Clinicogenomic landscape of morphological evolution in lung adeno-carcinoma

Lung adenocarcinomas (LUADs) encompass a broad spectrum of histological appearances. We use multi-region, prospective and longitudinal sampling from the TRACERx dataset to show the relationship between LUAD morphologies and their underlying evolutionary genomic landscape, as well as clinical risk and the nature of metastatic dissemination.

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The problem

LUAD is a form of cancer with significant inter- and intra-tumoural histological heterogeneity. Pathologists recognise six architectural growth patterns in LUAD and there is a correlation between 'high grade' patterns of growth (cribriform, solid, micropapillary) and poor patient outcome. Moreover, the presence of free-floating tumour cells that are spread through airspaces (STAS) is associated with risk of recurrence[1]. Most LUADs show a combination of multiple growth patterns, a complexity which hampers attempts to relate histological pattern to underlying tumour biology. The evolutionary trajectories and genomic underpinnings of these growth patterns are poorly understood and the potential clinical utility of combining histopathological findings and genomics to better predict patient outcome is yet to be fully realized.

The observation

Our analysis involved detailed histopathological annotation of LUAD samples from the TRACERx study (TRAcking non–small cell lung Cancer Evolution through therapy (Rx)) and integration of these morphological features with tumour genomic findings, evolutionary characteristics and patient outcome. TRACERx is a prospective observational study of surgically treated non-small cell lung cancer from diagnosis through to cure or relapse, with multi-region primary and metastatic tumour sampling alongside longitudinal circulating tumour DNA (ctDNA) analysis and detailed clinical information[2-5]. Whole exome sequencing data was generated from 805 primary tumour regions and 121 paired metastatic tumours, all derived from 248 primary LUADs, with RNA-Sequencing and pre-operative ctDNA data available for the majority of patients.

Somatic copy number alterations (SCNAs) in chromosome 3 were associated with growth pattern, with predominantly low or mid-grade pattern tumours demonstrating loss of 3q and 3p, whereas tumours with purely undifferentiated solid patterns demonstrated a higher frequency of truncal arm or focal 3q gains, encompassing several driver and differentiation-related genes. These findings suggest that early copy number alterations involving 3q are an evolutionary constraint associated with LUAD morphology. Of the high-grade patterns, solid and cribriform had elevated levels of chromosomal instability (CIN) and harboured large recent subclonal expansions, whereas micropapillary, also a high-grade pattern, was associated with higher subclonal diversity at the tumour regional level (Fig.1a). Furthermore, our findings suggest that different high-grade patterns may relate to the mode of metastasis. Indeed, solid and cribriform patterns were associated with pre-operative ctDNA positivity, histological necrosis and extra-thoracic recurrence, likely to reflect an increased risk of hematogenous cancer cell dissemination. Micropapillary pattern, however, was related to STAS and intra-thoracic recurrence which may reflect an increased risk of non-hematogenous spread. STAS positivity and pre-operative ctDNA were found to be independent prognostic indicators (Fig.1b), and associated with specific relapse sites. A combination of these features has the potential to risk-stratify post-operative patients.

The implications

Variable histological differentiation is a common feature of many tumour types and the evolutionary characteristics of high-grade disease described in our study may be relevant to other cancers. The findings also illustrate how linking morphological features to fundamental tumour biology can be used to predict outcome and mechanism of tumour dissemination. Although this study highlights novel correlations between histological, genomic and clinical characteristics, their correlation is still limited, such that genomic and histological subtypes are not entirely interchangeable and our understanding of how growth patterns are shaped by the tumour microenvironment remains limited. The biology underlying tumour evolution from preinvasive to invasive disease remains unclear, and in-depth analysis of earlier stage tumours is required to further that understanding. Future studies integrating multiregional sequencing alongside whole tumour spatial reconstruction may have greater power to fully resolve the relationship between clonal evolution, histological growth pattern and the tumour microenvironment.

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EXPERT OPINION

"In this study, the TracerX consortium reports associations between pathological and genomic features in Lung cancer. They leverage the previously reported impressive TracerX dataset, adding detailed histological subtyping. There is a comprehensive analysis with several interesting insights that will be of great use to the lung cancer community." **An anonymous reviewer**.

