Giles F. Filley Lecture

Field Cancerization in Non–Small Cell Lung Cancer
Implications in Disease Pathogenesis

Humam Kadara[1] and Ignacio I. Wistuba[1,2]

[1]Department of Thoracic/Head and Neck Medical Oncology, and [2]Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Lung cancer, of which non–small cell lung cancer (NSCLC) composes the majority, is the leading cause of cancer-related deaths in the United States and worldwide. NSCLCs are tumors with complex biology that we have recently started to understand with the advent of various histological, transcriptomic, genomic, and proteomic technologies. However, the histological and molecular pathogenesis of this malignancy, in particular of adenocarcinomas, is still largely unknown. Earlier studies have highlighted a field cancerization phenomenon in which histologically normal-appearing tissue adjacent to neoplastic and pre-neoplastic lesions display molecular abnormalities, some of which are in common with those in the tumors. This review will summarize advances in understanding the field cancerization phenomenon and the potential relevance of this knowledge to gain important and novel insights into the molecular pathogenesis of NSCLC as well as to subsequent development of biomarkers for early detection of lung cancers and possibly personalized prevention.

Keywords: lung cancer; field cancerization; pathogenesis; airway epithelium

Lung cancer is the leading cause of cancer deaths in the United States and worldwide in both developing and developed regions (1). The high mortality of this disease is in part due to our lacking knowledge of the molecular mechanisms governing lung cancer pathogenesis as well as the late diagnosis of the majority of lung cancers after regional or distant spread of the malignancy (2). Non–small cell lung cancer (NSCLC) represents the majority of diagnosed lung cancers (2) and is mainly composed of squamous cell carcinomas (SCCs) and lung adenocarcinomas (2, 3). Several major differences exist between adenocarcinomas and SCCs. For example, compared with SCCs that arise from the major bronchi and are mainly centrally located, pulmonary adenocarcinomas arise from small bronchi, bronchioles, or alveolar epithelial cells and are typically peripherally located, as reviewed elsewhere (2–5). Moreover, whereas SCC pathogenesis is strongly linked to smoking, lung adenocarcinoma is the more common histological subtype in never-smoker patients (6–9). Although the sequence of lesions in the pathogenesis leading to SCCs is well described, little is known about the sequential development of adenocarcinomas. Moreover, we are still lacking in our knowledge of differential mechanisms of molecular pathogenesis among both subtypes of NSCLC.

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Correspondence and requests for reprints should be addressed to Ignacio I. Wistuba, M.D., Departments of Thoracic/Head and Neck Medical Oncology and Pathology, the University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 085, Houston, TX 77030. E-mail: iiwistuba@mdanderson.org.

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In light of the postulated period of time and multiple stages required for the development of overt epithelial tumors, it is plausible to assume that early diagnosis of lung cancer or intraepithelial lesions coupled with effective prevention strategies will reduce the significant health burden associated with this disease (10). Despite recent encouraging findings from the National Lung Screening Trial (NLST) (11), early detection and prevention of lung cancer is challenging due to the lack of biomarkers for early diagnosis of the disease and to the presence of multiple neoplastic molecular pathways that mediate lung carcinogenesis. Earlier studies have highlighted a field cancerization phenomenon in which histologically normal-appearing tissue adjacent to neoplastic and pre-neoplastic lesions displays molecular abnormalities, some of which are in common with those in the tumors. It is plausible to assume that understanding early events in tumor development that commence in histologically normal epithelium would pave the way for unmet effective and personalized strategies for lung cancer prevention and treatment. This review mainly summarizes advances in understanding the field cancerization phenomenon and the potential relevance of this knowledge to gain important and novel insights into the molecular pathogenesis of NSCLC as well as to subsequent development of biomarkers for early detection of lung cancers and possibly personalized prevention.

