Smoking and Lung Cancer
The Role of Inflammation

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Worldwide over 1 million people die due to lung cancer each year. It is estimated that cigarette smoking explains almost 90% of lung cancer risk in men and 70 to 80% in women. Clinically evident lung cancers have multiple genetic and epigenetic abnormalities. These abnormalities may result in activation of oncogenes and inactivation of tumor-suppressor genes. Chronic inflammation, which is known to promote cancer, may result both from smoking and from genetic abnormalities. These mediators in turn may be responsible for increased macrophage recruitment, delayed neutrophil clearance, and increase in reactive oxygen species (ROS). Thus, the pulmonary environment presents a unique milieu in which lung carcinogenesis proceeds in complicity with the host cellular network. The pulmonary diseases that are associated with the greatest risk for lung cancer are characterized by abundant and deregulated inflammation. Pulmonary disorders such as chronic obstructive pulmonary disease (COPD)/emphysema are characterized by profound abnormalities in inflammatory and fibrotic pathways. The cytokines and growth factors aberrantly produced in COPD and the developing tumor microenvironment have been found to have deleterious properties that simultaneously pave the way for both epithelial–mesenchymal transition (EMT) and destruction of specific host cell–mediated immune responses. Full definition of these pathways will afford the opportunity to intervene in specific inflammatory events mediating lung tumorigenesis and resistance to therapy.

Keywords: smoking; inflammation; lung cancer; COPD; EMT

Lung cancer is the leading cause of cancer death, both in the United States and worldwide. It is estimated that lung cancer will cause over 160,000 deaths in the United States in 2007, and greater than one million deaths worldwide. The most important risk factor for lung cancer is tobacco smoking, and the data supporting this relationship are compelling (1). Compared with nonsmokers, smokers have as much as a 30-fold increased risk of developing cancer (1–3). Thirty-one percent and 26% of all cancer deaths in men and women, respectively, result from lung cancer in the United States. Overall 5-year survival is only 15%, and 1-year survival is approximately 42%. In total, lung cancer is responsible for more deaths than prostate, colon, pancreas, and breast cancers combined. Woloshin and coworkers have recently framed the health risks due to smoking status in a different context (4). For men age 60 and above who currently smoke, the chance of dying from lung cancer is of the same order of magnitude as the chance of dying from heart disease. After age 50, it is 10 times greater than the chance of dying from prostate or colon cancer. For women who currently smoke, the chance of death due to lung cancer exceeds the chance of dying from breast cancer from age 40 onward (4). The tobacco smoking–induced inflammatory response yields an array of deregulated cells, cytokines, and growth factors that are conducive to the development of both chronic obstructive pulmonary disease (COPD) and lung cancer. Inflammation has been suggested to promote lung cancer via several possible pathways. For example, inflammatory cell–derived reactive nitrogen or oxygen species may bind to DNA and thus lead to genomic alterations (5, 6).

Thus, pulmonary inflammation could play a role in cancer initiation or promotion. The pulmonary environment of COPD, including ongoing tissue repair with enhanced cellular proliferation, could be conducive to both DNA mutation and angiogenesis (6). In addition, the proinflammatory cytokines released in this milieu elevate epithelial apoptosis resistance.

SMOKING, INFLAMMATION, AND LUNG CANCER

Lung cancer evolves as a result of a series of mutational events that have been studied in detail by numerous investigators (7). However, the molecular pathogenesis of lung cancer remains incompletely defined. Because inflammation appears to play an important role in the pathogenesis of lung cancer, a thorough understanding of lung cancer pathogenesis requires consideration of the tumor microenvironment (TME) and the inflammatory pathways operative in carcinogenesis (8).

The tobacco-induced pulmonary cellular network presents a unique environment in which carcinogenesis proceeds in complicity with surrounding lung inflammatory, structural, and stromal cells. The pulmonary diseases that are associated with the greatest risk for lung cancer are characterized by abundant and deregulated inflammation (9–11). Pulmonary disorders such as COPD are characterized by profound abnormalities in inflammatory pathways (12–14). For example, among the cytokines, growth factors, and mediators released in these lung diseases and the developing TME, interleukin (IL)-1β, prostaglandin (PG)E_2, and transforming growth factor (TGF)-β have been found to have deleterious properties that simultaneously pave the way for both epithelial–mesenchymal transition (EMT) and destruction of specific host cell–mediated immune responses against tumor antigens (15–19).

The commonalities in smoking, COPD, and lung cancer begin with the profound alterations induced by cigarette smoke, which contains known carcinogens as well as high levels of reactive oxygen species (ROS). The ready induction of ROS after tobacco smoke exposure leads to impairment of epithelial and endothelial cell function as well as inflammation. The ongoing inflammatory processes in COPD may be persistent even after smoking cessation and have been quantified and related to disease progression (20). As COPD progresses, the percentage of the airways that contain macrophages, neutrophils,

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T cells, B cells, and lymphoid aggregates containing follicles increases (20).