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FIGURE

Fig.1 | Association between growth patterns, genomic variables and prognosis. **a**. Correlation between genomic variables and proportion of high-grade patterns (**left**) or proportion of each growth pattern within each tumour (**right**), with high-grade patterns indicated in bold. Asterisks indicate *q* value ranges * $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$, **** $q < 0.0001$. TMB, tumour mutational burden; wGII, weighted genome instability index; FLOH, fraction of the genome subject to loss of heterozygosity; SCNA, somatic copy number alteration; ITH, intra-tumour heterogeneity. **b**. Kaplan–Meier curves of disease-free survival, split by the positivity of STAS (spread through airspaces) and pre-operative circulating tumour DNA (ctDNA) detection. © 202x, XXX

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BEHIND THE PAPER

At the start of this project, we hypothesised that high-grade growth pattern tumours would demonstrate high genomic intratumour heterogeneity (ITH) and clonal diversity. Indeed high-grade tumours, in particular solid predominant tumours, demonstrated higher CIN and SCNA ITH compared with other subtypes. However, we also noticed that clonal diversity within each tumour region, defined by genetic mutations, was relatively low in solid predominant tumours. This finding suggested that high-grade tumours may harbour subclones that recently expanded and as a result dominate the region. In our companion manuscript[2], we further investigated subclonal selection and expansion in NSCLC, and developed the novel metric "recent subclonal expansion score", as defined by the maximum cancer cell fraction of mutational clusters terminus in phylogenetic trees. **T.K.**

The figure for this Briefing is composed of **2** panels.
- Panel a = Fig **1b,c** from the original paper
- Panel b = Fig **4f** from the original paper

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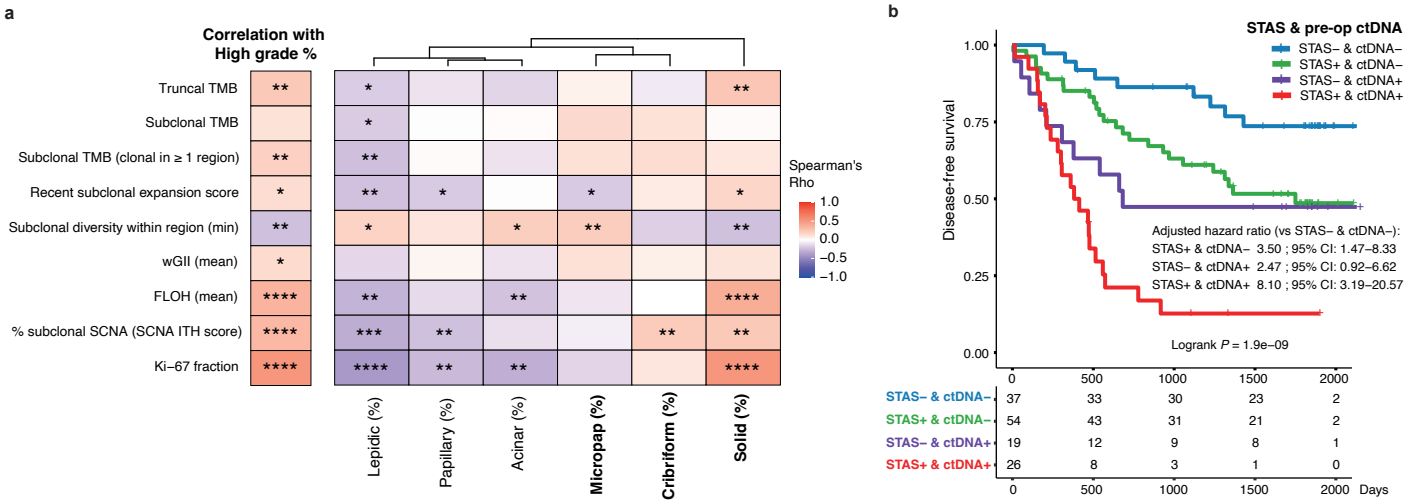
References only above this box

FROM THE EDITOR

"This study from the TRACERx Consortium, offers a deep dive into the histopathology of lung adenocarcinoma, yielding insights into how tissue morphology reflects tumour evolutionary history and disease progression in patients." **The Nature Medicine team, Nature Medicine.**


a
Correlation with High grade %
Truncal TMB
Subclonal TMB
Subclonal TMB (clonal in ≥ 1 region)
Recent subclonal expansion score
Subclonal diversity within region (min)
wGII (mean)
FLOH (mean)
% subclonal SCNA (SCNA ITH score)
Ki−67 fraction
Lepidic (%)
Papillary (%)
Acinar (%)
Micropap (%)
Cribriform (%)
Solid (%)
Spearman's Rho
1.0
0.5
0.0
−0.5
−1.0
b
STAS & pre-op ctDNA
STAS− & ctDNA−
STAS+ & ctDNA−
STAS− & ctDNA+
STAS+ & ctDNA+
Disease-free survival
1.00
0.75
0.50
0.25
0.00
Adjusted hazard ratio (vs STAS− & ctDNA−):
STAS+ & ctDNA− 3.50 ; 95% CI: 1.47–8.33
STAS− & ctDNA+ 2.47 ; 95% CI: 0.92–6.62
STAS+ & ctDNA+ 8.10 ; 95% CI: 3.19–20.57
Logrank P = 1.9e−09
0 500 1000 1500 2000
STAS− & ctDNA− 37 33 30 23 2
STAS+ & ctDNA− 54 43 31 21 2
STAS− & ctDNA+ 19 12 9 8 1
STAS+ & ctDNA+ 26 8 3 1 0
0 500 1000 1500 2000 Days