COPD, INFLAMMATION, AND LUNG CANCER

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ABSTRACT

Both lung cancer and COPD are associated with cigarette smoking, which by generating reactive oxidant species, induces a chronic inflammatory state in the lung. Activation, particularly of NF-kB occurs in both cancer and COPD, and expression of a number of genes is altered in both diseases. In lung cancer DNA damage, lack of DNA repair and genomic instability predominate whereas matrix degradation, lack of repair and an intense immune response predominate in COPD. The reasons for the different responses to a common inflammatory response induced by smoking remain to be determined, but likely lie in genetic polymorphisms in genes that regulate genome integrity in cancer and that regulate the immune response to tissue destruction in COPD.

Key Words: Reactive oxygen species, Inflammation, genomic instability, lung immune reponse.

It has long been known that cigarette smoke plays a causal role in both COPD and lung cancer. In addition, smokers who have COPD appear to be at increased risk for developing lung cancer, suggesting that there is some link between the processes that induce COPD and those that induce lung cancer [1;;2;;3]. The odds ratio, or relative risk for developing lung cancer, increases 1.4-2.7 fold with moderate COPD and 2.8-4.9 fold with severe COPD. The likelihood of developing lung cancer within 10 years is 3 fold greater in subjects with mild to moderate COPD vs smokers with normal lung function and close to 10 fold greater in subjects with severe COPD[1]. While cumulative smoking history increases the risk for developing both diseases, in trying to understand disease pathogenesis, it is important to recognize that the majority of smokers develop neither COPD nor lung cancer.

Chronic Inflammation

Cigarette smoke contains an extremely high concentration of oxidants along with a number of known carcinogens [4]. The reactive oxidant species (ROS) generated by smoking induce inflammation in the lung and its airways as well as causing mutations in airway epithelial cell DNA. The risk of developing lung cancer does not disappear after smoking has been discontinued. In the United States lung cancer now occurs in as many former smokers as current smokers [5]. We have recently shown that expression of a large number of genes is altered in the airway epithelial cells of smokers [6]. Expression of many of these genes, especially anti-oxidant and drug metabolizing genes, returns to normal within 2 years of smoking cessation, but expression of a number of putative oncogenes

and tumor suppressor genes remains altered for decades after smoking has been discontinued. These genes may be in part responsible for the occurrence of lung cancer years after individuals have stopped smoking. In a similar fashion, the local lung inflammation initiated by ROS in cigarette smoke, persists in COPD subjects after they have stopped smoking, generating smoking independent oxidant stress and explaining the persistence and progression of the disease after smoking has been discontinued [7].

Chronic inflammation has been shown to lead to cancer in a number of organs. Reflux esophagitis, H.pylori gastric inflammation, viral hepatitis, ulcerative colitis, and cigarette smoking are all associated with chronic inflammation and an increased incidence of local cancers [8]. In ulcerative colitis, DNA mutational “fingerprints” of dysplastic and normal colonic crypts show that DNA mutations spread by clonal expansion of proliferating cells and by fusion of adjacent crypts moderated in part by crypt cell turnover and cell death [9]. The clonal expansion of crypt cells occurs because of the growth advantage and apoptosis resistance of cells that has been induced by continued inflammation and generation of ROS.

There is considerable evidence that links chronic inflammation, the transcription factor NF-kB, and cancer [10]. Recent studies have also demonstrated the synergistic interaction of the classic mediator of inflammation, NFkB, and the classic tumor suppressor gene, p53, which acts as a general inhibitor of inflammation [10]. P53 acts as an inhibitor of transcription of a number of genes with NFkB-dependent promoters. As a result, cytokines, macrophage activation,

and markers of inflammation are increased in mice with absent p53 who are injected with lipopolysaccharide compared to wild type mice with functional p53. Since p53 is often mutated by cigarette smoke, in COPD and in lung cancer, one might expect that oxidant activation of NFkB-mediated inflammation might be excessive, absent the suppressive effect of p53.

The genetic link between chronic lung inflammation and lung cancer has recently been reviewed by comparing QTL (quantitative trait loci, or chromosomal locations of putative susceptibility genes) in mouse models of chronic lung inflammation and mouse models of lung cancer [11]. Combined susceptibility loci have been identified at 8 chromosomal sites for genes such as Kras, TNFalpha, etc. The authors also point out that a number of animal studies demonstrate the anti-tumor effects of anti-inflammatory drugs.

