likely track with poor patient outcomes
- TS gene signatures may serve as diagnostic biomarkers.

References:

1. Kartha et al, Genome Med 2018
2. Tumorsphere assay methods conducted according to Mammocult's instructions