On the Origin of Lung Cancers

It is widely accepted that early diagnosis is crucial for improving outcomes in lung cancer, which is currently the leading cause of cancer-related death in the United States (1). Computed tomography screening studies have demonstrated remarkable mortality benefits through a stage shift at diagnosis (2). Yet, our understanding of early carcinogenesis remains poor, and 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 heterogeneous within themselves (5). This complexity makes the identification of biomarkers and effective therapeutic targets extremely challenging; inhibitors of EGFR and ALK are effective, but although 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 occur in the lung, beginning with ADC *in situ* (AIS) followed by minimally invasive ADC (MIA). In a study presented in this issue of the *Journal*, Qian and colleagues (pp. 697–706) performed genomic sequencing on 21 AISs, 27 MIAs, and 54 invasive ADCs obtained from lung resections to identify early changes leading to cancerous transformation (7). Interestingly, these early lesions already displayed extensive molecular changes. Although the mutation burden was higher in ADCs, driver mutations and copy number changes were identified in AISs and MIAs, and heterogeneity was 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 that is mirrored in preinvasive studies of squamous lung cancers (8).

The authors applied a number of methods to tease out biological signals specific to early disease. They identified 21 genes that were significantly mutated across histologies, several of which showed a trend toward more mutations in more advanced disease. Copy number losses were more common in AISs/MIAs, and gains were more common in ADCs. An analysis of mutational signatures demonstrated enrichment of a DNA mismatch-repair–associated signature. This was a surprising finding, as ADCs tend to be dominated by smoking-related signature 4 mutations (9) (although this finding may have been skewed by the targeted sequencing approach used). Again, however, it was not possible to differentiate histological stages by their mutational signatures. Perhaps most intriguingly, the authors used a computational approach called Pipeline for Cancer Inference to compare mutations across successive histological subtypes in an effort to identify causative mutations. This analysis highlighted several putative early events, such as EPPK, KMT2C, and NOTCH3 mutation. This model generates several coherent hypotheses with clear clinical implications: understanding the sequencing of mutations in this way might allow effective development of therapies targeted toward the earlier changes, potentially arresting cancer development. In addition, as technologies for detecting mutations in circulating tumor DNA mature (10), it may become possible to detect these more-frequent early changes in blood samples, providing a powerful noninvasive screening tool. However, the small number of samples precludes us from drawing conclusions with any statistical certainty, and the study stops short of experimentally validating these findings.

Alongside these biological analyses, the authors sought to identify genetic signatures in these early lesions predictive of future survival. They found a five-gene signature associated with poor survival and a three-gene signature associated with improved survival, irrespective of histology. The authors suggest that such signatures may represent critical early driver events promoting tumor progression, although they lack validation in a larger cancer cohort. These results may have relevance in the growing field of computed tomography screening. With rapidly increasing numbers of early-stage ADC diagnoses, molecular biomarkers that can be used to stratify indolent versus aggressive disease could lead to improved patient pathways, for example, as indicators for adjuvant chemotherapy or appropriate follow-up protocols. On the population scale, even small improvements in screening pathways could potentially have a major impact.

To our knowledge, this is the largest study of its kind regarding precancerous AIS/MIA lesions, and the authors are to be applauded for their tenacity in making what were surely painstaking efforts to identify and capture these lesions. The study does suffer from a number of limitations, however. Working with preinvasive lung ADCs 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 can only be identified histologically after lung resection. Hence, we cannot truly know their clinical course—we cannot know whether, if left *in situ*, they would have undergone a malignant transformation, or, as happens in precursors to squamous lesions (11), some would have remained static or even spontaneously regressed under selective pressure from immune surveillance. The authors used a relatively limited technical approach and performed targeted sequencing of only 347 common cancer genes in single-region tumor samples. Although the results are certainly informative, many recent studies of advanced and preinvasive cancers have moved beyond this technology, for example, by using whole-genome or whole-exome sequencing (12) and thus increasing the power to detect rare mutations and resolve copy number changes. Other studies have integrated multiomics strategies, such as examining transcriptomic and epigenetic data (8), assessing clonality by multiregion profiling (5), and assessing the microenvironment alongside the profiled tumor (11, 13). Such

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detailed information will also likely impact patient outcomes. Finally, this study suffers from underpowering, as it includes just 102 samples, less than half of which are the preinvasive AIS/MIA lesions of interest. Atypical alveolar 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 looking back to earlier preinvasive lesions, and even to the "normal" airways of smokers, as has been done in other tissues (14).

Nevertheless, this study presents one of the largest cohorts published to date of preinvasive lung ADC, a rare disease state that 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 backward from invasive cancer into the earliest stages of carcinogenesis, this study represents an important step forward in our understanding of lung cancer evolution. ■

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An Event-driven Trial for Oral Treprostinil
Progress but Not the Holy Grail

Treatment of pulmonary arterial hypertension (PAH) with prostacyclin pathway agents is widely perceived among providers to be the most efficacious treatment compared with treatments acting via other implicated disease pathways such as nitric oxide–cyclic GMP and endothelin. In 1995, intravenous epoprostenol was the first specific PAH therapy approved by the U.S. Food and Drug Administration (FDA), based on a randomized controlled trial demonstrating improvement not only in 6-minute-walk distance (6MWD) but also in mortality compared with controls (1). In 2002, subcutaneous treprostinil (TRE), a prostacyclin analog with a considerably longer half-life (approximately 4 h) than epoprostenol (approximately 6 min), was approved on the basis of a small (16 m), but statistically significant, improvement in 6MWD compared with controls (2). Intravenous TRE was approved in 2004 on the basis of uncontrolled trials showing improved 6MWD in patients started *de novo* on intravenous TRE (3) and maintenance of benefit in patients switched from epoprostenol to TRE (4).

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