Review

The Airway Transcriptome as a Biomarker for Early Lung Cancer Detection

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Abstract

Lung cancer remains the leading cause of cancer-related death due to its advanced stage at diagnosis. Early detection of lung cancer can be improved by better defining who should be screened radiographically and determining which imaging-detected pulmonary nodules are malignant. Gene expression biomarkers measured in normal-appearing airway epithelium provide an opportunity to use lung cancer–associated molecular changes in this tissue for early detection of lung cancer. Molecular changes in the airway may result from an etiologic field of injury and/or field cancerization. The etiologic field of injury reflects the aberrant physiologic response to carcinogen exposure that creates a susceptible microenvironment for cancer initiation. In contrast, field cancerization reflects effects of "first-hit" mutations in a clone of cells from which the tumor ultimately arises or the effects of the tumor on the surrounding tissue. These fields might have value both for assessing lung cancer risk and diagnosis. Cancer-associated gene expression changes in the bronchial airway have recently been used to develop and validate a 23-gene classifier that improves the diagnostic yield of bronchoscopy for lung cancer among intermediate-risk patients. Recent studies have demonstrated that these lung cancer–related gene expression changes extend to nasal epithelial cells that can be sampled noninvasively. While the bronchial gene expression biomarker is being adopted clinically, further work is necessary to explore the potential clinical utility of gene expression profiling in the nasal epithelium for lung cancer diagnosis, lung cancer risk assessment, and precision medicine for lung cancer treatment and chemoprevention. *Clin Cancer Res; 24(13); 2984–92. ©2018 AACR.*

Introduction

Lung cancer is the leading cause of cancer-related death both in the United States and the world, with an estimated 222,500 new lung cancer cases and 155,870 deaths in the United States in 2017 (1). Lung cancer is most often lethal, with a 5-year survival rate, which was only 18% between 2005 and 2011 (1). Clearly, the improvements in survival realized in other malignancies have yet to be achieved in lung cancer. This is due, in part, to our frequent failure to detect lung cancer at its earliest and potentially curable stage; less than 20% of patients with lung cancer are diagnosed at stage 1 (2).

Although 90% of lung cancer cases in the United States occur in individuals with a history of tobacco smoke exposure, only 15% to 20% of lifetime smokers will develop lung cancer, and we are currently limited in our ability to identify those at highest risk (3–5). Over the past 5 years, the early detection landscape for lung cancer has been transformed by the results of the National Lung Screening Trial (NLST), which compared low radiation dose helical CT with chest radiography (6). They reported a 20.3% reduction in lung cancer mortality in the CT arm as well as a statistically significant reduction of 6.9% in all-cause mortality.

Currently, annual CT screening has been recommended for individuals at highest risk for developing lung cancer (National Comprehensive Cancer Network Criteria: ages 55–74 years old with ≥30 pack-years smoking history; ref. 7). However, over 70% of lung cancer cases in the United States occur in individuals who fall outside of the NLST criteria (8), suggesting that better methods are needed to identify those who ultimately will benefit from CT screening.

Beyond identifying those who should be screened for lung cancer, there is also the challenge of identifying which abnormalities detected by CT represent lung cancer. It is estimated that 1.5 million indeterminate pulmonary nodules are found in the United States annually, either incidentally or on screening chest CT, representing a significant diagnostic dilemma given that the overwhelming majority of these nodules will ultimately be determined to be benign (9). Recent studies have found that many of these subjects undergo unnecessary invasive biopsies that incur significant risks and cost (10, 11). There is thus an urgent need to develop approaches that can better discriminate benign versus malignant pulmonary nodules in this setting to aid decision-making with regard to appropriate follow-up.

Addressing these unmet needs would improve the early detection of lung cancer and thereby have the potential to decrease lung cancer mortality. Significant recent efforts have been made to develop molecular biomarkers for lung cancer detection primarily in the setting of screen-detected or incidental lung nodules using a variety of analytes (genomic, transcriptomic, epigenomic, and proteomic) measured in a number of different biospecimens (12–30). This review focuses on a promising approach using gene expression profiling of the relatively accessible airway epithelium as a window for sampling carcinogen-exposed tissue that could reflect the molecular signatures of cancer-associated processes. The use of genome-wide gene expression approaches as part of the