Gene Expression Profiling:

One way to explore the relation between COPD and lung cancer is to compare global gene expression in resected cancerous and emphysematous lung tissue using high-density gene expression arrays. A number of papers have detailed patterns of gene expression in various lung cancer cell types and stages of lung cancer [12]. Similar studies from other types of cancer and many in vitro studies have been summarized in a now classic “Hallmarks of Cancer” paper [13] (see Table 1). Until recently, there have been no such studies of lung tissue from COPD subjects. Three papers using different gene expression platforms in different COPD populations have generated somewhat similar findings in terms of functional categories of genes altered in emphysema (although individual

genes significantly vary due to differences in study design and data mining) [14-16]. Together with previous studies reporting expression of specific genes and proteins in lungs and bronchial biopsies of COPD subjects [17], a “Hallmarks of COPD” can be constructed (see Table 1). Despite the common inciting agent, cigarette smoke, that generates ROS and inflammation in both COPD and lung cancer, the resulting biologic processes differ considerably. In cancer uncontrolled cell proliferation, lack of cellular apoptosis, tissue invasion and angiogenesis predominate. In COPD, apoptosis, matrix degradation, inflammation and local and systemic immune responses predominate. The reasons for these rather different responses to a common causal agent, cigarette smoke, which presumably generates ROS and inflammation in all smokers, remain unclear.

In order to explore the pathogenetic events associated with cigarette smoke and subsequent initiation of COPD and lung cancer, we have begun to examine the ways in which relatively easily accessible airway epithelial cells respond to cigarette smoke and the differences that occur in smokers with and without COPD or lung cancer. Our studies are based on the well-established concept that smoking induces a field of epithelial cell injury [6;18]; i.e. all airway epithelial cells are exposed and react to cigarette smoke, and therefore the expression of genes in relatively accessible airway epithelial cells might provide insights into the COPD or cancer generating processes that smoking has initiated in the lung as a whole. A number of studies have documented various forms of DNA injury, such as loss of heterozygocity and DNA methylation, in bronchial

biopsies and brushings of airways in smokers both with and without lung cancer and some have found similar changes in buccal mucosal biopsies. Our laboratory has been using high-density oligonucleotide arrays to measure gene expression profiles of large airway epithelial cells obtained at bronchoscopy in normal non-smokers, current and former smokers with and without lung cancer and current and former smokers with COPD. Our initial studies defined the normal airway transcriptome, i.e. the genes expressed in large airway epithelial cells of normal non-smokers [6]. We next explored how the airway epithelial cell transcriptome changes in cigarette smokers. We found ~ 100 genes that differed between smokers and non-smokers at a highly significant level. Many the genes whose expression levels increased were drug metabolizing (xenobiotic) and anti-oxidant genes. Several of the genes whose expression increased were oncogenes and several whose expression decreased were putative tumor suppressor and immunomodulatory genes. Expression of a number of genes correlated with cumulative smoking history. We have also found that gene changes, corrected for smoking history, were greater and often different in African Americans than in Caucasians (manuscript in preparation) and lung cancer appears to be more frequent in African Americans [19]. Many of the genes that changed in smokers returned to non-smoker levels within several years of smoking cessation, although ~15% (mostly oncogenes and tumor suppressor genes) do not return to normal even 20-30 years after smoking cessation, consistent with the observation that risk for developing lung cancer remains high for decades after smoking cessation.

We have since extended these studies to current and former smokers with lung cancer and have begun to study current and former smokers with COPD. It is clear that subjects with lung cancer have a unique gene expression profile in airway epithelial cells that distinguish them from comparable subjects without lung cancer and this profile may have value as a diagnostic tool. Preliminary studies of subjects with COPD suggest that there may also be a gene expression profile that is characteristic of COPD. We have already identified a number of genes whose expression levels correlate in a negative or positive fashion with FEV1 and are not altered in subjects with lung cancer.

While our cancer studies may provide new tools for the early diagnosis of lung cancer, gene expression profiles will not replace the FEV1 or chest CT scans for the diagnosis of COPD. We believe that the ultimate value of such studies lies in the integrative analysis of expression data utilizing increasingly sophisticated bioinformatic approaches for defining transcriptional activation and protein-protein interaction networks and signaling pathways. It is this parsing of expression data that will begin to provide fundamental biologic insights into disease pathogenesis and will ultimately identify potential therapeutic targets for preventing or treating disease.

