# **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 epithelialmesenchymal transition (EMT) and destruction of specific host cellmediated 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

Proc Am Thorac Soc Vol 5. pp 811–815, 2008 DOI: 10.1513/pats.200809-100TH Internet address: www.atsjournals.org 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)-1 $\beta$ , prostaglandin (PG)E<sub>2</sub>, and transforming growth factor (TGF)- $\beta$  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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## 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

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are circumvented (48). A variety of pathways are now appreciated to impact EMT in cancer. For example, the TGF- $\beta$  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-1 $\beta$  and PGE<sub>2</sub> 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 tobaccoinduced 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-(nmethyl-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<sup>LA1</sup> 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<sup>LA1</sup> 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- $\kappa$ B 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 proangiogenic 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<sub>2</sub>.

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<sub>2</sub> 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<sub>2</sub>, 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-1ß and TGF-B, 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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#### References

- Youlden DR, Cramb SM, Baade PD. The international epidemiology of lung cancer: geographical distribution and secular trends. J Thorac Oncol 2008;3:819–831.
- Sasco AJ, Secretan MB, Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. *Lung Cancer* 2004;45:S3–S9.
- Proctor RN. Tobacco and the global lung cancer epidemic. Nat Rev Cancer 2001;1:82–86.
- Woloshin S, Schwartz LM, Welch HG. The risk of death by age, sex, and smoking status in the United States: putting health risks in context. *J Natl Cancer Inst* 2008;100:845–853.
- Weitzman SA, Gordon LI. Inflammation and cancer: role of phagocytegenerated oxidants in carcinogenesis. *Blood* 1990;76:655–663.
- Engels EA. Inflammation in the development of lung cancer: epidemiological evidence. *Expert Rev Anticancer Ther* 2008;8:605–615.
- Sato M, Shames DS, Gazdar AF, Minna JD. A translational view of the molecular pathogenesis of lung cancer. J Thorac Oncol 2007;2:327– 343.
- Prendergast GC. Inflammatory mediators in cancer etiology and targets for therapy and prevention. *Cancer Reviews Online* 2008;9:17–18.
- Taraseviciene-Stewart L, Voelkel NF. Molecular pathogenesis of emphysema. J Clin Invest 2008;118:394–402.
- O'Donnell R, Breen D, Wilson S, Djukanovic R. Inflammatory cells in the airways in COPD. *Thorax* 2006;61:448–454.
- Sevenoaks MJ, Stockley RA. Chronic obstructive pulmonary disease, inflammation and co-morbidity: a common inflammatory phenotype? *Respir Res* 2006;7:70.
- Reynolds PR, Cosio MG, Hoidal JR. Cigarette smoke-induced Egr-1 upregulates proinflammatory cytokines in pulmonary epithelial cells. *Am J Respir Cell Mol Biol* 2006;35:314–319.
- Smith CJ, Perfetti TA, King JA. Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. *Inhal Toxicol* 2006;18: 667–677.
- Kim V, Rogers TJ, Criner GJ. New concepts in the pathobiology of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2008;5: 478–485.
- Dohadwala M, Yang SC, Luo J, Sharma S, Batra RK, Huang M, Lin Y, Goodglick L, Krysan K, Fishbein MC, *et al.* Cyclooxygenase-2dependent regulation of E-cadherin: prostaglandin E(2) induces transcriptional repressors ZEB1 and snail in non-small cell lung cancer. *Cancer Res* 2006;66:5338–5345.
- Charuworn B, Dohadwala M, Krysan K, Sharma S, Escuadro B, Dubinett SM. Inflammation-mediated promotion of EMT in NSCLC: IL-1b mediates a MEK/Erk- and JNK/SAPK-dependent downregulation of E-cadherin [abstract]. *Proc Am Thorac Soc* 2006;3:D96.
