Chronic obstructive pulmonary disease and lung cancer: inflammation, the missing link

The link between chronic obstructive pulmonary disease (COPD) and lung cancer has been known for over 40 years. A total of 50–70% of patients diagnosed with lung cancer have COPD, and COPD is an important risk factor for lung cancer. Only recently have advances in both basic science and clinical studies shed light on the links between them, with dysregulated inflammation playing a central role in the pathogenesis of both diseases. The development of COPD and lung cancer share many common biochemical and cellular mechanisms. In addition, the critical importance of inflammation leading to cancer initiation and progression is becoming increasingly apparent. Understanding the common processes/molecules that are central to the pathogenesis of both COPD and lung cancer, and the interactions between chronic infection, inflammation and cancer, may help identify novel therapeutic strategies to prevent and treat lung cancer, in addition to identifying biomarkers to target those at high risk of developing this disease.

KEYWORDS: COPD ■ family history ■ infection ■ inflammation ■ lung cancer ■ screening

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Lung cancer is the leading cause of cancer death in the world, and chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality, representing the second and fourth leading cause of death worldwide. The overall 5-year survival for lung cancer is 15%, and 1-year survival is approximately 42%. In the UK, there may be as many as 1.5 million people with COPD [1]. COPD is responsible for 126,000 deaths in the USA [2], and 26,000 deaths in the UK [1]. With the increase in cigarette smoking in developing countries, especially China, COPD is expected to become the third leading cause of death worldwide by 2020 [1]. Both are major health problems and are likely to continue to be so for the foreseeable future.

A total of 50–70% of patients diagnosed with lung cancer also have COPD and a history of tobacco use. Compared with nonsmokers, smokers have as much as a 30-fold increased risk of developing cancer [3,4]. However, only a minority of long-term smokers develop COPD. Therefore, complex mechanisms and interactions determine an individual's own risk of developing COPD, lung cancer or both, including environmental exposures and host susceptibility factors.

COPD and low forced expiratory volume in 1 s (FEV_1) are major risk factors for lung cancer, increasing the risk 4.5-fold compared with comparative smokers with normal lung function [5]. As far back as 1863, Virchow hypothesized that malignant neoplasms occurred at sites of chronic inflammation. He believed that various 'irritants' caused tissue injury, inflammation and increased cell proliferation [6,7], with consequent cancer development. COPD is characterized by inflammation-mediated destruction of the extracellular matrix (ECM). Shared inflammatory pathways drive the pathogenesis of COPD and lung cancer (Figure 1). This review will explore the links between lung cancer and COPD at the clinical level, examine the basic biological commonalities between the two diseases, with emphasis on directly overlapping disease-generating pathways, and finish by exploring how this knowledge may impact on future prevention and treatment of lung cancer.

COPD & lung cancer

The association between COPD and lung cancer has been long known. In 1956, Francis Lowell and colleagues wrote that 'During the last 2 years a number of patients with chronic obstructive emphysema have been observed while under treatment with bronchodilator agents…, in the course of which our attention was drawn to an association between pulmonary emphysema, carcinoma of the lung and a high incidence of smoking' [8]. It was of course Sirs Richard Doll and Austin Bradford Hill who first highlighted the links between smoking and lung cancer [9]. It should be noted though that a link had already been suggested by Muller as far back as 1939 [10]. Doll and Hill asked 20 London hospitals to notify them of all patients admitted with lung cancer (as well as cancer of the stomach, colon or

