Defining the role of non-canonical PIK3CA mutations in head and neck squamous cell carcinoma

Michelle Ji-Eun Lee¹, Nan Jin, Janice Cho, Patrick Kwok-shing Ng, Gordon B. Mills, Daniel E. Johnson, and Jennifer R. Grandis
¹University Of California, San Francisco

OBJECTIVES/GOALS: To characterize the oncogenic potential of HNSCC cell lines harboring 17 non-canonical PIK3CA mutations.

METHODS/STUDY POPULATION: Non-canonical PIK3CA mutant constructs generated via site-directed mutagenesis are subcloned into doxycycline-inducible vector pLVX-Puro. Serum-dependent HNSCC cell line (PCI-52-SD1) is then stably transfected with vectors and undergo doxycycline-induction. Cell survival is determined by depriving cells of fetal bovine serum for 72 hours and quantifying remaining cells with 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. Cell proliferation and migration is evaluated with colony formation assays and transwell assays, respectively.

RESULTS/ANTICIPATED RESULTS: To date, the survival behavior of eight non-canonical mutants was assessed. Three mutants – Q75E, V71I, and E970K – exhibited 18.7–26.7% greater survival rate relative to cells transfected with wild-type. Five mutants – R519G, Y606C, W328S, C905S, and M1040I – demonstrated survival differences that differed only by -4.3% to +6.6% relative to wild-type. We hypothesize the three activating mutants that exhibited increased survival will also demonstrate increased cell proliferation and migratory behavior whereas the three neutral mutants will not differ from control.

DISCUSSION/SIGNIFICANCE OF IMPACT: Ongoing HNSSC PI3K hyperactivation results in a cyclic STAT3 decoy oligonucleotide. The remaining authors declare no conflicts.

Dissecting the role of microenvironment heterogeneity on metastatic tumor cell phenotype at an engineered metastatic niche

Sophia Orbach¹, Michael D. Brooks², Grace G. Bushnell², Max S. Wicha², Jacqueline S. Jeruss², and Lonnie D. Shea²
¹University of Michigan School of Medicine; ²University of Michigan

OBJECTIVES/GOALS: Breast cancer metastases are stochastic and difficult to detect. Therapy is often ineffective due to phenotypic changes of tumor cells at these sites. We engineered a synthetic metastatic niche to study the role of phenotypic transitions in the microenvironment on tumor cell phenotype.

METHODS/STUDY POPULATION: The engineered metastatic niche is composed of a porous polycaprolactone scaffold implanted subcutaneously in Balb/c mice. The mice received an orthotopic inoculation of 4T1 cells (murine triple negative breast cancer) in the fourth right mammary fat pad and the disease was allowed to progress for 7-21 days (pre-metastatic to overt metastatic disease). The scaffolds and lungs (native metastatic site) were explanted and analyzed by single cell RNA-seq via Drop-seq. Cell phenotypes were identified and tracked over time with the Seurat and Monocle3 pipelines. Assessment of the impact of these cell populations on tumor cell phenotype was conducted through Transwell co-cultures.

RESULTS/ANTICIPATED RESULTS: Healthy scaffolds are primarily composed of macrophages, dendritic cells, and fibroblasts – consistent with a foreign body response. Despite differences in the lung and scaffold prior to tumor inoculation, both tissues were marked by >5-fold increase in neutrophils/MDCSCs. Additionally, 79% of genes at the scaffold that significantly changed over time were also identified in the lung, indicating key similarities in niche maturation. However, many immune cells at the scaffold had distinct phenotypes, with pro-