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 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 lepideic regions isolated from OMP and LMP tumors (AUC = 0.81). Conclusion: We identified solid-, micropapillary-, and cribriform-specific gene expression among clinical stage 1 LUADs and developed a classifier predictive of aggressive histologic features using either lepideic (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 iNversion 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 Kras<sup>G12D</sup>P53<sup>−/−</sup> 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 H460) 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, 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-β1, and IP-10 were significantly increased after coculture but did not decrease after EGFR-TKI. PD-L1 expression on tumor cells increased after coculture (p = 0.08 in HCC827 and p = 0.09 in H460) but did not decrease after coculture with activated PBMC and EGFR-TKI treatment (p =
0.36 in HCC827 and p = 0.45 in H4006). PD-1 expression of CD8 T cell cocultured with HCC827 or H4006 did not change; however, proportion of regulatory T cell increased after coculture with HCC827 or H4006 (p=0.05 and p=0.08, respectively) and did not decrease during EGFR-TKI treatment. Proportion of regulatory T cell in cocultures with A549 or H1975 (erlotinib resistant cell line) did not change during coculture or EGFR-TKI treatment. Increase of IP-10 is mediated by IFN-γ in both EGFR mutant cell lines and PBMCs. The inhibition of IP-10 by si-RNA significantly decreased TGF-β1 expression and proportion of regulatory T cells in cocultured mutant EGFR lung cancer cell with EGFR-TKI treatment. Transcriptome analysis by RNA sequencing showed 1,747 gene sets were differentially expressed in EGFR-TKI treated EGFR mutant cell line cocultured with activated PBMC compared to EGFR-TKI treatment alone. Interferon gamma response pathway (NES 2.65, FDR q = 0.36 in HCC827 and p = 0.45 in H4006). Whole-slide lymphocyte level was similar although slightly lower in LUAD (9.9%+/-0.2% vs. 11.4%+/-0.2%). However, lymphocytes in LUAD samples were more likely to infiltrate tumor regions than those in LUSC (48.1%+/- 1.2% vs. 42.7%+/-0.7%), and/or were immediately adjacent to tumor regions (78%+/-1.2% vs. 74.2%+/-0.9%). Lymphocyte levels were more bimodal in LUAD than LUSC, with 28.6% (vs. 22.9%) having very high TIL (>60%) despite having lower overall lymphocyte counts. **Conclusions:** Despite lower overall TMB and lymphocyte levels, there exists a subset of LUAD samples with very high infiltrating lymphocyte counts, indicating a potentially anti-PD1-sensitive subpopulation. Further characterizing this subset and confirming differential IO response is warranted.

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**A30**

**Tumor-Infiltrating Lymphocytes (TILs) Found Elevated in Lung Adenocarcinomas (LUAD) Using Automated Digital Pathology Masks Derived from Deep-Learning Models**

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**Background:** Tumor mutation burden (TMB) is associated with increased response to anti-PD-1 therapy in non-small cell lung cancer (NSCLC) (Rizvi, 2015). Squamous cell carcinomas (LUSC) have higher average TMB than adenocarcinomas (LUAD) (Schumacher, Schreiber, 2015); however, meta-analyses show that in fact LUAD receive slightly more survival benefit from anti-PD1 therapy (Zhou, 2018). Here we explored whether lymphocyte distribution in the tumor microenvironment may give a rational explanation for this differential response to immune-oncology (IO) agents.