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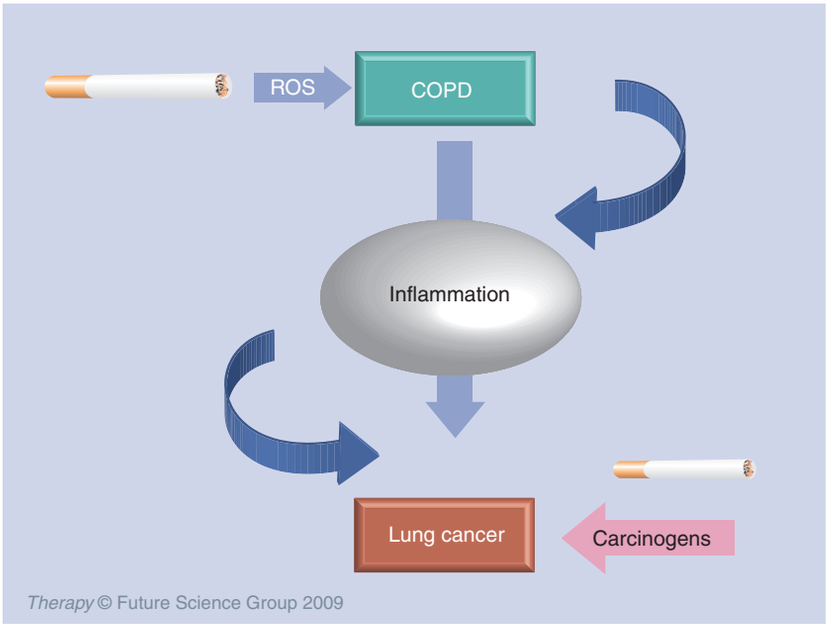

Figure 1. COPD and cancer: inflammation, the missing link.
COPD: Chronic obstructive pulmonary disease; ROS: Reactive oxygen species.

rectum). Initially an 'almoner' visited the hospital to interview the patient, but it soon became apparent that interviewing all patients was not feasible. In total, 709 patients with lung cancer were reported in their study, and after review of the data the authors concluded that 'there is a real association between carcinoma of the lung and smoking'.

Is there a link?

Clinical studies

The first published connection between COPD and lung cancer was a case report by Goldstein and colleagues in 1968 [11]. This was closely followed by a radiographic survey in males that showed that the prevalence of lung cancer in those with bullous disease of the lung was 61 per 1000, 32-times the rate in those without bullous disease [12]. The first prospective (unmatched) study of COPD and lung cancer was performed by Davis and colleagues [13]. They followed 835 patients at the Bellevue Emphysema Clinic in New York from 1955 to 1974, and found an incidence of lung cancer of 11.6 per 1000 man years, which was 4–5-times higher than was previously reported in smokers. Of the 32 patients diagnosed with lung cancer, only two survived more than 5 years. The two who survived both had lung resections and were alive when the paper was published, 11 and 13 years after the first recognition of their tumors. The first matched study was published by Skillrud and colleagues [5]. They followed patients at the Mayo Clinic, Minnesota, USA, for 10 years; there were 186 men and 40 women in the study aged between 45 and 59 years. Lung cancer developed in nine cases and two controls, giving a 4.4-times greater risk for lung cancer among those with COPD. However, the small sample size of this study did not permit controlling for the effects of cigarette smoking. The following year, Tockman and colleagues published a study that confirmed a greater risk of lung cancer associated with COPD [14]. The study population consisted solely of men who had previously been enrolled in the Intermittent Positive Pressure Breathing Trial and Johns Hopkins Lung project. Airways obstruction was defined as an FEV_1 of less than 60%, and all participants had a chest radiograph to exclude lung cancer prior to study entry. The presence of COPD was more of an indicator for subsequent development of lung cancer than age or quantity of smoking. The risk of lung cancer also increased in proportion to the degree of COPD. The proportion of risk was similar to that found in the Skillrud study. Further evidence of the risk of lung cancer among those with airway obstruction was reported in the results of the Lung Health Study [15]. Among nearly 6000 smokers with mild-to-moderate airway obstruction, lung cancer was the most common cause of death at the end of the 5 years of follow-up.