- Baratelli F, Lin Y, Zhu L, Yang SC, Heuzé-Vourc'h N, Zeng G, Reckamp K, Dohadwala M, Sharma S, Dubinett SM. Prostaglandin E<sub>2</sub> induces *FOXP3* gene expression and T regulatory cell function in human CD4<sup>+</sup> T cells. *J Immunol* 2005;175:1483–1490.
- Keshamouni VG, Michailidis G, Grasso CS, Anthwal S, Strahler JR, Walker A, Arenberg DA, Reddy RC, Akulapalli S, Thannickal VJ, et al. Differential protein expression profiling by iTRAQ-2DLC-MS/ MS of lung cancer cells undergoing epithelial-mesenchymal transition reveals a migratory/invasive phenotype. J Proteome Res 2006;5:1143– 1154.
- Leng Q, Bentwich Z, Borkow G. Increased TGF-β, Cbl-b and CTLA-4 levels and immunosuppression in association with chronic immune activation. *Int Immunol* 2006;18:637–644.
- Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, *et al.* The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.

- Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N Engl J Med 1961;265:253–267.
- Wistuba II. Genetics of preneoplasia: lessons from lung cancer. Curr Mol Med 2007;7:3–14.
- Wistuba II, Behrens C, Milchgrub S, Bryant D, Hung J, Minna JD, Gazdar AF. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. *Oncogene* 1999;18:643–650.
- 24. Wistuba II, Behrens C, Virmani AK, Mele G, Milchgrub S, Girard L, Fondon JW III, Garner HR, McKay B, Latif F, et al. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/ preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. *Cancer Res* 2000;60:1949–1960.
- Wistuba II, Berry J, Behrens C, Maitra A, Shivapurkar N, Milchgrub S, Mackay B, Minna JD, Gazdar AF. Molecular changes in the bronchial epithelium of patients with small cell lung cancer. *Clin Cancer Res* 2000;6:2604–2610.
- Wistuba II, Lam S, Behrens C, Virmani AK, Fong KM, LeRiche J, Samet JM, Srivastava S, Minna JD, Gazdar AF. Molecular damage in the bronchial epithelium of current and former smokers. J Natl Cancer Inst 1997;89:1366–1373.
- Franklin WA, Gazdar AF, Haney J, Wistuba II, La Rosa FG, Kennedy T, Ritchey DM, Miller YE. Widely dispersed p53 mutation in respiratory epithelium: a novel mechanism for field carcinogenesis. *J Clin Invest* 1997;100:2133–2137.
- Yashima K, Litzky LA, Kaiser L, Rogers T, Lam S, Wistuba II, Milchgrub S, Srivastava S, Piatyszek MA, Shay JW, *et al.* Telomerase expression in respiratory epithelium during the multistage pathogenesis of lung carcinomas. *Cancer Res* 1997;57:2373–2377.
- Mao L, Lee JS, Kurie JM, Fan YH, Lippman SM, Lee JJ, Ro JY, Broxson A, Yu R, Morice RC, *et al.* Clonal genetic alterations in the lungs of current and former smokers. *J Natl Cancer Inst* 1997;89: 857–862.
- Park IW, Wistuba II, Maitra A, Milchgrub S, Virmani AK, Minna JD, Gazdar AF. Multiple clonal abnormalities in the bronchial epithelium of patients with lung cancer. J Natl Cancer Inst 1999;91:1863–1868.
- Spira A, Beane J, Shah V, Liu G, Schembri F, Yang X, Palma J, Brody JS. Effects of cigarette smoke on the human airway epithelial cell transcriptome. *Proc Natl Acad Sci USA* 2004;101:10143–10148.
- Ammous Z, Hackett NR, Butler MW, Raman T, Dolgalev I, O'Connor TP, Harvey BG, Crystal RG. Variability in small airway epithelial gene expression among normal smokers. *Chest* 2008;133:1344–1353.
- 33. Landi MT, Dracheva T, Rotunno M, Figueroa JD, Liu H, Dasgupta A, Mann FE, Fukuoka J, Hames M, Bergen AW, et al. Gene expression signature of cigarette smoking and its role in lung adenocarcinoma development and survival. PLoS ONE 2008;3:e1651.