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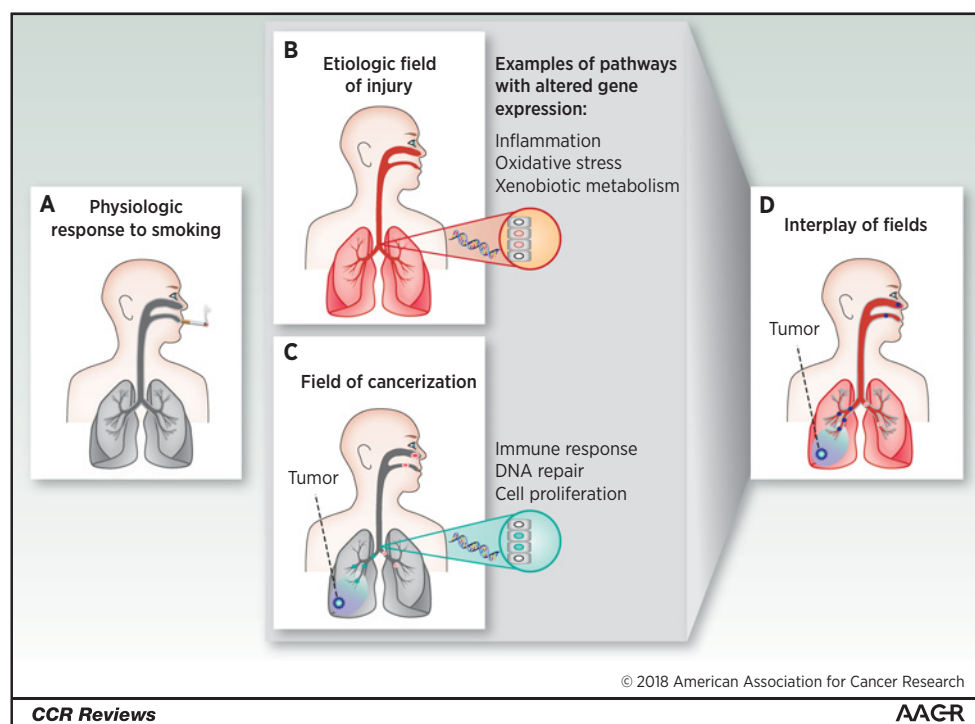
Figure 1.
Molecular alterations found throughout the respiratory tract can be attributed to field effects. **A,** The physiologic effect of smoking occurs predictably, commonly, immediately, and largely reversibly. This field of injury consists of molecular alterations present throughout the respiratory tract upon exposure to tobacco smoke. **B,** The etiologic field of injury consists of processes at the molecular level that contribute to lung carcinogenesis. Molecular alterations related to inflammation, oxidative stress, and xenobiotic metabolism are among these processes that potentiate the establishment and expansion of cells harboring the genetic or epigenetic alterations that are directly required for tumorigenesis. **C,** Field cancerization refers to tumor-associated abnormalities (e.g., gene expression pathways related to immune response, DNA repair, and cell proliferation) in tumor-adjacent normal-appearing cells. These abnormalities may reflect clonal expansion of cells harboring cancer-initiating alterations that are insufficient, on their own, to lead to transformation or may be a consequence of the tumor on surrounding tissue. **D,** These fields are not mutually exclusive, and histologically normal-appearing tissue exhibiting cancer-associated alterations due to the etiologic field of injury and/or field cancerization can be leveraged for developing gene expression–based biomarkers for the early detection of lung cancer.

biomarker discovery process allows for a broad search for genes with cancer-associated gene expression for further biomarker development, and it can generate hypotheses as to the biological processes responsible for many of the observed gene expression differences. The utility of airway epithelium is based on two distinct, yet related, concepts: the "Etiologic Field of Injury" and "Field Cancerization" (Fig. 1). We will focus on reviewing potential mechanisms underlying these two "field effects" and the clinical application of airway gene expression for the early detection of lung cancer, and highlight some of the critical challenges and opportunities that lie ahead for this field.

Field of Injury

Tobacco smoke induces molecular alterations in epithelial cells throughout the respiratory tract that are exposed to its carcinogens. These molecular alterations represent a smoking-related field of injury that reflects a complex physiologic response to toxins and mechanical injury (31) characterized by increased inflammation, oxidative stress, and xenobiotic metabolism (32). Several groups have characterized the gene expression correlates of this physiologic response. Hackett and colleagues demonstrated upregulation of antioxidant-related genes in active smoker epithelium compared with nonsmokers (33) that

generally fell into one of four categories: catalase/superoxide dismutase, glutathione metabolism (*GPX2* and *GSR*), redox balance (*ADH7* and *AKR1C3*), and the pentose phosphate cycle (*TALDO1* and *PGD*). Furthermore, they identified subgroups of smokers who differed in their expression levels of these antioxidant-related genes. Similarly, Spira and colleagues used genome-wide gene expression profiling to broadly characterize alterations in healthy current smokers and identified smoking-associated differences in genes involved in xenobiotic metabolism (*CYP1B1* and *DBDD*), oxidative stress (*GPX2* and *ALDH3A1*), and immune response (34). Subsequent analysis demonstrated that although the expression of the vast majority of these genes returns to normal after smoking cessation, a number of genes (e.g., *CEACAM5*, *SULF1*, and *UPK1B*) have persistently altered expression for years after smoking cessation (35). Chari and colleagues similarly found that the expression levels of many genes altered by tobacco smoking return to baseline levels after smoking cessation, including genes related to xenobiotic metabolism (e.g., *ALDH3A1*, *ALDH7*, *AKR1C2*, *CYP1B1*, and *NQO1*), whereas others (e.g., *GSK3B* and *COX2*) remain persistently altered (36). Overall, these studies suggest that the physiologic response to tobacco smoke occurs commonly, immediately, and largely reversibly in individuals who smoke. As such, these smoking-associated changes are not directly useful for assessing the

presence of lung cancer, and are distinct from those changes due to an etiologic field of injury associated with lung carcinogenesis.