The two major features of COPD are destruction of the alveoli in the lung resulting in airspace enlargement (emphysema), and narrowing of the breathing tubes resulting in airways obstruction (airways disease). With the advent of computerized tomography (CT) scanning, investigators were not only able to look at the links between COPD and lung cancer, they were able to tease out independent risks between emphysema or airways obstruction and lung cancer. The study by Kishi and colleagues [16] confirmed previous results showing an increased risk of lung cancer associated with airway obstruction. They recruited 1520 participants over the age of 50 years (52% male, 48% female) who were current or former smokers with a greater than 20 pack-year smoking history. They were recruited over a period of 1 year in 1999. They discovered that the likelihood of developing lung cancer was significantly increased for those with an FEV_1 of less than 40% of predicted. However, percentage of emphysema as determined by low-dose spiral CT was not associated with an increased risk of lung cancer. In contrast to this, the study by de Torres and colleagues found that the presence of

emphysema on low-radiation-dose CT (LDCT) was an independent risk factor for lung cancer [17]. In this study, 1166 participants (74% male, 26% female) over the age of 40 years with a greater than 10 pack-year smoking history were recruited. The incidence density of lung cancer among individuals with and without emphysema on LDCT was 25 per 1000 person-years and 7.5 per 1000 person-years, respectively. Multivariate analysis showed that the presence of emphysema on LDCT, but not airway obstruction, was associated with an increased risk of lung cancer after adjusting for potential confounders. The disparities between the Kishi and de Torres papers likely reflect methodological differences between the two studies [17]. Firstly, the Kishi study followed a case–control design in which only a small subgroup of individuals of the entire cohort were used in the analysis. In the de Torres study, the entire population was included in the analysis. Secondly, the proportion of subjects with airways obstruction that were included in the analysis was 67% and 79% in the control and lung cancer groups, respectively, in the Kishi study, and 25% in the de Torres study. Lastly, the method to determine the presence of emphysema was quantitative in the Kishi study and by visual assessment in the de Torres study. In the former, participants were subclassified into groups depending on the amount of emphysema, whereas in the de Torres study, only the presence or absence of emphysema was considered for the analysis. More recently, the study by Wilson and colleagues [18] has shown that in high-risk individuals, emphysema on CT scan and airflow obstruction on spirometry are related to lung cancer. A total of 3638 current and ex-smokers aged over 50 years were included in their study (51% male, 49% female). Patients were screened with CT and evaluated with spirometry. The authors correlated visually graded emphysema severity on CT with emphysema risk factors and airflow obstruction. They then examined lung cancer related to airflow obstruction and radiographic emphysema, and as previously shown by de Torres and colleagues, discovered that lung cancer is related to radiographic emphysema independent of airflow obstruction and smoking. They also showed that airflow obstruction was related to lung cancer independent of smoking.

Does the link exist in nonsmokers?

In the de Torres study, the association between emphysema detected on LDCT and lung cancer risk was found even among individuals with normal airway function. Their results are consistent with previous retrospective and epidemiological reports [19–22], and have recently been validated in a much larger study [18]. Interestingly, the study by Turner and colleagues suggests that there is an increased incidence of lung cancer in lifelong nonsmokers with emphysema, in keeping with evidence presented by Cohen as far back as 1980 [23]. The Turner study [22] used data from the Cancer Prevention Study cohort, a study established by the American Cancer Society. Nearly 1.2 million study participants were enrolled by over 77,000 volunteers in 1982. Participants were recruited in all 50 states of the USA, as well as the District of Columbia and Puerto Rico. Participants were at least 30 years of age at baseline and were followed up for 20 years. A total of 448,600 lifelong nonsmokers with physician-diagnosed chronic bronchitis and emphysema that were cancer-free at baseline were included in the analysis. Lung cancer mortality was significantly associated with both emphysema and with the combined end point of emphysema and chronic bronchitis in analyses that combined men and women. No association was observed with chronic bronchitis alone in the overall analysis, although the association was stronger in men than women. The association between emphysema and lung cancer was stronger in analyses that excluded early years of follow-up.