- 34. Wang IM, Stepaniants S, Boie Y, Mortimer JR, Kennedy B, Elliott M, Hayashi S, Loy L, Coulter S, Cervino S, *et al.* Gene expression profiling in patients with chronic obstructive pulmonary disease and lung cancer. *Am J Respir Crit Care Med* 2008;177:402–411.
- Choi IG, Kwon J, Kim SH. Local feature frequency profile: a method to measure structural similarity in proteins. *Proc Natl Acad Sci USA* 2004;101:3797–3802.
- 36. Spira A, Beane JE, Shah V, Steiling K, Liu G, Schembri F, Gilman S, Dumas YM, Calner P, Sebastiani P, *et al.* Airway epithelial gene expression in the diagnostic evaluation of smokers with suspect lung cancer. *Nat Med* 2007;13:361–366.
- Beane J, Sebastiani P, Whitfield TH, Steiling K, Dumas YM, Lenburg ME, Spira A. A prediction model for lung cancer diagnosis that integrates genomic and clinical features. *Cancer Prevention Research* 2008;1:56–64.
- Sridhar S, Schembri F, Zeskind J, Shah V, Gustafson AM, Steiling K, Liu G, Dumas YM, Zhang X, Brody JS, *et al.* Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. *BMC Genomics* 2008;9:259.
- Sidransky D. The oral cavity as a molecular mirror of lung carcinogenesis. *Cancer Prevention Research* 2008;1:12–14.
- Stearman RS, Dwyer-Nield L, Grady MC, Malkinson AM, Geraci MW. A macrophage gene expression signature defines a field effect in the lung tumor microenvironment. *Cancer Res* 2008;68:34–43.
- Hodge S, Hodge G, Ahern J, Jersmann H, Holmes M, Reynolds PN. Smoking alters alveolar macrophage recognition and phagocytic ability: implications in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2007;37:748–755.

- 42. Charles PC, Alder BD, Hilliard EG, Schisler JC, Lineberger RE, Parker JS, Mapara S, Wu SS, Portbury A, Patterson C, *et al.* Tobacco use induces anti-apoptotic, proliferative patterns of gene expression in circulating leukocytes of Caucasian males. *BMC Med Genomics* 2008;1:38.
- Huber MA, Kraut N, Beug H. Molecular requirements for epithelialmesenchymal transition during tumor progression. *Curr Opin Cell Biol* 2005;17:548–558.
- 44. Gotzmann J, Mikula M, Eger A, Schulte-Hermann R, Foisner R, Beug H, Mikulits W. Molecular aspects of epithelial cell plasticity: implications for local tumor invasion and metastasis. *Mutat Res* 2004; 566:9–20.
- Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003;112:1776–1784.
- Grunert S, Jechlinger M, Beug H. Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. *Nat Rev Mol Cell Biol* 2003;4:657–665.
- Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 2003;15:740–746.
- Dasari V, Gallup M, Lemjabbar H, Maltseva I, McNamara N. Epithelial-mesenchymal transition in lung cancer: is tobacco the "smoking gun"? Am J Respir Cell Mol Biol 2006;35:3–9.
- Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, *et al.* Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* 2005;436:123–127.
- 50. Thomson S, Buck E, Petti F, Griffin G, Brown E, Ramnarine N, Iwata KK, Gibson N, Haley JD. Epithelial to mesenchymal transition is a determinant of sensitivity of non-small-cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition. *Cancer Res* 2005;65:9455–9462.
- 51. Krysan K, Lee JM, Dohadwala M, Gardner BK, Reckamp KL, Garon E, St John M, Sharma S, Dubinett SM. Inflammation, epithelial to mesenchymal transition, and epidermal growth factor receptor tyrosine kinase inhibitor resistance. *J Thorac Oncol* 2008;3:107–110.