However, the identification of persistent effects of smoking on airway epithelium is intriguing, as it may relate to the persistent risk of many smoking-associated diseases (including lung cancer) among former smokers. Furthermore, variability in the response to tobacco smoke exposure and variability in the injury it induces (e.g., immune modulation, oxidative damage, and microbiome) might contribute to differences in risk for lung cancer (37). These processes reflect what has been referred to as an etiologic field of injury. The etiologic field of injury reflects the processes triggered by environmental exposures that potentiate the molecular alterations (e.g., genetic and epigenetic changes) that directly lead to cancer development (38). Markers of the etiologic field of injury may therefore be used to assess lung cancer risk or detect lung cancer. As one example, Blomquist and colleagues measured bronchial airway epithelium expression of 14 genes related to antioxidant and DNA repair pathways among smokers with suspect lung cancer, including *CEBPG*, *E2F1*, *ERCC4*, *ERCC5*, *GPX1*, and *GPX3* (39). Combining the levels of these genes into a single score resulted in a biomarker that could reliably differentiate individuals with and without lung cancer [80% accuracy, odds ratio (OR) = 12.8]. This biomarker is currently being validated as a screening tool in the Lung Cancer Risk Test trial, a prospective cohort study evaluating the clinical validity and utility of a 15-gene classifier from bronchial epithelial cells of individuals with and without screen-detected lung cancer (40). Leveraging the cancer-associated transcriptomic field of injury for the assessment of lung cancer risk and cancer detection could improve our ability to detect lung cancer earlier in the disease process.

Field Cancerization

Field cancerization, a concept first introduced by Slaughter and colleagues in the context of oral cancers, suggests that both the neoplasm and adjacent grossly normal–appearing tissue share some histologic abnormalities, such as epithelial hyperplasia and hyperkeratinization (41). This concept has evolved to broadly reflect tumor-related changes in adjacent normal tissue, including molecular alterations. These tumor-related changes can either be due to "first-hit" genetic alterations in a clone of cells from which the tumor ultimately develops or effects of the tumor on the local microenvironment. As an example of the former, Nelson and colleagues found K-ras mutations in both non–small cell lung cancer (NSCLC) tumor tissue and adjacent normal tissue (42). Similarly, Franklin and colleagues analyzed DNA from microdissected airway epithelial cells of sites along the entire tracheobronchial tree of an autopsy specimen in a patient with widespread dysplastic lesions and identified widespread p53 mutations despite the absence of an overt carcinoma. This suggests that "field carcinogenesis" (or at least the expansion of clones of cells harboring cancer-associated mutations), in which a single abnormal progenitor clone may expand to populate broad areas of the bronchial mucosa, can occur prior to tumor development (43). This hypothesis is supported by several observations. In 1997, Wistuba and colleagues identified genetic changes (i.e., loss of heterozygosity, allelic loss, and microsatellite alterations) in normal and abnormal bronchial epithelium from current smokers compared with former and never smokers (44). Similarly, Mao and colleagues investigated microsatellite DNA from airway

biopsies of chronic smokers and identified genetic alterations in putative tumor suppressor genes (45). Furthermore, Tang and colleagues found EGFR mutations in histologically normal respiratory epithelium that were concordant with EGFR mutations found within the actual adenocarcinoma tissue (46). Also, PI3K pathway activation has been demonstrated in the cytologically normal bronchial airway epithelium of smokers with lung cancer (including patients with adenocarcinoma or squamous cell carcinoma) and smokers with airway dysplasia, a process thought to reflect an early event in tumorigenesis (47).

Several other studies provide evidence of field cancerization. Kadara and colleagues conducted serial expression profiling on the airway epithelium from patients with lung cancer following resection and found that gene expression patterns in airway epithelium adjacent to previously resected tumors persisted for at least 3 years after definitive surgery. These differences could reflect field cancerization arising from somatic mutations in the adjacent field, the persistence of the etiologic field even after tumor resection, or some combination of the two (48). However, this study did not include presurgical baseline specimens, and it is difficult to determine whether the genes were altered due to the malignancy or in response to the surgery (49). A more definitive demonstration of the impact of field cancerization on gene expression comes from another study by Kadara and colleagues that performed gene expression profiling on resected lung tumor tissue, matched uninvolved normal lung tissue, and adjacent airways at varying distances from the tumor (50). They found a number of genes with tumor-associated changes in gene expression (e.g., *TGFBR2*, *VIPR1*, *NETO2*, and *LAPTM4B*) that are similarly changed in the matched airway samples. For some of these genes, the extent of altered airway expression was greater in more tumor-proximal airways (presumably reflecting field cancerization), whereas for other genes, airway expression was consistently altered regardless of tumor proximity (presumably reflecting the etiologic field of injury). These observations illustrate a relationship between molecular changes present in airway epithelium and the tumor and provide a basis for using less invasive tissue sampling for lung cancer detection remote to the actual tumor. Key studies related to the etiologic field of injury and field cancerization are summarized in Table 1.