α1-antitrypsin deficiency

α1-antitrypsin deficiency (α1-ATD) is a common genetic disorder that especially affects populations of European extraction. It can lead to early-age onset of emphysema in homozygous individuals [24]. The potential link between α1-ATD and lung cancer was first described by Harris, Primack and Cohen in 1974 [25]. They found elevated serum levels of α-1 antitrypsin in 73 patients with nonresectable lung cancer. α1-ATD carriers (heterozygous individuals) do not normally have severe α1-ATD-related diseases, and most of them are not aware of their carrier status. These carriers may be more vulnerable to carcinogen-containing tobacco smoke than noncarriers [24]. A study by Yang and colleagues sought to evaluate three risk factors (α1-ATD, COPD and cigarette smoking) together and their interplay in lung cancer development, and to estimate their relative contributions to lung cancer risk in the general population [26]. During the period 1997–2003, 1856 patients with lung cancer were enrolled in the study and matched with unrelated controls

and with full sibling controls. Among never smokers, they found that α1-ATD allele carriers were at a 2.2-fold increased risk for lung cancer compared with noncarriers after adjusting for age, sex and history of unspecified COPD. Among moderate to heavy smokers (≥20 pack-years), the α1-ATD allele-associated lung cancer risk was estimated at 2.3-fold, whereas among light smokers (<20 pack-years), the α1-ATD allele-associated lung cancer risk was twofold. Having a history of COPD increased lung cancer risk significantly for all three groups of smokers (from 2.5- to 5.9-fold), with the largest effect on never smokers. Stratified analysis by tumor histological subtypes showed a significant increase for adenocarcinoma and squamous cell carcinoma (SCC) among α1-ATD carriers.

Family history

When looking at any component of lung cancer, it is important to comment on family history and predisposition to lung cancer. In 1963, Tokuhata and Lilienfeld [27] provided the first epidemiological evidence of familial aggregation of lung cancer, suggesting the interaction of genes, shared environment and common lifestyle factors in the etiology of the disease. There is now a substantial body of evidence for a hereditable component in lung cancer risk based on animal models [28,29], family studies [30,31], linkage analysis [32] and candidate gene association/genome-wide association studies [33,34]. A family history of lung cancer has been consistently shown to be associated with increased lung cancer risk among nonsmokers [35,36], as well as smokers. In a recent large case–control study with 1946 lung cancer cases, 2116 controls and their first-degree relatives, Gao and colleagues [37] found that a mother's, father's and sibling's history of lung cancer were significantly associated with increased lung cancer risk after adjusting for the matching variables, education and, most notably, personal history of smoking.

Chronic inflammation & lung cancer

What then are the links between two diseases that seem to be diametrically opposed? Lung cancer arises as a result of uncontrolled cell proliferation, whereas COPD is characterized by inflammation-mediated destruction of the ECM and cell death.

Cell proliferation in COPD & lung cancer

As far back as the early 1960s, Passey hypothesized that it was the irritating properties of tobacco smoke, resulting in inflammatory destruction of lung tissue, which were of pathogenic significance in causing lung cancer, rather than any direct action by particulate carcinogens in tobacco smoke [38]. In the mid-1980s, Tockman [14] and Skillrud [5] independently demonstrated that lung cancer incidence increased in individuals with COPD as their FEV_1 declined, even after correction for lifetime cigarette smoke dosage. Thus the stage was set for a link between inflammation and lung cancer, despite the mechanistic processes appearing to be completely unrelated.

One hypothesis linking lung cancer and COPD has recently been proposed by Houghton, Mouded and Shapiro [39]: emphysema usually arises as a consequence of low-grade inflammation induced by cigarette smoking. There is resultant production of matrix-degrading proteases, particularly elastases, and recruitment of inflammatory cells such as neutrophils and macrophages. If the ECM destruction and cell death exceeds reparative capacity leading to airspace enlargement, then emphysema results. Bronchoalveolar stem cells (BASCs) are located in the mouse small airway and have been hypothesized to replenish the peripheral portions of the lung where emphysema develops [40]. As emphysema is caused by chronic, persistent inflammation rather than a single inflammatory insult, the otherwise quiescent BASCs are under constant pressure to repopulate the damaged areas of the lung, proliferate, and ultimately restore the lung architecture. This repeated lung injury and repair driven by chronic inflammation and tissue damage results in increased BASCs proliferation in conjunction with smoking/inflammation-related genetic damage and epithelial to mesenchymal transition (EMT), and may result in cancerous transformation of lung epithelial cells. BASCs are already known to give rise to lung adenocarcinoma in the mouse model of K-ras-induced malignancy [40], and it is tempting to speculate [39] that these cells are a key link between COPD and lung cancer. Furthermore, recent studies have suggested that inhaled corticosteroids may reduce the risk of lung cancer in patients with COPD [41,42].