NSCLC PRE-NEOPLASIA AND MOLECULAR PATHOGENESIS

From biological and histopathological perspectives, NSCLC is a complex malignancy that develops through multiple pre-neoplastic pathways. Lung adenocarcinoma, a major subtype of NSCLC, has been increasing in incidence globally in both smokers and nonsmokers (9), with a concurrent decrease in SCC frequency. It has been suggested that the increasing incidence of lung adenocarcinomas compared with SCCs is in part due to the change in the type of cigarettes used (lower nicotine and tar) and smoking habits and behavior (7). Anatomical differences in the location of diagnosed lung adenocarcinomas and SCCs strongly suggest that both NSCLC subtypes develop through different histopathological and molecular pathways and have different cells of origin; however, the specific respiratory epithelial cell type from which each lung cancer type develops has not been established with certainty (3). However, it is noteworthy that Clara cells and the type II pneumocytes are believed to be the progenitor cells of the peripheral airways, and peripherally arising adenocarcinomas often express markers of these cell types (12, 13).

The multistage stepwise fashion of tumor development has been demonstrated in various anatomical organs exemplified by the operational stages occurring during skin carcinogenesis (14). Carcinogenesis of the skin is initiated by a carcinogen-induced mutational event, promoted by clonal outgrowth, which may be dependent on tumor promoters, followed by progression of premalignant lesions (e.g., papillomas) and their conversion to malignant tumors (14).

Importantly, earlier genetic studies indicate that nonrandom, sequential chromosomal aberrations (trisomies of chromosomes 6 and 7) are associated with premalignant progression of mouse skin papillomas (14–17). The sequence of histopathological and molecular changes in bronchial epithelia that precede the development of lung SCCs have been characterized, demonstrating that sequentially occurring allele-specific molecular changes commence in dispersed foci signifying a multistage fashion of squamous lung cancer pathogenesis (4, 18). At least in this subtype of NSCLC, it has been shown that specific genomic alterations (mainly 3p21, 3p22–24, 3p25, and 9p21) occur in histologically normal bronchial epithelia from resected specimens (18). Moreover, and notably, these alterations persisted in hyperplasias, dysplasias, carcinomas *in situ*, and tumors that exhibited different commencing genomic aberrations (18). In addition, loss of heterozygosity (LOH) in the 3p region was demonstrated in normal bronchial epithelia of cancer-free smokers, further highlighting the early role this specific genomic alteration exerts in lung cancer pathogenesis (19, 20). It is noteworthy that McCaughan and colleagues demonstrated that no low-grade lesions, but all high-grade lesions, exhibited 3q amplification targeting the sex determining region Y-box 2 (*SOX2*) lineage-specific oncogene (21). These previous findings and reports, through highlighting associations between histopathological sequences and specific molecular aberrations, pinpoint to a multistage and multistep manner of lung carcinogenesis.

On the other hand, one cannot neglect the alternative hypothesis that the sequence of genetic and epigenetic alterations is irrelevant to lung cancer pathogenesis, but rather the accumulation of molecular abnormalities beyond a certain threshold mediates development of the malignant phenotype. It has been suggested that at least two molecular pathways, the Kirsten rat sarcoma viral oncogene (*KRAS*) and epidermal growth factor receptor (*EGFR*) pathways, are involved in the development of smoker and never-smoker adenocarcinomas, respectively (2, 9). Moreover, and as reviewed by Yatabe and colleagues, atypical adenomatous hyperplasias (AAHs), which are considered to be precursor lesions for peripheral lung adenocarcinomas (3, 5) and the only sequence of morphologic change identified so far for the development of invasive lung adenocarcinomas, exhibit *KRAS* mutations more frequently than invasive adenocarcinomas (5). Conversely, our group has previously demonstrated that *EGFR* mutations commence in histologically normal bronchial epithelia adjacent to lung adenocarcinomas and precede copy number increase of the oncogene (22, 23). *EGFR* mutations also are persistent throughout the different phases of lung adenocarcinoma development (5), which harbor different genomic alterations (24). It is plausible to surmise that only after increasing our knowledge of the pre-neoplastic changes as well as the corresponding molecular abnormalities leading to the development of lung adenocarcinomas would we then be able to more confidently determine whether adenocarcinomas follow a linear progression mechanism or not (5). However, and based on the aforementioned previously reported observations by our group and others, we believe that it is not counterintuitive to speculate that development of lung malignant phenotype, including that of adenocarcinomas, is due to stepwise, sequence-specific, and multistage molecular pathogenesis as well as accumulation and combination of genetic and epigenetic abnormalities.

