known vertebrate genomes retain a functional copy of both. USP4 was found to be consistently overexpressed in primary tumor tissue from small-cell carcinomas and adenocarcinomas of the lung. Despite their similarity, high expression of USP4 is correlated with decreased overall survival in lung adenocarcinoma, whereas high expression of USP15 is correlated with increased survival. Both USPs are known to be involved in some of the same signaling pathways such as Wnt/β-catenin; however, subfunctionalization has occurred such that they each regulate the stability of distinct substrates. To better understand each USP’s role, we are analyzing mice in which one or both genes have been inactivated and have found that the absence of both USPs results in a lethal phenotype. Although USP4 and USP15 have diverged over evolutionary time, we hypothesize that there may still be some level of functional redundancy. We found that embryos null for both genes die at midgestation and are physically smaller than embryos heterozygous for both genes. They have underdeveloped livers, indicating a possible defect in hematopoiesis. Proper fetal hematopoiesis requires signaling through Wnt/β-catenin pathway, and a systematic analysis of the components of this pathway has been undertaken by Western blot and qPCR. Current data indicate that there are deficiencies in at least some USP4 substrates, and that the TCF transcriptional complex is greatly reduced. Published reports assert a role for USP4 in metastatic spread of lung cancer to the brain, mediated by its effects on the Wnt/β-catenin pathway. Potential functional compensation by USP15 must be evaluated before targeted therapies can be considered. Our studies will establish the extent and mechanism of such compensation.

A27
Stage I Lung Adenocarcinoma Gene Expression Associated with Aggressive Histologic Features for Guiding Precision Surgery and Therapy


Background: Stage I lung adenocarcinomas (LUADs) show heterogeneity in histologic patterns that correlate with malignant behavior. Solid, micropapillary, and cribriform patterns are associated with worse survival whereas lepidic (in situ) predominance has the best prognosis. In this study, we sought to characterize histologic pattern-specific gene expression in resected clinical stage I LUADs. We also aimed to train and validate a genomic biomarker predictive of histologic aggressive patterns with the ultimate goal of being able to impact surgical and therapeutic decision making for post-biopsy management.

Methods: A training cohort of 56 tumors from patients with stage I LUAD was included for pathologic annotation and whole-exome RNA sequencing. Histologic pattern subtyping in 5% increments including all diagnostic slides was performed. A single representative FFPE block was chosen for RNA sequencing. Negative binomial models were used to identify gene expression differences associated with percent solid, cribriform, or micropapillary histology, and EnrichR was used for pathway enrichment analysis. A random-forest classifier predicting aggressive histologic patterns was trained using 5-fold cross-validation. An independent set of ≤2.0 cm clinical stage I LUAD was macrodissected into 32 paired components (lepidic + non-lepidic regions) and subjected to RNAseq. Six tumors were defined as low malignant potential (LMP: lepidic + acinar/papillary) and ten tumors were defined as overtly malignant potential (OMP: lepidic + solid/micropapillary/cribriform).

Results: In the training cohort, we identified 1,322 genes associated with tumor histologic composition (FDR q <0.05 and fold-change > 2). Genes whose expression differs with solid histology% were enriched for involvement in DNA replication, cell cycle regulation, and inflammation (FDR q <0.001). Genes associated with microcapillary% were enriched for involvement in tRNA-aminoacylation and decrease of T-cell activity (FDR q <0.001). The functional enrichment of genes associated with cribriform% was less informative. A gene expression classifier was trained to predict the presence of aggressive histology. We validated this classifier on a set of 16 tumor specimens from which we macrodissected and analyzed tissue from the most aggressive histologic pattern (AUC = 0.92). We also found that this classifier could differentiate between lepidic regions isolated from OMP and LMP tumors (AUC = 0.81).

Conclusion: We identified solid-, micropapillary-, and cribriform-specific gene expression among clinical stage I LUADs and developed a classifier predictive of aggressive histologic features using either lepidic (in situ) or nonlepidic components. This biomarker has the potential to predict histologic aggressiveness even from presurgical tumor biopsies where all histologic patterns may not be represented. Such a biomarker may be useful in guiding clinical decision making, including extent of surgical resection.

A28
Investigating Antitumor T-Cell Responses Using NINJA: An Inducible Genetic Model for Creating Neoantigens

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Historically, attempts to generate inducible neoantigens in mouse models have been hindered by leaky expression of the antigen in the thymus, leading to central tolerance in developing CD8+ and CD4+ T cells. We have developed the iTNversion INducible Joined NeoAntigen (NINJA) model to resolve the existing problems of tolerance and leakiness using RNA splicing, DNA recombination, and three levels of regulation to control induction of neoantigen. Furthermore, this inducible model system is compatible with existing Cre-driven models of cancer, and we have generated a NINJA-antigen-inducible tumor cell line from a KrasG12D;P53−/− mouse lung tumor. Antigen expression in this model is temporally controlled via systemic drug delivery, and generates responses in both transgenic and endogenous CD8 T cells. We will use this model to investigate specific T-cell responses to tumors and to assess how therapies such as checkpoint blockade impact T-cell response.

A29
Immune-Suppressive Microenvironment Induced by Increased Treg During EGFR-TKI Mediated IP-10 and TGF-β

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Background: Studies on the immune microenvironment of EGFR mutant lung cancer have been limited. We analyzed the effect of immune microenvironments on the development of EGFR-TKI resistance in EGFR-mutated lung cancer. Methods: The EGFR mutant lung cancer cell lines (HCC827 and H4406) were cocultured with activated PBMC for 72 hours with EGFR-TKI. Changes of cytokines/chemokines in the media, PD-1 expression of CD8+ T cells, regulatory T cells fraction, and transcriptome analysis of tumor cells were analyzed. We also performed immune profile analysis of fresh tissues of 21 surgically resected NSCLC (7 EGFR mutant and 14 EGFR wild) by multicolor FACS. Results: IFN-γ, IL-6, VEGF, TGF-β, and IP-10 were significantly increased after coculture but did not decrease after EGFR-TKI. PD-1/L1 expression on tumor cells increased after coculture (p = 0.08 in HCC827 and p = 0.09 in H4406) but did not decrease after coculture with activated PBMC and EGFR-TKI treatment (p =