Infection in COPD & lung cancer

It has been known for some time that chronic infections causing inflammation predispose to cancer. Chronic infection with hepatitis B virus or hepatitis C virus is causally associated with more than 80% of the global incidence of hepatocellular carcinoma [43]. Epstein–Barr

virus, a herpes DNA virus, has been implicated in the pathogenesis of Hodgkin's disease and non-Hodgkin's lymphoma [44]. *Helicobacter pylori* has been identified as a major cause of multifocal atrophic gastritis and gastric carcinoma [45]. In all of these instances there is a chronic inflammation that precedes the onset of cancer.

In contrast to the sterile airways of a healthy lung, in stable COPD there are usually respiratory pathogens present. Microbial colonization and associated inflammation develop early in the course of the disease in smokers with non-obstructive chronic bronchitis and may persist in ex-smokers with COPD [46,47]. Maintenance of a pathogen-free environment in a healthy lung is dependent on an efficient innate lung defense system. Many disruptions of innate lung defense occur in COPD [48]. It is the failure of the innate immune system in the lung that allows the establishment of chronic infection. As chronic infection takes hold, respiratory pathogens further disrupt the innate lung defense, setting up a vicious circle of infection, inflammation and COPD progression [48]. Experiments in mice have shown that infecting the lung with nontypeable *Haemophilus influenza* caused a COPD-like bronchial inflammation, which promotes lung cancer development [49].

Nonbacterial pathogens may also increase the risk of lung cancer as a result of persistent inflammation. For example, the co-existence of TB and lung cancer has been described for nearly 200 years, having initially been reported by Bayle in 1810 [50]. Since then, it has been reported that pulmonary TB may co-exist with a variety of histological types of lung cancer. For example, close association of metastatic SCC of the lung with TB has been documented [51]. A study in India reported that TB lesions, either active or healing, were found in the lungs of 30–33% of patients with bronchogenic tumors as compared with 7% in the general population [52]. An interesting finding was described by Zheng and colleagues who reported that TB-infected patients were at increased risk of lung cancer that tended to arise on the same side of the chest as the TB [53]. It has been tempting to speculate that low-grade inflammation as a consequence of the TB infection has resulted in the increased risk of the development of lung cancer, and this in fact has recently been demonstrated experimentally: in an elegant study by Nalbandian and colleagues [54], they found that chronic TB infection in the lungs was sufficient to cause a multistep transformation of cells associated with TB lung lesions through squamous cell dysplasia to malignant SCCs. Furthermore they showed TB-infected macrophages produce not only DNA-damaging reactive oxygen and nitrogen species (ROS/RNS), but also a potent member of the EGF family called epiregulin, which they believe may serve as a paracrine growth factor at the initial steps of tumorigenesis. They also identified a powerful genetic modifier of TB-induced lung tumorigenesis in their model: the genetic locus *sst1*, which specifically controls tissue damage and TB progression in the lungs.

Finally, there has been a debate for several years about the association between HIV and lung cancer. It was recognized in the pre-highly active antiretroviral therapy era that there was an increased rate of lung cancer in patients with HIV [55,56], and it has been speculated that this increase is not related to immune suppression or HIV *per se*, but merely to an increased proportion of HIV-positive patients smoking cigarettes [57,58], which in itself has been recognized for some time [59,60]. While the cause of this phenomenon remains unclear, work with tumor samples shows that HIV-related lung cancers have increased genetic instability, which, in turn, could predispose to the development of lung cancer [61].