FIELD CANCERIZATION

Smoking-Damaged Epithelium and the Field Cancerization Phenomenon

Although the majority of lung cancer patients are current or former smokers, a relatively small proportion of these smokers (approximately 15%) develop primary lung tumors. Patients with early-stage NSCLC commonly exhibit recurrence or second primary tumor development after definitive treatment by resective surgery. There is a large body of evidence that heavy smokers and patients who have survived lung cancer compose a high-risk population that may be targeted for early detection and chemoprevention efforts (4). Although the risk of developing lung cancer decreases after smoking cessation, the risk never returns to baseline. Pre-neoplastic changes have been used as surrogate endpoints for chemopreventive studies. However, it was suggested that this "shooting in the dark" approach may explain the reasons behind the general failures of clinical chemoprevention studies (10). Therefore, novel approaches to identify the best population to be targeted for early detection and chemoprevention should be devised, and risk factors for lung cancer development or relapse need to be better defined. For these important purposes, a better understanding of the biology and molecular origins of lung cancer is warranted.

Earlier work by Danely Slaughter in patients with oral cancer and oral premalignant lesions has suggested that histologically normal-appearing tissue adjacent to neoplastic and pre-neoplastic lesions display molecular abnormalities, some of which are in common with those in the tumors (25). In 1961, a seminal report by Auerbach and colleagues suggested that cigarette smoke induces extensive histological changes in the bronchial epithelia in the lungs of smokers and that premalignant lesions are widespread and multifocal throughout the respiratory epithelium, suggestive of a field effect (26). This phenomenon, coined "field of cancerization," was later shown to be evident in various epithelial cell malignancies, including lung cancer. Some degree of inflammation and inflammatory-related damage is almost invariably present in the central and peripheral airways of smokers and may precede the development of lung cancer (4). Thus, the field of cancerization may also be explained by both the direct effect of tobacco carcinogens and the initiation of inflammatory response. In this context, different theories for the origin of the field cancerization or smoking-related field of injury have been put forward and extensively reviewed elsewhere by Steiling and coworkers (27).

Multiple altered foci of bronchial epithelium are present throughout the airway in patients with lung cancer and smokers (18, 28, 29). As mentioned before, detailed analysis of histologically normal, premalignant, and malignant epithelia from patients with lung SCC indicated that multiple, sequentially occurring allele-specific chromosomal deletions of LOH commence in clonally independent foci early in the multistage pathogenesis of SCCs (18, 28). Notably, 31% percent of histologically normal epithelium and 42% of mildly abnormal (hyperplasia/metaplasia) specimens had clones of cells with allelic loss at one or more regions examined. Moreover, these molecular aberrations were also found in carcinomas *in situ* and SCCs and at a more advanced level (18). Molecular changes involving LOH of chromosomal regions 3p (e.g., fragile histidine triad gene/*FHIT*), 9p (e.g., *CDKN2A/p16*), genomic instability (increased microsatellite repeats), and *p16* methylation have been demonstrated in histologically normal bronchial epithelia in patients with SCC and in the sequence of pathogenesis of the disease (3). Moreover, Nelson and colleagues demonstrated that *KRAS* is also mutated in histologically normal lung tissue adjacent to lung tumors (30). In addition, similar epigenetic and gene methylation patterns between tumors and adjacent histologically normal epithelia were described. Belinsky and colleagues reported aberrant promoter methylation of *p16*, which was described to be commonly methylated in lung tumors (31), in at least one bronchial epithelial site from 44% of lung cancer cases examined (32). Moreover, *p16* and death-associated protein kinase (*DAPK*) promoter

methylation were frequently observed in bronchial epithelium from smoker but not from never-smoker patients with lung cancer and persisted after smoking cessation (32).