Bronchial Airway Gene Expression Biomarker for Lung Cancer Detection

The etiologic field of injury and field cancerization are potentially relevant for the diagnosis of lung cancer as they suggest the opportunity for detecting lung cancer via abnormalities in more accessible nontumor tissue. This could have considerable clinical utility in settings where the probability of lung cancer is too low to justify the risk of directly assessing the suspicious lesion. In the current diagnostic algorithm for indeterminate pulmonary nodules, the American College of Chest Physicians (ACCP) recommends that clinicians estimate the pretest probability of malignancy either qualitatively using their clinical judgment and/or quantitatively using a validated model (3, 4, 51, 52). In those individuals with a lower pretest probability, surveillance with serial CT scans may be appropriate. In those individuals with a high pretest probability, further workup is indicated and typically involves direct pathologic assessment of lesion tissue obtained via bronchoscopy, needle biopsy, or surgical resection (Fig. 2A). Individuals with an intermediate pretest probability often pose

**Table 1.** Summary of key findings from select articles in the field

Article	Study population	Tissue	Analytes	Significant genes/pathways	Major findings
Slaughter et al. 1953 (41)	Oral cancer cases	Resected oral tumor	Histology	Not applicable	Nonmalignant epithelium surrounding tumors is abnormal.
Blomquist et al. 2009 (39)	Ever smokers undergoing bronchoscopy for suspect lung cancer	Bronchial airway epithelial cells via bronchoscopy	mRNA	Antioxidant and DNA repair pathways (*CAT, CEBPG, E2F1, ERCC4, ERCC5, GPX1, GPX3, GSTM3, GSTP1, GSTT1, GSTZ1, MGST1, SOD1,* and *XRCC1*)	Variability in the expression of antioxidant, DNA repair, and transcription factor genes in normal airway epithelium is associated with the presence of lung cancer.
Spira et al. 2007 (54)	Ever smokers undergoing bronchoscopy for suspect lung cancer	Bronchial airway epithelial cells via bronchoscopy	mRNA	Inflammation (*PLA2G4A, DEFB1, C6, FCGR3A, IL8,* and *SERPINA1*) Cell cycle (*PPBP, DUSP6,* and *TOB1*) Antioxidant (*BACH2* and *DUOX1*)	An 80-gene classifier derived from cytologically normal large airways of ever smokers can distinguish lung cancer vs. benign disease.
Franklin et al. 1997 (43)	Lung premaligancy	Bronchial epithelial cells at multiple sites during autopsy	DNA	p53	A p53 tumor suppressor gene mutation was identified at several sites throughout the airway epithelium that exhibited only squamous metaplasia and mild to moderate atypia by histology.
Tang et al. 2005 (46)	Lung cancer cases	Resected lung tumor	DNA	EGFR mutations	EGFR mutations are found in normal epithelium of patients with EGFR-positive lung cancer.
Kadara et al. 2014 (50)	Lung cancer cases	Resected lung tumor	mRNA	*TGFBR2, VIPR1, NETO2,* and *LAPTM4B*	Histologically normal-appearing adjacent airway exhibits a gradient of tumor-associated gene expression that decreases with increasing distance from the tumor as well as a set of genes that are consistently altered regardless of tumor proximity.
Silvestri et al. 2015 (57)	Ever smokers undergoing bronchoscopy for suspect lung cancer	Bronchial airway epithelial cells via bronchoscopy	mRNA	Immune response (*BST1* and *CD177*) Tumor related (*TSPAN2, NOVA1, CDR1,* and *MCAM*) Xenobiotic metabolism (*EPHX3*) Cell cycle (*SDC2, NKX3-1,* and *CGREF1*) Others (*RPS4Y1, SLC7A11, CLND10, TKT, RUNX1T1, AKR1C2, CD177.2, ATP12A, GABBR1, CLND22, LYPD2, MIA,* and *RNF150*)	A 23-gene expression classifier measured in cytologically normal mainstem bronchus improved the diagnostic performance of bronchoscopy for the detection of lung cancer.

a diagnostic challenge, although current guidelines recommend functional imaging (e.g., PET) to help direct further management, such as invasive biopsy versus surveillance imaging. Bronchoscopy to collect cells from the lesion is often used in the setting of intermediate-risk indeterminate pulmonary nodules, as it is only minimally invasive. However it is challenging to acquire lesion tissue from peripheral nodules by bronchoscopy, and its sensitivity for lung cancer is estimated to be 50% to 60% (53). When bronchoscopy is nondiagnostic, molecular biomarkers that leverage cancer-associated abnormalities in histologically normal epithelium provide an opportunity to detect malignant nodules with increased sensitivity. Furthermore, molecular changes in airway epithelium could also play a role in lung cancer risk assessment, as some might occur at early stages of lung tumorigenesis before an incipient tumor is readily detectable by imaging (Fig. 2B).