DNA damage, inflammation & lung cancer

Human lung is highly vascularized and provides an enormous surface area that is vulnerable to a wide range of toxic and infectious agents with the potential to induce oxidative damage. An average adult inhales approximately 10,000 liters of air per day, polluted with cigarette smoke, vehicle exhaust fumes, soot, sulfur dioxide, nitrogen dioxide and other pollutants [62]. Inhalation of such toxic pollutants and microorganisms results in lung injury and generation of ROS/RNS such as superoxide, hydrogen peroxide and nitric oxide, leading to cascades of signaling events that trigger production of pro-inflammatory cytokines and chemokines [63]. These reactive species help in killing the pathogen, and the inflammation often subsides after the assaulting agent is removed or following completion of the repair process. However, repeated tissue damage and regeneration produce increased ROS/RNS from inflammatory cells, which then interact with DNA in proliferating epithelium, resulting in permanent genomic alterations such as point mutations, deletions or re-arrangements [64]. Although cells respond to DNA damage by activating p53-controlled genes associated

with cell cycle and DNA repair, when the rate of ROS/RNS-mediated DNA damage is extensive, it leads to chronic inflammation and subsequent transformation of cells to a malignant phenotype [65], increasing the risk of developing lung cancer.

Remodeling of chromatin may induce posttranslational modifications of core histone proteins and DNA methylation, which have been shown to regulate pro-inflammatory gene expression during the development of both COPD and lung cancer. Increased histone acetylation occurs on the promoters of pro-inflammatory genes in alveolar macrophages and airway epithelial cells in patients with COPD. The amount of acetylation is positively correlated with disease severity [66]. The mechanisms that result in hyperacetylation of histones and nonhistone proteins in lungs of patients with COPD have been shown to be associated with reduced levels and activity of histone deacetylase (HDAC)2 [66,67]. This has also been reported in the lungs of rodents exposed to cigarette smoke [67,68]. Therapeutic strategies aimed at increasing levels and activity of HDAC2, such as by phenolic antioxidants and theophylline, are being investigated with regards to the lung inflammatory response and to ascertain whether they reduce corticosteroid resistance in patients with COPD [69]. Methylation of the p16 promoter is commonly observed in the sputum of patients with COPD, and is positively correlated with heavy cigarette smoking, suggesting the involvement of DNA methylation in COPD.

Lung cancer demonstrates major alterations in chromatin structure. Genome-wide DNA demethylation with site-specific hypermethylation has been shown to occur in lung cancer cells with resultant silencing of a variety of tumor-suppressor genes by the recruitment of HDACs. The mechanisms by which these processes may occur include aberrant expression or activity of DNA methyltransferases and histone demethylases in cancer cells. Methylation in the promoters of several genes has been reported in adenocarcinomas and non-small-cell lung carcinoma (NSCLC), and is associated with tumorigenesis and recurrence [70]. As a result, identification of DNA methylation on specific genes may serve as useful biomarkers for early detection or chemoprotective intervention in lung cancer. Modifications of core histone proteins increases the amount of epigenetic alterations mediated by aberrant DNA methylation in cancer cells. Increased HDAC1 correlates with advanced stage of disease and outcome in patients with lung cancer. DNA demethylating agents and HDAC inhibitors may synergistically cause apoptosis of lung cancer cells, and therefore prevent lung cancer genesis in animals exposed to tobacco carcinogens [71]. There is already clinical data available for HDAC inhibitors such as *N*-acetyldinaline and vorinostat in the treatment of advanced NSCLC [72], and these compounds are being investigated in randomized Phase III trials.

The mismatch repair (MMR) pathway plays an important role in repairing mismatches, which are small insertions and deletions that take place during DNA replication [73]. Failure of the MMR pathway commonly results in a type of genetic instability termed microsatellite instability, which is manifest as increased rates of DNA replication errors throughout the genome. Inflammation downregulates MMR proteins by a variety of mechanisms [74,75]. Inflammation also downregulates components of the nucleotide excision repair [76] and base excision repair [77] pathways, both of which help to protect against DNA damage.

