Heterogeneity in lung cancers by single-cell DNA sequencing

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Dear Editor,

Lung carcinoma genomes are heterogeneous. We probe heterogeneity origins by sequencing 13,343 single-cell genomes from seven lung adenocarcinomas (LUAD), seven lung squamous cell carcinomas (LUSC), and two small-cell lung carcinomas (SCLC). Our findings

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reflect lung tumors holding huge subclone diversity on copy number variations (CNVs) and complex structure variations (cSVs).

Lung cancer tops global cancer deaths, with intra-tumor heterogeneity (ITH) contributing to recurrence and resistance. Single-cell DNA sequencing (scDNA-Seq) provides a precise ITH perspective by profiling individual cells in multiple cancers, but limited in lung cancer. Most lung cancer studies examine subclonal CNVs, leaving cSV’ ITH, and their role in lung cancer progression remains incompletely understood.

This study analyzed 13,343 single-cell genomes from 16 lung tumors: 7 LUAD, 7 LUSC, and 2 SCLC (Figure 1, Table S1, Supporting Information Tables and Supporting Information Methods). We investigated the CNV landscape across all tumors. Tumor cell groups were obtained from hierarchical clustering (HC) using cell ranger-DNA. We assigned cells grouped by a leaf node in the HC cut-dendrogram as cell clusters; that is, cells inside one cell cluster sharing similar CNVs. We identified 16 to 33 cell clusters per tumor (Figures S1–S3), yielding three to nine subclones per tumor and 72 subclones overall (Table S2). All tumors display polyclones, meaning they have at least two subclones. Subclones are denoted by dominant amplified (A), diploid (D), or lost (L) copy numbers (Table S2). “LX” indicates subclone has loss of heterozygosity in chromosome X. The largest subclone populates 1,273 diploid cells (LUAD002-D), whilst we detected 23 small cell populations, that is, subclones under 10 cells (Table S2). We calculated the Gini index per subclone, with higher values reflecting greater CN dispersion across genomic regions in the subclone. Overall, cell numbers and Gini indices vary between subclones, illustrating CNV tumor heterogeneity in LUAD, LUSC, and SCLC (Figure 1 and Table S2). Moreover, hierarchical clustering of subclone CNVs revealed inter-patient similarities (Figures S4 and S5, Table S3 and Supporting Information Results).

Punctuated copy number evolution (PCNE) hypotheses subclonal CNVs arise in short bursts of crisis.
while branching copy number evolution (BCNE) hypothesizes subclonal CNVs are intermediate accumulated over evolution.\textsuperscript{3–5} We manually infer CNV evolutionary trees (Figure 2A) from tumor cell- and subclone-level phylogenies, alongside subclone-level consensus CNVs (Figures S6–S8). We posit most lung tumors experience PCNE, with certain subclones subsequently undergoing BCNE (Figures S6–S8 and Supporting Information Methods). We hypothesize copy number evolution begins in normal tissue and then diverges into A-, D- or L-dominated subclones, which may continue evolving. We suffixed indices to subclone names to indicate their occurrence in the evolution process (e.g. A2 occurs after A1). All lung tumors exhibit PCNE signatures, except LUAD02T and LUSC01T, forming one dominant group with minor clones under ten cells. Likewise, six lung tumors (LUAD05T, LUAD06T, LUAD07T, LUSC07T, LUSC08T and SCLC01T) show BCNE evidence derives post-PCNE subclones (≥10 cells) (Figures S6–S8). SCLC02T-A features MYC and ASCL1 amplification, while SCLC02T-L exhibits MYCN amplification. Both MYC and MYCN promote SCLC in mice.\textsuperscript{10} Distinct amplified genes in subclones A and L suggest differing evolutionary paths. APC is specifically amplified in LUAD06T-A2 and LUAD07T-A2. APC ampli-
Subclone-level deletions dominate across cohorts (Table S4). Many genetic alterations (10–70%) co-exist in tumor subclones formed by PCNE (Figure 3B, Figures S9 and S10, Table S5, and Supporting Information Result). Furthermore, we detected two breakage-fusion bridges in LUSC, duplicating oncogenes PLA2G4A and GBE1, which may occur accompanying PCNE (Figures S14 and S15 and Supporting Information Results). Interestingly, InDels, SVs, and cSVs recurrently hit Human leukocyte antigens (HLA) genes in subclones (Figure 3C and Figure S16 and Table S6). We detected cSVs harboring in MHC-II genes. LUSC shows more SV breakpoints in the HLA-DRB gene than LUAD. These findings suggest cSVs in MHC-II genes coincide with or precede CNV bursts (Figures S17–S20 and Supporting Information Results). Despite tumor heterogeneities, we identified recurrent genetic alterations in LUAD- or LUSC-related genes (Figure 3C and Supporting Information Results).

In brief, we used 10x scDNA-Seq to reveal extensive subclone diversity in CNVs and cSVs across LUAD, LUSC and SCLC. The number of cell clusters and subclones aligns with previous breast cancer scDNA-Seq studies.\(^3\)–\(^5\) We suggest PCNE in three lung cancer subtypes, characterized by early genomics gains and losses, followed by BCNE. Two breakage-fusion-bridges duplicating oncogenes PLA2G4A and GBE1 were detected in LUSC, potentially linked to PCNE. cSVs are identified in lung cancers, especially with high frequency (75%) in LUSC, affecting two MHC-II genes (HLA-DRB5 and HLA-DRB1). Evolutionary analysis suggests these cSVs may occur before or during PCNE. Hence, our findings reflect extensive subclone diversity in lung tumors concerning CNVs and cSVs.

One study limitation is subclone detection dependent on 10x scDNA-Seq cell profiling,\(^6\) partially repenting subclone diversity in tumors. Average single-cell coverage was low (Supporting Information Tables), potentially concealing SVs and cSVs in minor subclones. The issue is prevalent in scDNA-Seq, we aim to sequence more lung tumors to enrich findings. PCNE and BCNE hypotheses rely solely on observing cell phylogenies and subclone CNVs. Looking forward, we plan to mathematically model PCNE and BCNE processes for quantitative answers.

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Not applicable.

**CONFLICT OF INTEREST STATEMENT**
Not applicable.

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**DATA AVAILABILITY STATEMENT**
The data that support the findings of this study are available at https://doi.org/10.57760/sciencedb.08329.

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REFERENCES

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.