Pathways To Lung Cancer and COPD:

Despite all of the studies noted above, the reason that some smokers develop COPD, some develop lung cancer, and some are free of disease remains unclear. Figure 1 depicts similarities and differences in the major biologic and molecular events that lead to each disease and provides some ideas

about pathogenetic differences between COPD and lung cancer. Smoke contains high concentrations of oxidants and free radicals (ROS) along with thousands of particulates. Local antioxidant and metabolizing enzymes inactivate many potentially toxic species and in the process often generate more reactive oxidant species. As noted earlier, NF-kB activation and subsequent transactivation of inflammation-related genes appears to play a central role in both COPD and cancer. These events occur to some extent in all smokers, even those without evident lung disease. Which genes are activated and which are suppressed in smokers may be a major determinant of whether a smoker remains disease free, or develops cancer or COPD. In COPD, matrix degradation and excessive apoptosis, with loss of blood vessels and incomplete tissue repair predominates. In lung cancer, excessive DNA damage and incomplete DNA repair predominate. The diseases diverge further with genomic instability causing further chromosomal abnormalities resulting in clonal expansion of cells that have a growth advantage occurring in cancer, while an intense immune response and further inflammation predominate in COPD. The process by which these events diverge is unclear; it may be a consequence of random mutations in DNA. More likely, heritable genetic polymorphisms influence susceptibility to DNA or connective tissue damage, efficiency of DNA or connective tissue repair, the intensity of immune responses to constituents of tobacco smoke or to degraded connective tissue, or genomic instability, determine the disease pathway taken. It is also likely that genetic factors explain the absence of either disease in most smokers. We can certainly learn as much

from studying the genomics and genetics of individuals who have substantial smoking histories and no evidence of either COPD or cancer as we can from studying smokers with cancer or COPD.

The recent focus on smoking-related lung disease and on COPD and lung cancer, and the advances that have been made in defining important pathogenetic mechanisms hold promise for clarifying the relation between inflammation, cancer, and COPD, and for identifying potential therapeutic targets in the next decade.

TABLE 1: Unique Biologic Features of Lung Cancer and COPD

HALLMARKS OF CANCER( )	HALLMARKS OF COPD
Evading apoptosis	Increased apoptosis
Self-sufficiency of growth	Matrix degradation
Insensitivity to anti-growth	Ineffective tissue repair
Tissue invasion	Intense immune/inflammation
Sustained angiogenesis	Limited angiogenesis
Limitless replication	

Figure 1: Inflammation-related Pathways in Lung Cancer and COPD

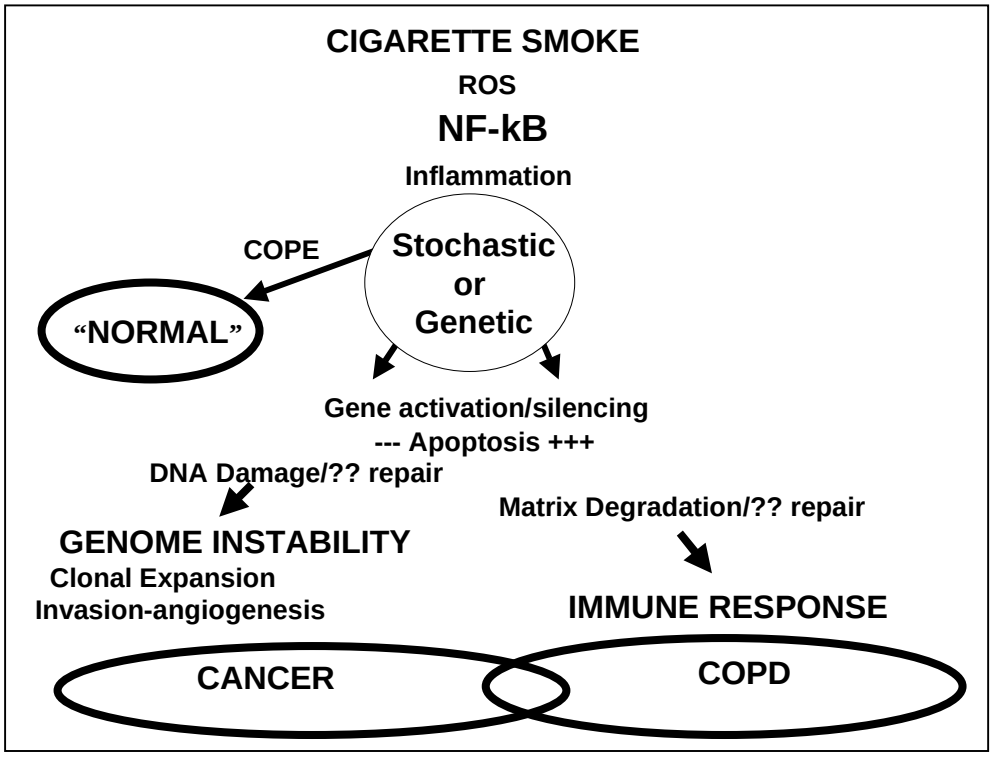

Figure 1: Pathways to lung cancer or COPD.
See text for explanation

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