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Sequencing Lung Cancer's Sequence

Non–small cell lung cancers (NSCLCs) are becoming increasingly diagnosed at early stage as the diagnostic modalities for detecting small lung lesions have improved in quality over time and the implementation of screening has increased across the world. Although this is good news for patients, we still face the challenge of understanding whether we can push the envelope further and detect and eradicate tumors before they are evident on diagnostic imaging studies. In breast cancer, recent research from Hosseini and colleagues (1) and Harper and colleagues (2) using murine models and analysis of human blood specimens indicates that circulating tumor cells can disseminate before development of a clinically detectable primary tumor. Presumably, these cells derive from microscopic tumors that are clinically silent because of dominant dormancy pathways and/or because of effective immune response. In this issue of the *Journal*, Kadara and colleagues (pp. 742–750) present a comprehensive deep sequencing analysis of tumor and nonmalignant airway epithelium specimens from 48 patients with cancer to examine the sequence of the sequence of spatial mutations in the lung (3). This work sheds important light on molecular and genetic processes involved in lung carcinogenesis, especially during an early phase of its evolution.

More comprehensive genomic analyses have been conducted for a similar biological context, Barrett's esophagus, which is thought to be a premalignant precursor lesion for esophageal adenocarcinoma. These studies showed that nondysplastic metaplastic Barrett's lesions can harbor mutations commonly observed in esophageal adenocarcinomas; however, phylogenetic analyses of multiple lesions of Barrett's esophagus and esophageal adenocarcinomas revealed distinct genomic alterations patterns suggestive of parallel carcinogenic progression (4).

In the hematopoietic system, comprehensive genomic analyses of blood samples collected from a population without hematopoietic malignancy have revealed clonal mutations in specific genes that are frequently observed at a relatively high prevalence in leukemia (5). Although the vast majority of those cases do not progress to

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leukemia, the patients with clonal hematopoiesis of indeterminate potential, or CHIP, do develop overt malignancy at the rate of 0.5–1% per year, suggesting a biological state as a premalignant precursor. Longitudinal analyses of myelodysplastic syndromes suggest that overt clones can arise from nonclonal cell population even in the presence of CHIP (6). It is important to note that CHIP is not a hyperplastic or dysplastic state, as their hematopoiesis is functionally normal. Therefore, the prevalence of CHIP, recognized as a result of advancement in genomic technologies, raises the question as to whether clonal expansion serving as a precursor to malignancy similarly occurs in solid tissues with normal gross appearance.

The study by Kadara and colleagues describes targeted genomic analyses of the somatic mutational landscape on cancer-panel genes and allelic imbalance of the normal airway epithelium in patients with early-stage NSCLC (3). The study uniquely uses brushing samples from distant airway and nasal epithelia, in addition to multiple normal-appearing airways adjacent to NSCLCs. The analysis identified somatic mutations in adjacent normal-appearing airways in the majority of cases at a lower mutation burden of 1.5 mutations per megabase. The study design provides a sufficient sequence depth to allow for detection of mutations at a low variant allele frequency, predominantly reflecting a proportion, or clonality, of the mutant cells in a given specimen. The variant allele frequency was observed at a lower level in adjacent airways with decreasing order by distance from the tumor. The analyses on allelic imbalance provided more robust relationships between tumor genomes and cancer field genomes by identifying shared chromosomal events in decreasing order by distance from the tumor. These results are consistent with the notion of field cancerization and increased clonal mosaicism in the tumor area. Moreover, some of the mutations identified in adjacent normal-appearing airways were not shared by the matched tumor samples, suggesting clonal heterogeneity in the cancer field.

Perhaps the most significant finding in this study is the identification of somatic mutations of the cancer-panel genes in distant nonmalignant airways in eight of 47 patients. These mutations include recurrently mutated genes in NSCLCs, namely, *RB1*, *RET*, *TSHR*, and *AKT1*. Because these samples were taken from uninvolved normal airways, and because these mutations are callable with default thresholds even at a very high sequence depth, the data suggest that unrelated clonal airway epithelial regeneration, but not hyperplasia, occurs in the uninvolved airways. Whether these events are enriched in patients with lung cancers under a broader definition of field cancerization effect, or they are more frequent events than previously thought that can be observed in any population exposed to carcinogens, similar to CHIP, is an intriguing question.

The study by Kadara and colleagues provides additional insight into a classic cancer genetics theory. The study identified shared somatic alterations between adjacent airway and tumor with additional somatic event on the same genomic region, effectively enriching an oncogenic allele or losing both alleles of a tumor suppressor only in the tumor, supporting the original Knudson's two-hit hypothesis (3). However, if these mutations are observed in normal-appearing airways without hyperplasia or dysplasia, presumably because of insufficiency by one hit for overt proliferative phenotype, why are these first events not observed at a similar frequency in the distant or uninvolved airways? This may be explained by field cancerization effects demonstrated by the phylogenetic analyses. The results from this study imply that carcinogenic insults occur in a relatively confined spatial unit in the airway and that multiple consecutive genetic alterations happen in a burst or in a long period of time if the carcinogen persists over time in that confined space.

Kadara and colleagues relied on control DNA from individual subjects' hematopoietic cells to develop a study design such that each patient served as his or her own control. This is an effective strategy, yet the study lacks control subjects without cancer. The analysis of smoking and nonsmoking subjects without cancer would add important insights into the specificity of the findings. Kras and BRAF are among driver mutations that have been detected in the lungs of individuals without cancer (7, 8), suggesting that a comprehensive survey of individuals without cancer will identify these and other alterations; the challenge will be to better understand the biology and clinical significance of these findings.