On the basis of both the etiologic field of injury and field cancerization, there has been considerable effort to determine if there are gene expression alterations in normal-appearing bronchial airway epithelium that might have clinical utility for lung cancer detection. Enthusiasm for this approach stems from an early study by Spira and colleagues that profiled gene expression in bronchial airway epithelium from the mainstem bronchus of current and former smokers undergoing bronchoscopy for suspect lung cancer using microarrays (54). These patients were then followed prospectively until a definitive diagnosis of cancer or not was established.

Spira and colleagues identified a number of gene expression differences between patients with and without cancer in normal-appearing airway cells from the mainstem bronchus, establishing the existence of a cancer-associated gene expression signature in the proximal airway (54). In support of the biological relevance of these gene expression changes, Kadara and colleagues showed that these genes are enriched for genes whose expression is altered both in the tumor and airways regardless of the distance from the tumor (48). Beyond demonstrating cancer-associated gene expression alterations in the mainstem bronchus, Spira and colleagues also demonstrated that they could function as a biomarker that achieved high sensitivity and negative predictive value in two independent test sets (54). As these airway epithelial biospecimens were collected during a clinical procedure for suspect lung cancer that has modest sensitivity, the high sensitivity of the bronchial airway genomic biomarker suggested the potential clinical utility of combining the biomarker with routine bronchoscopy to serve as a rule-out test for lung cancer. Supporting this potential application, Spira and colleagues found that the performance of the gene expression biomarker is independent of whether bronchoscopy resulted in a diagnosis, and Beane and colleagues showed that the biomarker is independent of a number of clinical risk factors for lung cancer (55).

Key to the further refinement and validation of this approach were two large, multicenter clinical cohorts of current and former

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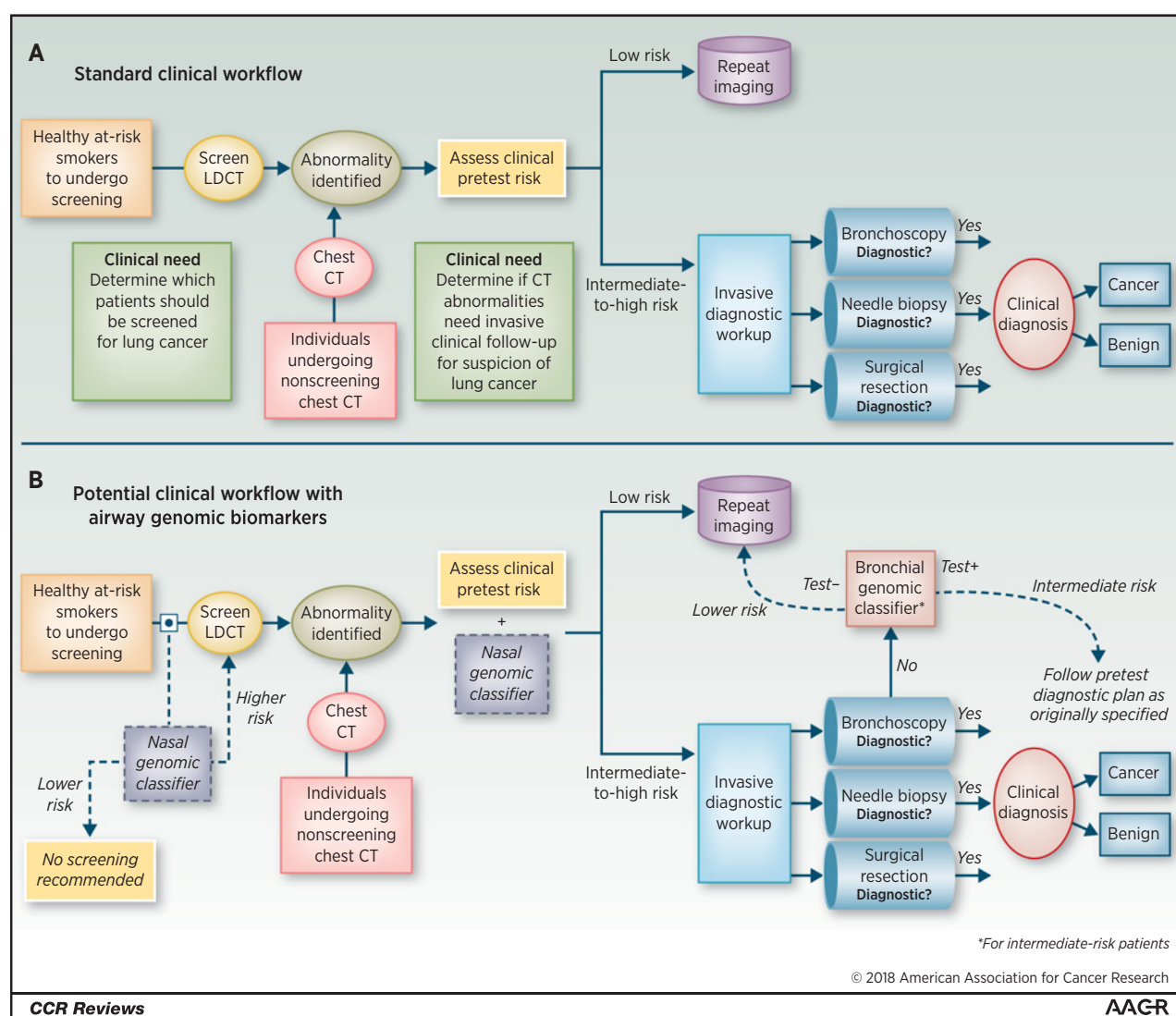
Figure 2.
Potential clinical applications of airway molecular biomarkers for the early detection of lung cancer. **A,** In current practice, pulmonary nodules are discovered either due to lung cancer screening based on clinical risk factors, as findings incidental to other imaging studies, or during the workup of symptomatic patients. In the current diagnostic algorithm for indeterminate pulmonary nodules established by the ACCP, based on the pretest probability of malignancy, patients determined to be low risk will typically undergo continued surveillance, whereas higher risk patients will undergo invasive testing. However, there is a clinical unmet need to better stratify those subjects at intermediate pretest risk into CT surveillance versus invasive biopsy as well as an unmet need to identify which subjects should undergo CT screening. **B,** Molecular biomarkers have the potential to impact the current diagnostic workflow of patients with indeterminate pulmonary nodules. Current biomarkers measured in histologically normal-appearing bronchial airway tissue can serve to identify patients with nondiagnostic bronchoscopies who are at low risk for lung cancer and can be followed with continued imaging surveillance. Future biomarkers measured in histologically normal-appearing nasal airway tissue could help identify at-risk individuals for CT-based lung cancer screening or direct diagnostic workup in individuals with suspect lung nodules who are not otherwise undergoing bronchoscopy. LDCT, low-dose computed tomography.