The aforementioned molecular abnormalities were detected in histologically normal epithelia adjacent to archival surgically resected tumors from patients with primary lung cancer. LOH and microsatellite alterations in multiple foci were also detected in distal histological normal bronchial epithelia of smokers without cancer (19, 20). Moreover, and importantly, these molecular abnormalities were detected in bronchial epithelia of cancer-free former smokers and appeared to have persisted for many years after smoking cessation. In addition, LOH was detected in DNA obtained from bronchial brushings of normal and abnormal lungs from patients undergoing diagnostic bronchoscopy and was detected in cells from the ipsilateral and contralateral lung (33). Mutations in *TP53* were also described to occur in bronchial epithelia of cancer-free smokers in a widely dispersed manner (34). Similar evidence also exists for promoter methylation and epigenetic changes in smoking-damaged lung epithelium of cancer-free patients. Methylation of various genes, including retinoic acid receptor 2 β (*RAR-β2*), *H-cadherin*, adenomatous polyposis coli (*APC*), *p16*, and Ras association (RalGDS/AF-6) domain family member 1 (*RASSFF1A*) has been described in bronchial epithelial cells of heavy smokers (35). Moreover, methylation of *p16*, glutathione S-transferase pi 1 (*GSTP1*), and *DAPK* was reported to be evident in bronchial brushings of one-third of cancer-free smokers examined (36). In the study by Belinsky and colleagues, as mentioned before, methylation of *p16* was detected in epithelia of cancer-free smokers (32). A more detailed list of aberrant gene promoter methylation in patients with lung cancer and cancer-free smokers is well summarized and explained in the review by Heller and coworkers (37).

Transcriptomic Studies of Lung Field Cancerization

High-throughput microarray profiling was shown to be useful to study the transcriptome of lung airways. Hackett and colleagues studied the expression of 44 antioxidant-related genes using bronchial brushings from cancer-free current smokers and never-smokers and found significant up-regulation of 16 of the antioxidant genes in the airways of smokers compared with nonsmokers (38). Later, Spira and colleagues described global alterations in gene expression between normal-appearing bronchial epithelium of healthy cancer-free smokers and that of nonsmokers (39). Importantly, irreversible changes in expression in airways of former smokers after years of smoking cessation were described that were believed to underlay the increased risk former smokers exhibit for developing lung cancer (39, 40). Alterations in the expression of microRNAs were also demonstrated between large airways of current and never-smokers (41). Notably, an 80-gene signature was derived from the transcriptome of large airway epithelial cells that can distinguish smokers without overt cancer from smokers with lung cancer despite originating from normal bronchial epithelia (42). More recently, Gustafson and coworkers derived a phosphoinositide-3-kinase (*PI3K*) pathway activation signature by using recombinant adenoviruses to express the 110α subunit of *PI3K* in primary human epithelial cells (43). The *PI3K* pathway activation signature was elevated in cytologically normal bronchial airways of smokers with lung cancer and, importantly, was decreased in the airways of high-risk smokers whose dysplastic lesions regressed after treatment with the *PI3K* inhibitor myoinositol (43). Microarray and gene expression profiling methodologies were also used to demonstrate the wide anatomical spread of the lung field cancerization. Common gene expression alterations were identified in bronchial, nasal, and buccal epithelia of smokers (44), and in a separate study, the expression of 119 genes was