Adaptive immune responses in COPD & lung cancer

COPD is associated with the infiltration of macrophages, neutrophils, $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, B cells and eosinophils into the bronchi. Macrophages, neutrophils and lymphocytes orchestrate the chronic inflammation seen in the airways of patients with COPD, and are the main amplifiers in the progression of COPD and emphysema. Inflammatory cells cause alveolar wall destruction and mucin hypersecretion as a result of the release of oxidants, proteases and granzymes. Adaptive immunity also plays an important role in the pathogenesis of COPD. Mature lymphoid follicles with germinal centers and separated T and B cell zones occur in lungs of patients with COPD [78,79]. These follicles are rare in the lungs of nonsmokers and their presence correlates with the severity of COPD [78]. The cause of these follicles is unclear, but may be due to the large antigen load associated with viral and bacterial infections, or to increased neoantigens from degraded ECM or carbonyl-modifying proteins by cigarette smoke resulting in autoimmune impairment in severe COPD [80,81]. The presence of these inflammatory cells can be subverted by lung cancer cells to generate a tumor-promoting microenvironment.

An inflammatory cell infiltrate, varying in size, composition and distribution, is present in most, if not all, tumors (Figure 2). Its components

include tumor-associated macrophages (TAMs) and related cell types, as well as mast cells and T cells. Studies have been published suggesting that these bone marrow-derived components may be involved in tumorigenesis [82–84].

There has been much interest recently in the role of TAMs in tumor development. They have been described as 'obligate partners for tumor-cell migration, invasion and metastasis' [85]. Macrophages display a high degree of heterogeneity and can adapt or alter their phenotype to suit the microenvironment in which they reside.

Several papers have looked to address the role of TAMs in lung cancer. In murine models of lung cancer, macrophages are typically elevated compared with control mice during the promotion stage and thereafter [86,87]. Depletion of macrophages from the two-stage carcinogenesis model using 3-methylcholanthrene (initiator) and butylated hydroxytoluene (promoter) significantly reduced tumorigenesis, implying a critical role for these inflammatory cells in tumorigenesis [88]. In an interesting study by Stearman and colleagues [89], the authors used a novel microarray approach to define a gene-expression signature from the lung tumor microenvironment in the murine A/J-urethane model of human lung adenocarcinoma. They describe a 'Field Effect' around the tumor that is indicative of the composition of the cell types present. Using this approach they correctly classified the bronchoalveolar lavage (BAL) cells more than 94% of the time. As TAMs generate a unique gene-expression signature within the tumor microenvironment, the authors suggest that this signature could potentially be used for identifying lung cancer from BAL cells and/or fluid.

In humans, tumor islet macrophage infiltration has been tentatively identified as a favorable independent prognostic marker for survival in patients with resected NSCLC [90].

Epithelial to mesenchymal transition

During recent years, the EMT, where cells undergo a developmental switch from a polarized, epithelial phenotype to a highly motile fibroblastoid or mesenchymal phenotype, has emerged as a central process during embryonic development and chronic inflammation and fibrosis [91,92]. Deregulated inflammation, as well as promoting progression of COPD, enables human bronchial epithelial cells to undergo EMT, a process that is critical for

