E5 Single Cell sequence / spatial biology and beyond - early-stage lung adenocarcinomas

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To comprehensively capture LUAD neoplastic heterogeneity and cellular plasticity, we performed single-cell RNA-sequencing (scRNA-seq) of 257,481 enriched epithelial cells (EPCAM+ sorting) from 16 early-stage LUADs, each with 3 matched normal lung (NL) samples at defined spatial proximities to the tumor (n=47). 29,076 LUAD-derived cells clustered by patient and harbored distinct gene expression features), signifying interpatient LUAD heterogeneity. Clusters of malignant cells were overall segregated based on driver mutations (e.g., KRAS, EGFR). Malignant cells from KRAS-mutant LUADs (KM-LUADs) had increased activation of NF-kB, estrogen and hypoxia signaling, comprising a unique gene module (GM) that correlated with a less differentiated state. Notably, cells from one EM-LUAD and its 3 multiregion NL tissues clustered closely and had activated pro-tumor lymphoid signatures (CD4 naïve, Treg). Our analysis of a large number of lung epithelial cells from LUAD patients reveals in-depth insights into LUAD taxonomy which can help identify epithelial heterotypes, unravel the continuum of early differentiation events and expand our understanding of early LUAD pathogenesis.

While T cells have been a central focus of cancer immunopathology and immunotherapy, the roles of tumor-infiltrating B and plasma cells (TIBs) in the activity of the adaptive immune response along the pathogenic course of solid tumors such as LUAD are extremely poorly understood. To fill these voids, we conducted pan-cancer single-cell RNA sequencing (scRNA-seq) analysis of TIBs using public and in-house cohorts of >15 cancers and matched normal samples. By comprehensively defining transcriptional profiles, somatic hypermutation (SHM) and antibody repertories, as well as cellular interactions of TIBs at single-cell resolution, our results map out the geospatial landscape of TIBs in early-stage LUAD and provide a valuable resource to leverage targets for innovative immunomodulatory strategies.

In addition, we collected, from the same tumour and normal regions, a set of tissues for genomic

profiling by whole exome sequencing (WES), and another set for high-resolution spatial transcriptome and protein analyses. Among the 16 patients, 7 showed gradually reduced AT2 fractions with increasing tumour proximity (P = 0.004 by ordinal regression analysis), 6 had reduced AT2 fractions in LUADs relative to NL, and only 2 showed fluctuated AT2 fractions in geospatial samples.

This atlas of epithelial and inflammatory cells in human LUADs underscores new cell-specific states that underlie inception of LUADs, and that can thus guide novel strategies to prevent the initiation and development of this morbid disease.

1 細胞シークエンス/空間的分析と次の展開
ー早期肺腺癌について

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今後の早期肺腺癌に対しての治療戦略を考えると、複雑な分子機構や細胞プロセスをさらなる理解が必要となる。シークエンス技術の進展により 1 細胞レベルでの分析が可能となった。我々のグループでは、16 名の患者からの 257481 の EPCAM 陽性上皮細胞の単細胞シークエンス(scRNA-seq)を行った。それぞれの腫瘍に対応した 3 種類の正常肺組織を腫瘍からの距離に応じて採取し、腫瘍への空間的な近接性を分析しています。またこれまでは T 細胞が癌の免疫病理学での中心的なトピックであった。しかしながら、固形腫瘍における腫瘍浸潤性 B 細胞と形質細胞(tumor-infiltrating B and plasma cells (=TIBs))の役割は重要である割に、これまで論じられることは少なかった。我々は先述した症例の EPCAM 陰性細胞の 1 細胞シークエンス(scRNA-seq)と 1 細胞 B 細胞受容体シークエンス(scBCR-seq)を行なった。上皮細胞と非上皮細胞のそれぞれの 1 細胞シークエンスから得られる情報を報告し、さらにシークエンス情報を病理学的な観点からの理解を深めるため、サブセルラーレベルで mRNA の発現データの組織学的な局在を示す空間分析方法（Spatial analysis）手法を駆使して得られる情報や現在複数のプラットフォームでβテスト確認しているセルラーレベルでの空間分析の中間報告を紹介する