smokers undergoing bronchoscopy for suspect lung cancer: AEGIS1 and AEGIS2. These studies were similar in study design to the original study by Spira and colleagues and included collection of prediagnostic bronchial epithelial cells from the mainstem bronchus and prospective follow-up of patients for up to 1 year after bronchoscopy until a final diagnosis was obtained. Samples were profiled using a substantially different microarray platform, so a portion of the samples from AEGIS1 were used to derive a new 23-gene biomarker. Consistency with the prior study was demonstrated by showing that the new biomarker could

distinguish patients with and without lung cancer in the previous dataset from Spira and colleagues. The strategy used by Whitney and colleagues to develop the 23-gene biomarker is noteworthy in that in addition to including 17 genes with cancer-associated expression, they included genes that serve as surrogates of clinical risk factors, including sex, current smoking status, and cumulative tobacco exposure (56). These gene expression correlates were incorporated to capture the physiologic effects of these clinical factors that might more accurately reflect their effect on disease risk and minimize the effect of inaccurate patient self-report. For

the important risk factor of patient age, they were unable to identify a robust gene expression surrogate, so the clinically reported value is used directly in the calculation of the biomarker score. For determining predicted cancer status, they chose a score threshold that resulted in a sensitivity of 93% and specificity of 57% in the training set. In addition to demonstrating that this biomarker had similar performance in the original dataset from Spira and colleagues, it was subsequently validated by Silvestri and colleagues in two additional independent datasets: the remainder of the samples from AEGIS1 that were not used for biomarker development ($n = 298$) and all of the samples from AEGIS2 ($n = 341$; ref. 57). This study demonstrated that the combination of bronchoscopy with the gene expression classifier performed well (97% sensitivity) compared with bronchoscopy alone (75% sensitivity). The performance of the gene expression classifier is striking in those individuals with an inconclusive bronchoscopy and intermediate pretest physician-assessed risk of lung cancer (88% sensitivity and 91% negative predictive value).