THE FIELD CANCERIZATION EFFECT

Beginning with the groundbreaking investigations of Auerbach and colleagues (21), an extensive literature documents that tobacco smokers' respiratory epithelium often contains multifocal premalignant lesions that can occur throughout the bronchial tree. These findings have been referred to as the field cancerization effect, and implicate the capacity of tobacco carcinogens to mutagenize the respiratory epithelium extensively (22). In analyzing premalignant and malignant epithelium from patients with squamous cell carcinoma, Wistuba and coworkers found multiple, sequentially occurring allele-specific chromosomal deletions (loss of heterozygosity) in widely dispersed, apparently clonally independent foci, early in the multistage pathogenesis of lung squamous cell carcinoma (23–25). The bronchial epithelium in current and former smokers also demonstrates multiple foci of genetic changes, as seen in patients with lung cancers. Importantly, these changes may persist for many years after smoking cessation (26–28). These persistent abnormalities serve as a driving force for increased risk in a growing population; there are more than 45 million former smokers in the United States, and the majority of new lung cancer diagnoses now occurs in former smokers.

In addition to premalignant lesions that are visible by histologic inspection, studies document that smoking induces field effect abnormalities even in histologically normal lung epithelium (26, 29, 30). High-density gene expression arrays have been used to define genes in human airway epithelial cells that are altered by cigarette smoking (31–35). The data obtained in these studies are expected to provide insights into lung cancer risk in smokers, with or without COPD. Spira and colleagues recently reported that gene expression profiles in histologically normal large airway epithelial cells could serve as a biomarker for the presence of lung cancer (36). These findings provide a strong case for the presence of a diffuse airway response to tobacco smoke that is not necessarily demonstrable by conventional histologic assessments. Because the airway gene expression profiles provide important information about the possible development of lung cancer that is not adequately predicted by clinically defined risk alone, Beane and coworkers have recently proposed a clinicogenomic model that has a higher prediction accuracy (37).

The tobacco smoking–induced changes in gene expression and cellular functions are not confined to the pulmonary airway epithelium but have also been reported in the nasal and buccal epithelium (38, 39), alveolar macrophages (40, 41), and peripheral blood (42). These findings are consistent with the previously presented hypotheses regarding the systemic inflammatory process operative in patients with COPD as well as in those with lung cancer.

EPITHELIAL–MESENCHYMAL TRANSITION

EMT has been initially described as a process in embryonic development. EMT is composed of a developmental shift from a polarized, epithelial phenotype to a highly motile fibroblastoid or mesenchymal phenotype (43). In addition to embryonic development, EMT has been implicated in chronic inflammation, fibrosis, and cancer development (44–47). In normal development, EMT is a tightly regulated process (47). In contrast, in cancer development and progression, EMT is unregulated, with selective elements of the process amplified while other aspects are circumvented (48). A variety of pathways are now appreciated to impact EMT in cancer. For example, the TGF-β pathway, PI3K/Akt, ROS, receptor tyrosine kinase/Ras signaling, and Wnt pathways have been among those implicated (43, 44, 49). Thus, EMT is operative in a variety of malignancies (50), including lung cancer (48).

The connection between inflammation and EMT progression in lung cancer development and resistance to therapy has recently been emphasized (15, 51). For example, IL-1β and PGE_2 have the capacity to decrease E-cadherin expression and promote EMT. These inflammatory mediators have the capacity to up-regulate the zinc-finger E-box–binding transcriptional repressors of E-cadherin, including Zeb1, Snail, and Slug, thus leading to EMT progression (15, 52). Recent work from Robert Weinberg's laboratory suggests a direct link between EMT and gain of epithelial stem cell properties (53). Thus, inflammation may impact stem cell properties via EMT-dependent events in the pathogenesis of lung cancer. While EMT-induced alterations have been widely implicated in the epithelial malignancy metastatic process, the work of Mani and colleagues suggests that the EMT genetic program may also regulate early events in carcinogenesis, therefore implicating the inflammatory pulmonary environment in both lung cancer initiation and progression. The fact that tobacco and tobacco-specific carcinogens may be involved by directly or indirectly promoting EMT adds additional importance to these relationships. For example, Yoshino and coworkers (54) found that benzo[a]pyrene induced EMT-related genes in lung cancer cells; while fibronectin and Twist were induced, E-cadherin expression was deceased. In support of these findings, and in the context of another tobacco-induced malignancy, Fondrevelle and colleagues (55) found that the expression of Twist was influenced by smoking status in patients with bladder cancer. Tobacco-specific carcinogen 4-(n-methyl-n-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has also been found to promote EMT via induction of E-cadherin transcriptional repressors in human bronchial epithelial cells (56).