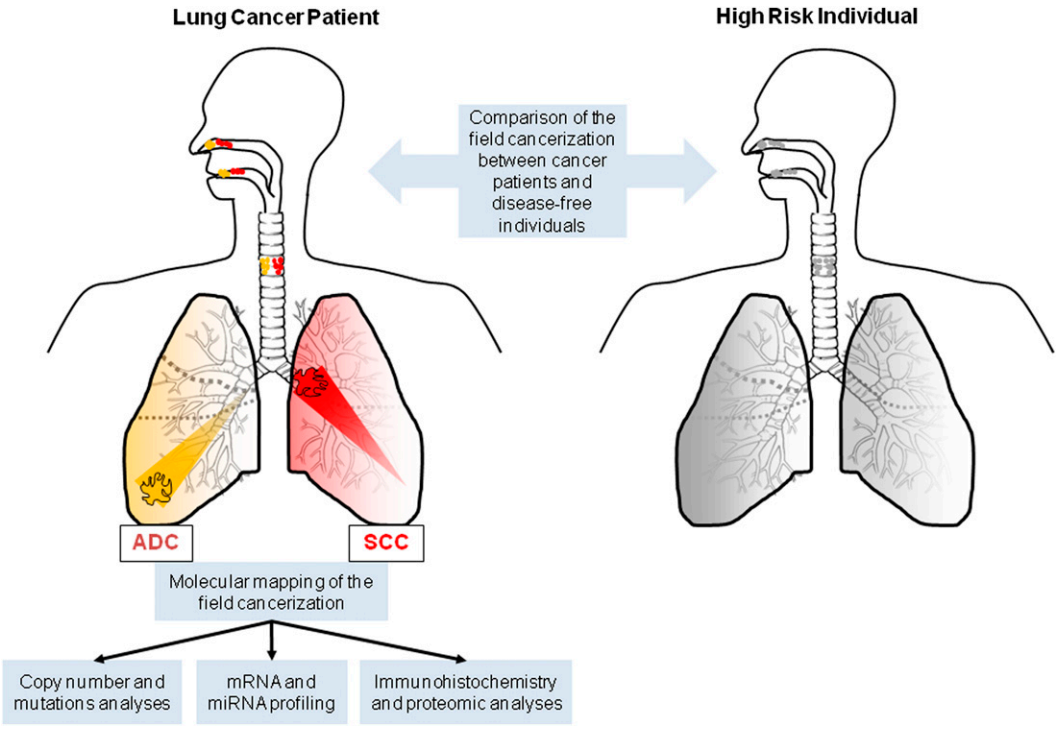

Figure 1. Molecular mapping analysis of the field cancerization in non–small cell lung cancer (NSCLC). The relevance of the lung field cancerization to the development of a particular subtype of NSCLC (i.e., adenocarcinoma compared with squamous cell carcinoma [SCC]), is still unknown, yet possible. Analyzing local and distant field of cancerization by analysis of the transcriptome of airway brushings from multiple sites independently for lung adenocarcinoma (*yellow spots*) and SCC (*red spots*) cases may shed light on events common or unique to the molecular pathogenesis of the two major subtypes of NSCLC. A "compartmental" approach coupled to a gradient or detailed molecular mapping method, which spans the tumor up to the nasal or buccal epithelium, to studying the field of cancerization may unravel biomarkers that can guide personalized prevention strategies suitable for each different NSCLC subtype. In addition, a comparison of the distant field cancerization in patients with cancer (*left*) to the expression patterns of the corresponding anatomical location in disease-free individuals (e.g., high-risk heavy smokers; *right*) would facilitate the development of efficacious markers for the detection of NSCLC. ADC = adenocarcinoma; miRNA = microRNA.

demonstrated to be affected by smoking similarly in both bronchial and nasal epithelium (45).