**Methods:** 867 subtyped NSCLC high-resolution diagnostic whole-slide images were obtained from TCGA sources. Images were tiled into 100micron 2D color patches. To ensure subtypes were visually distinct at this scale, a LUAD/LUSC classifier was developed as follows: Samples were randomly split into 80% training and 20% testing samples. Cells were counted in each image patch, and used to bin into 12 ranges of cell counts (0-10 cells per patch, 10-20, etc., up to >110 cells per patch). 2D color patches were transformed into 1D descriptive vectors using the ResNet54 deep learning framework, and used to train 12 separate support-vector machines (SVMs). An ensemble of these 12 SVMs was used to classify unseen samples. To detect tumor regions and lymphocyte infiltration, 2D color patches were used to train convolutional neural networks (InceptionV3) based on gold-standard masks generated with pathology assistance, then used to detect tumor and lymphocytes in all unseen patches. Patches that simultaneously classified as positive for tumor and lymphocyte area were considered evidence of TILs. Lymphocyte-positive patches immediately adjacent to tumor patches (i.e., lymphocytes within 100microns of tumor) were also analyzed. **Results:** LUAD and LUSC were highly classifiable using this system, with a ROC AUC of 0.95 and precision of 0.95 in test samples. The total tumor tissue area was similar between samples classified as LUAD and LUSC (48.3%+/-1.1% vs. 46.5%+/-1.1%). Whole-slide lymphocyte level was similar although slightly lower in LUAD (9.9%+/-0.2% vs. 11.4%+/-0.2%). However, lymphocytes in LUAD samples were more likely to infiltrate tumor regions than those in LUSC (48.1%+/-1.2% vs. 42.7%+/-0.7%), and/or were immediately adjacent to tumor regions (78%+/-1.2% vs. 74.2%+/-0.9%). Lymphocyte levels were more bimodal in LUAD than LUSC, with 28.6% (vs. 22.9%) having very high TIL (>60%) despite having lower overall lymphocyte counts. **Conclusions:** Despite lower overall TMB and lymphocyte levels, there exists a subset of LUAD samples with very high infiltrating lymphocyte counts, indicating a potentially anti-PD1-sensitive subpopulation. Further characterizing this subset and confirming differential IO response is warranted.

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**A31**

**A Reservoir of Tumor-Specific CD8 T Cells in Lung Cancer Resides in the Draining Lymph Node**

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Recent work has described the population of CD8 T cells that respond to anti-PD-1 therapy (marked by TCF1 and PD-1), but it remains unclear how these T cells are maintained within the immunosuppressive tumor microenvironment (TME) of lung cancer. To understand this, we developed a genetically engineered model in which Kras-G12D expressing p53 deficient lung adenocarcinomas express a known neoantigen called the iNversion INDuced joined neoAntigen (NINJA). NINJA allows us to follow neoantigen-specific CD8 T cells over the course of tumor development. We find that ~20% of tumor-specific T cells in early 8-wk tumors are TCF1+, but by 17-20 wks, this TCF1+ has significantly shrunk, and there has been a concomitant increase in the expression of markers of T-cell terminal differentiation (Tim3). This is consistent with the idea that T cells receive signals in the TME that drive terminal differentiation and restrict responses to immunotherapy. We reasoned that if the signals driving terminal differentiation were provided in the TME, neoantigen-specific T cells in the tumor-draining lymph node (dLN) may remain less differentiated over the course of tumor development. Analysis of tumor-specific T cells in the dLNs of 8-wk and 17-wk tumors showed that they were mostly TCF1+. Moreover, single-cell transcriptional analyses suggested that these cells were less differentiated than their counterparts in tumors. T-cell receptor (TCR) signals are a major driver of terminal differentiation, and we observed that tumor-specific T cells in the dLN were not receiving TCR signals, while all T cells in the TME were positive for TCR signals. This suggested at least two models for how antitumor cells function: 1) tumor-reactive T cells in dLNs and TME could function independently of one another, or 2) tumor-reactive T cells might have a role in sustaining the antitumor T-cell response over the course of lung-tumor development through migration. Consistent with the latter model, TCR sequencing of dLN and TME neoantigen-specific T cells showed a close clonal relationship; 13 of the top 15 clones in the TME were present in the dLN, and the hierarchy of clonal dominance was maintained. This was also true in 17-wk tumor-bearing mice, suggesting that the population of tumor-specific CD8 T cells in the dLN serves as a reservoir of less differentiated cells that can continuously replenish the T cells in the TME, helping to sustain the antitumor T-cell response over the course of tumor development. Critically, it is unclear whether current immunotherapeutic strategies leverage this reservoir of T cells, raising the possibility that this population of dLN tumor-specific T cells could be targeted to provide significant additional benefit for patients receiving immunotherapy.