- 52. Heinrich E, Dohadwala M, Charuworn B, Dubinett S. Inflammationdependent regulation of epithelial-mesenchymal transition in nonsmall cell lung cancer: the role of interleukin-1b [abstract 5366]. In: *Proceedings of the 99th Annual Meeting of the American Association* for Cancer Research; 2008 Apr 12–16; San Diego, CA: AACR; 2008.
- Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, *et al.* The epithelialmesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704–715.
- 54. Yoshino I, Kometani T, Shoji F, Osoegawa A, Ohba T, Kouso H, Takenaka T, Yohena T, Maehara Y. Induction of epithelial-mesenchymal transition-related genes by benzo[a]pyrene in lung cancer cells. *Cancer* 2007;110:369–374.
- 55. Fondrevelle ME, Kantelip B, Reiter RE, Chopin DK, Thiery JP, Monnien F, Bittard H, Wallerand H. The expression of Twist has an impact on survival in human bladder cancer and is influenced by the smoking status. *Urol Oncol* (In press)
- 56. Lee G, Dohadwala M, Dubinett S. Chronic exposure to tobacco-specific 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces epithelial to mesenchymal transition in non-small cell lung cancer [abstract]. Am J Respir Crit Care Med 2008;177:A543.
- Abate-Shen C, Brown PH, Colburn NH, Gerner EW, Green JE, Lipkin M, Nelson WG, Threadgill D. The untapped potential of genetically engineered mouse models in chemoprevention research: opportunities and challenges. *Cancer Prevention Research* 2008;1:161–166.
- 58. Wislez M, Fujimoto N, Izzo JG, Hanna AE, Cody DD, Langley RR, Tang H, Burdick MD, Sato M, Minna JD, et al. High expression of ligands for chemokine receptor CXCR2 in alveolar epithelial neoplasia induced by oncogenic Kras. *Cancer Res* 2006;66:4198–4207.
- Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* 2004;6: 447–458.
- 60. Komarova EA, Krivokrysenko V, Wang K, Neznanov N, Chernov MV, Komarov PG, Brennan ML, Golovkina TV, Rokhlin OW, Kuprash DV, et al. p53 is a suppressor of inflammatory response in mice. FASEB J 2005;19:1030–1032.
- 61. Huang M, Stolina M, Sharma S, Mao JT, Zhu L, Miller PW, Wollman J, Herschman H, Dubinett SM. Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. *Cancer Res* 1998;58:1208–1216.

- Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimaki A. Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res* 1998;58:4997–5001.
- 63. Hida T, Yatabe Y, Achiwa H, Muramatsu H, Kozaki K, Nakamura S, Ogawa M, Mitsudomi T, Sugiura T, Takahashi T. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. *Cancer Res* 1998;58:3761–3764.
- Brabender J, Park J, Metzger R, Schneider PM, Lord RV, Holscher AH, Danenberg KD, Danenberg PV. Prognostic significance of cyclooxygenase 2 mRNA expression in non-small cell lung cancer. *Ann* Surg 2002;235:440–443.
- 65. Achiwa H, Yatabe Y, Hida T, Kuroishi T, Kozaki K, Nakamura S, Ogawa M, Sugiura T, Mitsudomi T, Takahashi T. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. *Clin Cancer Res* 1999;5:1001–1005.
- 66. Hosomi Y, Yokose T, Hirose Y, Nakajima R, Nagai K, Nishiwaki Y, Ochiai A. Increased cyclooxygenase 2 (COX-2) expression occurs frequently in precursor lesions of human adenocarcinoma of the lung. *Lung Cancer* 2000;30:73–81.
- Khuri FR, Wu H, Lee JJ, Kemp BL, Lotan R, Lippman SM, Feng L, Hong WK, Xu XC. Cyclooxygenase-2 overexpression is a marker of poor prognosis in stage I non-small cell lung cancer. *Clin Cancer Res* 2001;7:861–867.