Field Cancerization Compartmentalization

In light of the prevalence of mutations in the *EGFR* oncogene in adenocarcinomas and in particular those occurring in never-smokers, Tang and colleagues investigated the presence of *EGFR* mutations in normal bronchial and bronchiolar epithelium adjacent to *EGFR* mutant tumors (22). *EGFR* mutations were detected in histologically normal peripheral epithelia in 44% of patients with lung adenocarcinoma with mutations but none in patients lacking mutations in the oncogene (22). Moreover, the same study highlighted more frequent *EGFR* mutations in normal epithelium within the tumor (43%) than in adjacent sites (24%), suggesting a localized field effect phenomenon for this abnormality in the respiratory epithelium of the lung (22). In addition, a higher frequency of mutations in cells obtained from small bronchi (35%) compared with bronchioles (18%) was detected (22). More recently, EGFR protein overexpression, similar to mutation of the gene, also exhibited a localized field effect, as it was more frequent in normal bronchial epithelia sites within tumors than in sites adjacent to and distant from tumors (23). Interestingly, *EGFR* copy number alteration was not evident in normal bronchial epithelia, which is in accordance with findings that *EGFR* copy number is a relatively late event in pathogenesis of adenocarcinomas (5, 23). These findings suggest that adenocarcinomas may be associated with a field cancerization dissimilar from that linked to SCCs.

The low frequency of molecular abnormalities detected in the centrally located bronchial respiratory epithelium in patients with peripheral lung adenocarcinomas, compared with specimens from patients with SCCs (28), suggests the presence of two compartments in the lung with different degrees of smoking-related genetic damage. Thus, smokers who develop SCCs display more smoking-related genetic damage in the respiratory epithelium of the central airway, whereas patients who develop adenocarcinomas exhibit molecular and histological damage mainly in the peripheral airways. Although some molecular changes (e.g., inflammation and signaling pathways activation) have been detected throughout the lung airway and include both compartments (central and peripheral airway), other aberrations have been more frequently altered in either central (e.g., LOH, genetic instability evidenced by microsatellite repeats) or peripheral (e.g., *EGFR* mutations as mentioned above) airways. These interesting observations indicate a possible compartmentalization of field cancerization and its dissimilarity between adenocarcinomas and SCCs, which may well reflect the differential mechanisms of pathogenesis of both NSCLC subtypes.

FUTURE DIRECTIONS AND CONCLUSION

Applying the same advanced high-throughput methodologies currently used in studying established tumors for the genetic analysis of lung NSCLC pre-neoplasia and histologically normal adjacent regions is expected to expand our understanding of the biology of this prevalent disease. Next-generation sequencing technology, through whole-genome, whole-exome, and whole-transcriptome approaches, holds great promise for providing invaluable insights into NSCLC biology, diagnosis, prevention, and therapy (46). An important step in this direction was a recent study in which RNA of bronchial airway epithelial cell brushings from healthy never-smokers and smokers with and without lung cancer was analyzed by RNA sequencing (47) and provided additional insight besides that provided when using microarray technology.

Earlier findings demonstrated that centrally located lung SCCs and peripherally located lung adenocarcinomas elicit and perpetuate differential effects on the airway epithelia (4). Changes in expression in the lung field of injury have been shown to be similar in the large and small airways, and it is unknown whether they are associated with the development of the particular subtype of NSCLC. Addressing this question may be highly important, because both NSCLC subtypes display different genomic features (2) and, therefore, are clinically managed by significantly dissimilar treatment strategies, let alone differences among various subtypes of lung adenocarcinomas. Moreover, revisiting the field cancerization effect using a compartmental coupled with a gradient or detailed molecular mapping approach in patients with cancer and disease-free individuals (Figure 1) will shed light on events in the early pathogenesis of lung adenocarcinomas and SCCs and unravel biomarkers that can guide targeted and personalized chemoprevention strategies suitable for each different NSCLC subtype as well as detection efforts, in particular using less invasive sites.

Despite numerous efforts that have centered on increasing our understanding of the biology of lung cancer, this malignancy still composes the biggest share of cancer-related deaths in the United States and worldwide. Compared with advances in targeted and personalized therapy of NSCLC, little progress has been made in the tailored prevention of this fatal malignancy. This may change with the recent encouraging and significant findings of the National Lung Screening Trial (11). Various molecular markers and expression classifiers previously described in the lung airways and in less-invasive sites of field cancerization (e.g., nasal epithelium) can aid in selecting high-risk individuals best suited for computed tomography screening, for example. A comprehensive analysis of early molecular events in NSCLC pathogenesis will undoubtedly unravel biomarkers that can guide future chemoprevention strategies.

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