SYIT: Modeling the interplay of SYstemic Immunity and Tumor heterogeneity

Translational

Lungs

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ABSTRACT

We performed a comparative analysis of single-cell RNAseq analysis of blood samples and paired single cell spatial transcriptomic analysis of tumor samples from non-small cell lung cancer patients treated with immunotherapy. We first analysed the datasets separatly and then combined to develop a model to predict immunotherapy response, progression-free survival and overall survival. For the blood samples, we developed three levels of annotations using the Azimuth analytical pipeline. We performed k-means and hierchical clustering to identify patients with shared immuno phenotypes. We selected k = 4 cluster as an ideal comparison across the three annotation levels (8-30 and 53 cell types). With the most coarse annotation the four immune type of patients included: 1. Monocyte dominant, 2. B cell dominant 3. CD8 T cell high dominant CD4 T cell dominant. Although this coarse annotation did not provide clear separation in PFS/OS or response that the cluster enriched for CD8 T cells showed the longest survival and 100 response rate. Non responders had higher monocyte and lower CD8 T cell counts. Both Level 1 and Level 2 clustering identifed clinically meaningful patient subgroups, with Level 1 being more parsimonious and Level 2 potentially capturing additional granularity in cell subtype composition. The T cell-enriched phenotype (especially high CD8 T cells) remains the strongest predictor of favorable outcomes at both resolution levels. The T cell enriched cluster 3 showed HR of 0.32 (68% lower risk, p = 0.097) - approaching significance, while overall survival significantly differed with the more detailed annotation. Interestingly, cluster 3 (Cd8 high) was also associated with enriched PD-L1 tumor expression potentially explaining ther results. With level 3 annotations suggested further refinement of outcomes and biological insights with cluster 3 patients not only enriched for CD8 Tcells but also for MAIT and γδT cells. Interestingly, each annotation level captured a fundamentally different aspects of immune composition. The near-zero agreement between levels suggests they provide complementary, not redundant information. Still level 3 annotation provided superior prognostic value compared to Level 1 and 2. The detailed subtype composition matters - not just broad categories. 100% ORR in Level 3 Cluster 2 suggests that these patients are "super-responders" with 41.9 month median OS which is exceptional for metastatic NSCLC on immunotherapy. This cluster (23% of cohort) could guide patient stratification for clinical trials.

In the analysis of tumors we found 3 main patient groups: 1. Malignancy-dominant group, 2. Fibroblast-rich group and 3. airway-associated outliers. We mapped 30 cell types and 21 out of 30 cell types show significant differences between clusters. High malignant cell burden correlated with worse outcomes, immune infiltration by macrophages showed trend toward better survival, while B cell infiltration was linked to worse survival. We compared tissue and blood single cell composition however the two appears to be independent with little potential equilibration between the compartments. Finally we aimed to developed a combined predictor for outcomes. While we did see improved AUC for combining blood and tumor based parameters (from AUC of 0.69 to 073) this effect was not substantial. This could be also due to the relatively small size of the cohort

SCIENTIFIC IMPACT

We provide evidence for combined blood and tumor truly single cell analysis for predictors of outcomes. We show the power but also the limitations primarly driven by the need to analyse larger cohorts of patients