These data suggest that combining the gene expression classifier with bronchoscopy has several potential benefits. By detecting most cancers (i.e., high sensitivity and high negative predictive value), a negative test result in patients with an intermediate pretest risk of lung cancer lowers the posttest probability of lung cancer sufficiently that repeat imaging could be pursued as a follow-up strategy rather than invasive diagnostic procedures (Fig. 2B), which may lower both cost and the risk of iatrogenic complications, including respiratory failure, bronchospasm, bleeding, or in the case of CT fine-needle aspiration, an over 20% rate of pneumothorax (58, 59). However, given the modest specificity and positive predictive value of the biomarker, this test cannot reliably "rule in" a diagnosis of cancer. Therefore, Silvestri and colleagues caution that a positive result should not be used to alter the decision between an invasive or conservative diagnostic approach. Moreover, in patients with a high pretest assessed risk of lung cancer, a negative biomarker result may not lower the posttest risk of lung cancer sufficiently to safely alter the pretest diagnostic plan. The authors noted several other limitations that narrow the intended-use population including exclusion of lifetime nonsmokers and individuals with a concurrent cancer or a prior history of lung cancer. It should also be noted that the biomarker does not incorporate a number of lung cancer risk factors that have been used in risk calculators such as comorbid chronic obstructive pulmonary disease (COPD) or emphysema, nodule fluorodeoxyglucose-PET (FDG-PET) avidity, size, location, spiculation, or texture; but these factors contribute to the physician's pretest risk assessment.

The genomic classifier developed and validated in the AEGIS trials has since been commercialized as the Percepta Bronchial Genomic Classifier, a Clinical Laboratory Improvement Amendments (CLIA)–based 23-gene genomic classifier. It is possible that this biomarker captures the effects of the etiologic field of injury, field cancerization, or some combination of the two. However, because these gene expression changes are readily detected in the airway epithelium, this biomarker may be used as a clinical decision-making tool in the evaluation of a subset of patients undergoing bronchoscopy due to suspect lung cancer. Clinicians are encouraged to follow a more conservative strategy of serial imaging in those bronchoscopy-negative patients with an intermediate pretest probability of disease whose Percepta test, with a high sensitivity and high negative predictive value, is negative; the clinical utility of the biomarker comes from reducing the burden of procedures in patients without cancer. A reanalysis of the AEGIS

data by Vachani and colleagues suggests that 50% of the invasive biopsies performed in AEGIS participants who were then determined not to have lung cancer could have been avoided by using Percepta negativity to reassign intermediate-risk bronchoscopy-negative patients to repeat imaging (60). Encouragingly, data from a decision impact study by Ferguson and colleagues based on a survey of pre- and posttest diagnostic management plans suggests the willingness of physicians to alter their management plans in intermediate-risk patients based on a negative Percepta result: The rate of recommended invasive procedures was 57% when the surveyed clinicians were blinded to the Percepta result but only 18% when they were unblinded (61). Finally, a recent study has provided an estimate of the cost-effectiveness of this diagnostic test (62).

Recent work has extended these lung cancer–associated transcriptomic alterations to miRNA, a class of small noncoding RNA that can regulate gene expression and protein translation. Previous work has demonstrated the effects of smoking on miRNA expression and the potential for these smoking-related differences in miRNA expression to regulate cigarette smoking–related gene expression (63–65). More recent work has identified four miRNA (miR-146a-5p, miR-324-5p, miR-223-3p, and miR-223-5p) with decreased expression in the bronchial airway of patients with lung cancer. The predicted mRNA targets of each of these miRNA are enriched among the genes most increased in the bronchial airway of patients with lung cancer, suggesting that these miRNA may be regulators of genes with cancer-associated expression patterns (66). Furthermore, of the four isoforms identified, incorporating miR-146a-5p into the 23-gene biomarker led to significantly improved performance, suggesting a potential benefit to a multiomic biomarker strategy.

Gene Expression Biomarkers for Lung Cancer in Extrathoracic Airway Epithelium

Although the bronchial genomic classifier may serve an important role in determining the clinical management of patients undergoing bronchoscopy for suspect lung cancer, there are patients with suspect lesions who do not undergo bronchoscopy (e.g., patients with small peripheral nodules), and the risks and costs associated with bronchoscopy preclude using it to establish cancer risk in the setting of broad lung cancer screening. One hypothesis that might provide an alternative approach is that the etiologic field of injury and/or field cancerization that give rise to the molecular signature of lung cancer detectable via bronchial airway epithelial gene expression might extend into the extrathoracic airway epithelium in the nose and mouth that can be collected less invasively.

Early work demonstrated that the majority of genes whose expression is altered in current smokers are altered similarly in bronchial and nasal epithelium (67), suggesting that if the lung cancer etiologic field effect results from the long-term consequences of the response to tobacco smoke, it might evolve similarly in bronchial and nasal epithelium. More recent work has directly addressed whether there is a gene expression signature of lung cancer in nasal epithelium. Perez-Rogers and colleagues profiled gene expression in nasal epithelium samples collected from patients in the AEGIS trials and identified a number of genes whose expression differed between patients with and without lung cancer (68). Moreover, there was a high degree of concordance between nasal and bronchial cancer–associated gene

expression, as determined using gene set enrichment analysis: Genes with either increased or decreased expression in nasal epithelium of patients with lung cancer were significantly enriched among the genes most significantly increased or decreased in bronchial epithelium of patients with cancer. These findings support the hypothesis that there is a common gene expression signature of lung cancer that extends from the bronchial epithelium into the nose. Samples from AEGIS1 were used to define a 30-gene biomarker that differed significantly between patients with and without lung cancer. When combined with known clinical risk factors and applied to patients from AEGIS2, the resulting clinico-genomic model performed better in identifying patients with and without lung cancer than a model containing only clinical risk factors, achieving an ROC curve AUC of 0.81, a sensitivity of 91%, and a specificity of 52%.