- Kim HS, Youm HR, Lee JS, Min KW, Chung JH, Park CS. Correlation between cyclooxygenase-2 and tumor angiogenesis in non-small cell lung cancer. *Lung Cancer* 2003;42:163–170.
- Tsubochi H, Sato N, Hiyama M, Kaimori M, Endo S, Sohara Y, Imai T. Combined analysis of cyclooxygenase-2 expression with p53 and Ki-67 in nonsmall cell lung cancer. *Ann Thorac Surg* 2006;82: 1198–1204.
- Campa D, Zienolddiny S, Maggini V, Skaug V, Haugen A, Canzian F. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. *Carcinogenesis* 2004;25:229–235.
- Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology* 1994;5: 138–146.
- Krysan K, Merchant FH, Zhu L, Dohadwala M, Luo J, Lin Y, Heuze-Vourc'h N, Pold M, Seligson D, Chia D, et al. COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB J 2004; 18:206–208.

- Pold M, Krysan K, Pold A, Dohadwala M, Heuze-Vourc'h N, Mao JT, Riedl KL, Sharma S, Dubinett SM. Cyclooxygenase-2 modulates the insulin-like growth factor axis in non-small-cell lung cancer. *Cancer Res* 2004;64:6549–6555.
- Sharma S, Stolina M, Yang SC, Baratelli F, Lin JF, Atianzar K, Luo J, Zhu L, Lin Y, Huang M, et al. Tumor cyclooxygenase 2-dependent suppression of dendritic cell function. *Clin Cancer Res* 2003;9:961–968.
- Pold M, Zhu LX, Sharma S, Burdick MD, Lin Y, Lee PP, Pold A, Luo J, Krysan K, Dohadwala M, *et al.* Cyclooxygenase-2-dependent expression of angiogenic CXC chemokines ENA-78/CXC Ligand (CXCL) 5 and interleukin-8/CXCL8 in human non-small cell lung cancer. *Cancer Res* 2004;64:1853–1860.
- Dohadwala M, Batra RK, Luo J, Lin Y, Krysan K, Pold M, Sharma S, Dubinett SM. Autocrine/paracrine prostaglandin E2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclooxygenase-2-dependent invasion. J Biol Chem 2002;277:50828–50833.
- Subbaramaiah K, Altorki N, Chung WJ, Mestre JR, Sampat A, Dannenberg AJ. Inhibition of cyclooxygenase-2 gene expression by p53. *J Biol Chem* 1999;274:10911–10915.
- Csiki I, Yanagisawa K, Haruki N, Nadaf S, Morrow JD, Johnson DH, Carbone DP. Thioredoxin-1 modulates transcription of cyclooxygenase-2 via hypoxia-inducible factor-1alpha in non-small cell lung cancer. *Cancer Res* 2006;66:143–150.
- Mao JT, Cui X, Reckamp K, Liu M, Krysan K, Dalwadi H, Sharma S, Hazra S, Strieter R, Gardner B, *et al.* Chemoprevention strategies with cyclooxygenase-2 inhibitors for lung cancer. *Clin Lung Cancer* 2005;7:30–39.
- Heuze-Vourc'h N, Zhu L, Krysan K, Batra RK, Sharma S, Dubinett SM. Abnormal interleukin 10Ralpha expression contributes to the maintenance of elevated cyclooxygenase-2 in non-small cell lung cancer cells. *Cancer Res* 2003;63:766–770.
- Cui X, Zhang L, Luo J, Rajasekaran A, Hazra S, Cacalano N, Dubinett SM. Unphosphorylated STAT6 contributes to constitutive cyclooxygenase-2 expression in human non-small cell lung cancer. *Oncogene* 2007;26:4253–4260.
- Subbaramaiah K, Marmo TP, Dixon DA, Dannenberg AJ. Regulation of cyclooxgenase-2 mRNA stability by taxanes: evidence for involvement of p38, MAPKAPK-2, and HuR. J Biol Chem 2003;278: 37637–37647.