Overall, Kadara and colleagues conducted the first comprehensive genomic analysis on adjacent and distant airways from the patients with NSCLC, which adds important data to genomic repositories and provides strong evidence for field carcinogenesis at the genomic level and potential evidence for clonal epithelial regeneration. Further investigation of the genome-scale mutational landscape may provide a more complete view of cancer evolution of NSCLC. In addition, genomic analyses at a significant sequence depth or even single-cell genomic DNA analyses on airways from patients without cancer may provide new insights into how somatic alteration landscapes are built in the airways and which of these alterations could potentially impart advantage for clonal expansion and, most important, identify opportunities for treatment intervention. ■

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Bronchopulmonary Dysplasia: A Continuum of Lung Disease from the Fetus to the Adult

The definitions of bronchopulmonary dysplasia (BPD), the lung injury that results from high oxygen exposure and mechanical ventilation of preterm infants, which was first described over 50 years ago by Northway and colleagues (1), have evolved to include very premature infants and changing care strategies (2). Each new definition was sequentially viewed as inadequate for the varied needs of epidemiology, clinical care, pathophysiology, and outcome predictions for evaluating new treatments. Dissatisfaction with Shennan and colleagues' 1988 definition of oxygen exposure at 36 weeks gestation and the 2000 NIH workshop definition of a graded severity of disease has resulted in a flurry of reports and editorials seeking to establish an ideal definition of BPD (2–5).

In a study presented in this issue of the *Journal*, Jensen and colleagues (pp. 751–759) used an evidence-based approach to determine which BPD definition best predicts respiratory and neurodevelopmental outcomes at 18–24 months (6). They parsed the elements of the NIH workshop definition and included newer care strategies that confound previous definitions. These elements included low- and high-flow nasal cannulas, levels of invasive respiratory support, and specified periods of oxygen support. They used a contemporary Eunice Kennedy Shriver National Institute of Child Health and Human Development neonatal research network data set of 2,677 infants to test the predictability of 18 definitions for death or serious respiratory morbidity (tracheostomy, initial hospitalization at >50 wk postmenstrual age, oxygen or respiratory support, or two or more respiratory hospitalizations) at follow-up at 18–26 months. The surprising result was that a graded severity of BPD based only on respiratory support at 36 weeks best predicted both respiratory and neurodevelopmental outcomes. This is surprising because oxygen use was the core variable for all previous definitions of BPD. The analysis has merit because of its statistical rigor resulting from the use of a large and relevant patient population. But the claim for the "best" definition with a C-statistic of 0.785 must be tempered by the five next best definitions with C-statistics of 0.784–0.780. The C-statistic for the worst definition was quite high at 0.741. Similarly predictive accuracy for neurodevelopmental impartment ranged narrowly from 0.747 to 0.725.

The cohort established as part of the Prematurity and Respiratory Outcomes Program has also been used to assess a composite measure of respiratory morbidity severity over the first year after very preterm birth with regard to outcome predictions (7). The aggregate of primarily nonpulmonary perinatal associations of male sex, intrauterine growth restriction, maternal smoking, race/ethnicity, intubation at birth, and public insurance was equivalent to BPD for the prediction of 1-year respiratory outcomes. When looked at from 36,000 feet, these attempts to predict outcomes for very preterm infants are all reasonably good, but not much different from each other. There are many pathways to BPD, including perinatal variables and postnatal adverse exposures that range from oxygen use and mechanical ventilation to necrotizing enterocolitis and sepsis. They all contribute to whatever BPD diagnosis one chooses and to adverse outcomes. The oxygen use and ventilatory support elements of a BPD diagnosis are simply linked fellow travelers—both physiologically and statistically.

A further consideration is the more recent realization that the lung injuries that result in BPD are not uniform. A recent report in the *Journal* by Tingay and colleagues (8) demonstrates that even gentle attempts to inflate the very preterm and surfactant-deficient lung cause nonuniform injury. Recent imaging studies using computed tomography or magnetic resonance imaging have demonstrated the extreme variability of parenchymal lung injury. Some infants have primarily emphysema and cysts, whereas others have fibrous interstitial opacities and mosaic lung attenuation or mixtures of abnormalities (9). Severe BPD also includes infants with glottic injury from endotracheal tubes, tracheal and bronchial malacia, control-of-breathing abnormalities, and pulmonary hypertension (10, 11).

A substantial criticism of all these definitions is that the elements of the definitions are simply therapies for BPD (12). In a recent report in the *Journal*, Svedenkrans and colleagues proposed the use of a measurement of gas exchange as a continuous indicator of disease severity (13). Oxygenation status is measured as oxygen saturation versus the oxygen pressure curve. Impaired oxygenation is indicated by a shift of the saturation curve from normal, by ventilation/perfusion, and by calculating shunt. For preterm infants with mild BPD, the complete test requires the use of oxygen concentrations of <21%, but a single measurement with a saturation of 86–95% at a known oxygen concentration may suffice. Of course, this test uses oxygenation only, with no assessment of ventilatory support.

Another criticism of current definitions that assess BPD at 36 weeks gestation is that the infant is still premature. However, Isayama and colleagues (14) demonstrated that an assessment at any week from 36 weeks to 44 weeks showed very similar risks for adverse respiratory or neurodevelopmental outcomes.

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