Although the study by Perez-Rogers and colleagues demonstrates the ability to detect lung cancer on the basis of nasal gene expression, considerable work remains to be done before a nasal biomarker could be used clinically. Importantly, the reported nasal classifier was derived in the context of patients undergoing bronchoscopy for suspicion of lung cancer. It is unclear how the classifier would perform in a setting where the prevalence of lung cancer is lower, which is the clinical context in which a nasal biomarker would have most utility relative to a bronchoscopy-based test. Furthermore, in the context of low disease prevalence, there may be more clinical utility for a test with high specificity that could trigger appropriate invasive follow-up (Fig. 2B). Additional work, especially in cohorts that more closely mirror the potential intended-use population(s) (e.g., at-risk individuals prior to screening and individuals with screen-detected indeterminate pulmonary nodules), will be necessary to (i) identify biomarkers and/or combinations of biomarkers and clinical factors that work well in these settings and (ii) determine intended uses based on the biomarker's performance in these settings.

Another site for minimally invasive biomarkers within the airway transcriptome is the mouth. Although this site is challenged by the degree of RNA degradation present in buccal epithelial cells obtained by scraping the cheek, recent studies suggest that there may be relevant gene expression signatures that can be measured at that site. Although smoking-related gene expression alterations in the buccal epithelium are less pronounced that those in the nose and bronchus, there is a common set of smoking-related gene expression changes across all three sites (69). Furthermore, Wang and colleagues recently profiled buccal gene expression in a cohort of women from rural China who have an elevated risk of lung cancer due to indoor household cooking with smoky coal (70, 71). They identified buccal gene expression alterations associated with exposure to smoky coal with similar molecular changes as those seen with tobacco smoke exposure. The similarity between the field of injury induced by smoky coal and tobacco smoke in buccal epithelium suggests that there may be potential to develop clinically relevant biomarkers at this site.

Conclusions and Future Directions for the Field

Identifying lung cancer–associated differences in normal-appearing tissue throughout the lung and airway has created an opportunity to detect lung cancer via sampling of nontumor tissue. Understanding the processes that give rise to these lung cancer–associated molecular signatures is challenging, but two leading

hypotheses are that (i) they are related to an etiologic field of injury in which processes that promote carcinogenesis (including the response to carcinogen exposure) occur throughout the lung and that the progression of these processes can therefore be measured in nontumor tissue like airway epithelium, and/or (ii) the effects of field cancerization can extend beyond the immediate tumor microenvironment and can be detected in nontumor tissue like airway epithelium. The airway gene expression biomarkers described in this review may reflect a molecular signature resulting from either an etiologic field of injury, field cancerization, or both (Fig. 1).

Further work may lead to a better understanding of the processes that give rise to these airway gene expression signatures and/or potentially expand their clinical applications (Fig. 2B). For instance, little is known about how the field is affected by surgical resection that might have practical implications for the utility of these biomarkers in patients with a prior history of lung cancer. It is also unclear whether there is an etiologic field of injury or field cancerization in never smokers who develop lung cancer that could be leveraged to develop lung cancer biomarkers for this patient population. Recent studies have identified gene expression alterations in the normal-appearing airway of smokers who have premalignant squamous cell lesions and that changes in the magnitude of these gene expression alterations is associated with lesion progression (72), but little is known about the time course during which these airway molecular changes occur relative to when these lesions appear. This might have implications for the role of an etiologic field of injury as a cause of these molecular changes as well as practical implications for the utility of these biomarkers in the setting of assessing lung cancer risk.

Further downstream is determining how the lung cancer signature detectable in the airways differs between patients with different types of lung cancer. This could ultimately provide information about field cancerization and/or how different etiologic fields of injury predispose patients to different types of lung cancer. It would also enable using airway gene expression for precision medicine approaches to lung cancer treatment (or chemoprevention) by serving as a biomarker for selecting therapy. Although there is certainly much to be learned about the processes that contribute to lung cancer–associated changes in airway gene expression, and many of the potential clinical applications that can be envisioned are yet to be explored, the initial successful translation of this approach into a tool with clinical utility for the diagnostic evaluation of patients with suspect lung cancer is a promising sign that there will be other tools to improve lung cancer outcomes that leverage the ability to detect cancer-associated molecular changes in airway epithelium.

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