PULMONARY EPITHELIAL CELLS CAN SERVE AS TARGETS FOR INFLAMMATION AS WELL AS INDUCERS OF ABNORMAL INFLAMMATORY RESPONSES

Smoking-induced epithelial abnormalities can serve both as targets for abnormal inflammatory responses and as initiators of deregulated inflammation. Cytokines, chemokines, and growth factors released by alveolar macrophages, lymphocytes, neutrophils, endothelial cells, and fibroblasts may act to promote epithelial dysfunction and malignant progression. Some of these relationships are most clearly demonstrable in genetically engineered murine models (57). For example, Wislez and coworkers used $Kras^{LA1}$ mice, which develop lung adenocarcinoma due to somatic activation of the KRAS oncogene, to study the importance of ligands for chemokine receptor CXCR2 in the pathogenesis of lung cancer (58). Vascular endothelial cells and neutrophils with high expression of CXCR2 ligands and CXCR2 were found in premalignant alveolar lesions of $Kras^{LA1}$ mice. Importantly, CXCR2 inhibition blocked the expansion of early alveolar neoplastic lesions. These studies are consistent with other recent findings indicating that the CXCR2 ligand CXCL8 plays a critical role in Kras-induced tumorigenesis (59). By implicating CXCL8, these findings highlight another common pathway in the pathogenesis of COPD and lung cancer.

Epithelial cells can also serve as a site of deregulated inflammatory responses in pulmonary tumorigenesis. For example, chronic exposure to tobacco compounds can lead to loss of p53 and Kras mutation. These in turn can lead to deregulated

inflammation and angiogenesis. Komarova found that p53, by acting to suppress NF-κB activity, could serve as a "buffer" for inflammatory responses (60). This is consistent with the p53 tumor suppressor functions. As noted above, Kras mutations can serve as a driving force for the generation of the pro-angiogenic CXC chemokines such as CXCL8. In addition, Kras mutations are one of the stimuli known to induce constitutive elevation of cyclooxygenase-2 (COX-2) in epithelial cells, resulting in high-level production of PGE_2.

Several studies have documented high constitutive expression of COX-2 in precursor lesions as well as established human lung cancer (61–66). In the initial report of COX-2 expression in human lung cancer, Huang and colleagues assessed COX-2 expression by immunohistochemistry in tumors and adjacent normal lung tissue (61). Both adenocarcinomas and squamous carcinoma showed cytoplasmic staining for COX-2 in tumor cells. In subsequent reports, elevated COX-2 expression has been shown with greater staining in lymph node metastases than in the primary tumor (62, 63), and tumor COX-2 expression has been found to be a poor prognostic indicator (64, 65, 67). These findings, along with studies documenting increased COX-2 expression in precursor lesions (67–69), an association between a common polymorphism in the COX-2 gene and increased risk of lung cancer (70), and epidemiological studies indicating a decreased incidence of lung cancer in individuals who regularly use aspirin, support involvement of COX-2 and its enzymatic products in the pathogenesis of lung cancer (71). Thus, in lung cancer development and progression, elevations of COX-2 and PGE_2 are driving forces for the hallmarks of malignancy including apoptosis resistance (72), proliferation (73), immunosuppression (74), angiogenesis (75), invasion (76), and EMT (15). Ongoing chemoprevention studies in patients at risk for lung cancer are now assessing blockade of the eicosanoid pathway.

Whereas the COX enzymes are expressed at low constitutive levels in the normal lung, a variety of factors may contribute to up-regulation of COX-2 in the developing lung cancer environment. This elevation in COX-2 leads to enhanced production of deleterious PG products, including PGE_2, which has well established pro-tumorigenic effects. Contributors to persistent elevation of COX-2 in epithelial stromal and lung cancer cells include cytokines such as IL-1β and TGF-β, growth factors including epidermal growth factor, oncogenic events such as mutant Kras or loss of p53, hypoxia, and tobacco-specific carcinogens (61, 77–79). Once COX-2 is up-regulated in lung cancer cells, its elevation may be maintained by abnormalities in signaling pathways required to down-regulate COX-2. Two such abnormalities are loss of IL-10 receptor expression and constitutive nuclear localization of STAT-6 (80, 81). Chemotherapy including taxanes also can stabilize COX-2 mRNA, thus leading to its prolonged and unregulated expression (82).

CONCLUSIONS

Lung cancer is often intimately linked to tobacco smoking and inflammation. The investigation of these relationships will lead to a more complete picture of the pulmonary environment at risk for the development of lung cancer. New investigations will revisit the original findings of Auerbach and coworkers with the application of the powerful tools of current genomics, proteomics, and imaging. The refined definitions of pulmonary inflammation and pre-malignancy will afford new opportunities for advances in risk assessment and prevention.

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