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On the Origin of Lung Cancers

Adam Pennycuick[1], Sam M Janes[1]

1. Lungs for Living Research Centre, University College London

It is widely accepted that early diagnosis is crucial to improving outcomes in lung cancer, currently the leading cause of cancer-related death in the US(1). CT screening studies have demonstrated remarkable mortality benefits through a stage-shift at diagnosis(2). Yet our understanding of early carcinogenesis remains poor; the precise mechanisms by which a smoke-exposed lung cell undergoes malignant transformation are mysterious. Studies of advanced lung cancers have shown highly complex genomic landscapes, beset with thousands of somatic mutations and copy number aberrations(3, 4). Moreover, individual cancers are highly heterogenous within themselves(5). This complexity makes the identification of biomarkers and effective therapeutic targets extremely challenging; inhibitors of EGFR and ALK are effective, but while response rates are reasonable relapse is universal(6).

To detect and treat cancer earlier, we must understand its origins. Before lung adenocarcinoma (ADC) becomes manifest, early histological changes can be found in the lung: first adenocarcinoma in-situ (AIS), followed by minimally invasive adenocarcinoma (MIA). Here, a new study by Qian et al.(7) performed genomic sequencing on 21 AIS, 27 MIA and 54 invasive ADC obtained from lung resections to

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identify early changes leading to cancerous transformation. Interestingly, these early lesions already display extensive molecular changes; whilst mutation burden is higher in ADC, driver mutations and copy number changes are identified in AIS and MIA, and heterogeneity is observed even at these early stages of carcinogenesis. As the authors state, "*AIS, although preinvasive, has the full genomic alteration profile displayed in invasive cancer*" – a finding mirrored in preinvasive studies of squamous lung cancers(8).

The authors apply a number of methods to tease out biological signals specific to early disease. 21 genes are identified which are significantly mutated across histologies, several of which show a trend towards more mutations in more advanced disease. Copy number losses are more common in AIS/MIA and gains more common in ADC. Mutational signatures were analysed, demonstrating enrichment of a DNA mismatch-repair associated signature – a surprising finding as adenocarcinomas tend to be dominated by smoking-related signature 4 mutations(9) (though this finding may be skewed by the targeted sequencing approach used). Again however, differentiating histological stages by their mutational signatures was not possible. Perhaps most

intriguingly, the authors apply a computational approach, Cancer Inference (PiCnlc), to compare mutations across successive histological subtypes in an effort to identify causative mutations. This analysis highlighted several putative early events in adenocarcinoma progression, such as EPPK, KMT2C and NOTCH3, as well as suggesting the order in which co-occurring pairs of mutations occur, such as KEAP1 selection by STK11 and ATM selection by KRAS. This model generates several coherent hypotheses with clear clinical implications – understanding the sequencing of mutations in this way might allow effective development of targeted therapies towards the earlier changes, potentially arresting cancer development. Additionally, as technology matures for mutation detection in circulating tumour DNA(10), it may become possible to detect these more frequent early changes in blood samples, providing a powerful non-invasive screening tool. However, the sample numbers are too small to draw conclusions with any statistical certainty, and this study stops short of experimentally validating these findings.

Alongside these biological analyses, the authors seek to identify genetic signatures in these early lesions predictive of future survival. They find a 5-gene signature

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associated with poor survival, and a 3-gene signature associated with improved survival, irrespective of histology. The authors suggest that such signatures may represent critical early driver events promoting tumor progression, though they lack validation in a larger cancer cohort. These results may have relevance in the growing field of CT screening; with rapidly increasing numbers of early-stage adenocarcinoma diagnoses, molecular biomarkers able to stratify indolent vs aggressive disease could lead to improved patient pathways, for example as indicators for adjuvant chemotherapy or appropriate follow up protocols. At population scale, even small improvements to screening pathways have the potential for major impact.

This to our knowledge is the largest study of pre-cancerous AIS/MIA lesions of its kind and the authors are to be applauded for their tenacity in what were surely painstaking efforts in identifying and capturing these lesions. The study does suffer from a number of limitations. Working with preinvasive lung adenocarcinoma is inherently challenging. Unlike precursors to proximal squamous cell carcinomas, which occur in the airways and can be sampled repeatedly by bronchoscopy, these lesions are distal and only identified histologically following lung resection. Hence we cannot truly know

their clinical course; we cannot know whether, if left in situ, they would have undergone malignant transformation or – as happens in precursors to squamous lesions(11) – some may remain static or even spontaneously regress under selective pressure from immune surveillance. The technical approach is relatively limited, performing only targeted sequencing of 347 common cancer genes on single-region tumour samples. Whilst certainly informative, many recent studies of advanced and preinvasive cancer have moved beyond this technology, for example by sequencing whole-genome or whole-exome(12), increasing power to detect rare mutations and resolve copy number changes. Other studies integrate multiple omics strategies such as transcriptomic or epigenetic data(8), assess clonality with multi-region profiling(5), or assess the microenvironment alongside the profiled tumor(11, 13). Detailing these missing areas will also likely impact patient outcomes. Finally, this study does suffer from underpowering, including as it does just 102 samples, less than half of which are the preinvasive AIS/MIA lesions of interest. Alveola atypical hyperplasia, a presumed precursor of AIS, was not studied. Indeed, given the extensive genomic changes found in AIS/MIA, to truly understand early carcinogenesis future studies must consider

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looking back to earlier preinvasive lesions, and even to the “normal” airways of smokers, as has been done in other tissues(14).

This said, this study presents one of the largest cohorts published to date of preinvasive lung adenocarcinoma, a rare disease state which is of great scientific interest given what it can teach us about cancer development. Several putative pathways for carcinogenesis are identified providing candidates for experimental validation, and the implications for screening, diagnosis and detection are significant. By stepping backwards from invasive cancer into the earliest stages of carcinogenesis, this study represents an important step forwards in our understanding of lung cancer evolution.

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