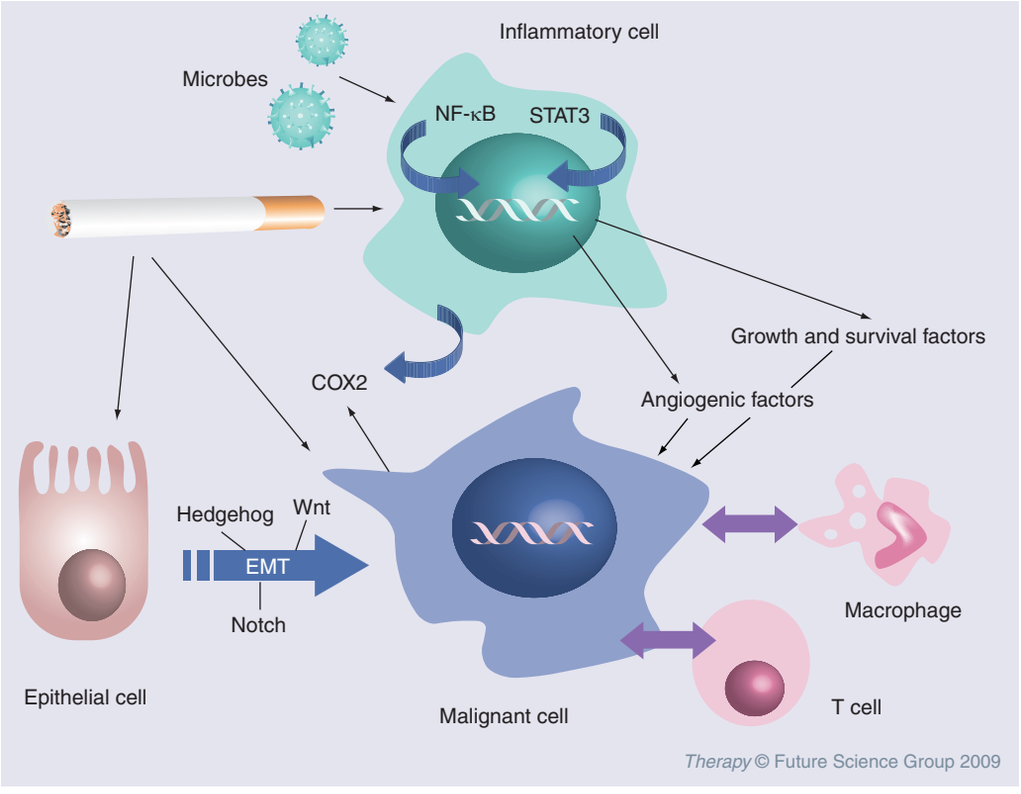

Figure 2. Cells and mediators involved in inflammation and lung cancer.
COX2: Cyclo-oxygenase-2; EMT: Epithelial–mesenchymal transition; NF-κB: Nuclear factor-κB; STAT3: Signal transducer and activator of transcription 3.

lung carcinogenesis and the malignant phenotype [93]. The role of EMT in cancer progression has recently been a topic of hot debate in the scientific community [94,95]. Despite this ongoing discussion, it is clear that several of the key pathways driving EMT in the highly controlled process of development are also aberrantly activated in cancer. Examples of such pathways include Hedgehog, Wnt/β-catenin and Notch signaling (FIGURE 2).

The Hedgehog pathway has been shown to be essential for normal mammalian development [96], and it is clear that Hedgehog signaling is important in early lung formation [97]. Both small-cell lung carcinoma (SCLC) and NSCLC have been shown to require an active Hedgehog signaling pathway [98,99]. Furthermore, an antagonist of the Hedgehog pathway has been shown to inhibit proliferation of NSCLC cell lines containing a hedgehog autocrine loop [99].

Wnt-1 was first identified from retroviral integration that caused mammary tumors in mice [100], and was found to be upregulated in various human cancers [101,102]. Furthermore, cancer cells expressing Wnt-1 are resistant to therapies that mediate apoptosis [103]. It has recently been demonstrated that Wnt-1 is overexpressed in NSCLC cell lines and primary tumor tissues [104]. Furthermore, the monoclonal anti-Wnt-1 antibody suppressed tumor growth. Wnt-2 has also been shown to be overexpressed in NSCLC [105].

Knockout murine studies have demonstrated a requirement for Notch signaling in lung development [106]. Both Notch ligand and receptor have been shown to be elevated in NSCLC lines [107,108]. The relevance of Notch signaling to NSCLC pathogenesis is highlighted by data demonstrating increased apoptosis and serum dependence, and reduced *in vitro* and *in vivo* NSCLC tumor growth in response to blockade of pathway activation using a γ-secretase inhibitor [108].

In the context of tumorigenesis, EMT has been studied with increasing intensity in tissue culture models of epithelial cells and transgenic mouse tumor models [109,110]. EMT can require the cooperation of oncogenic Ras or receptor tyrosine kinases, both of which induce downstream, hyperactive Raf/MAPK signaling, with endogenous TGFβ receptor signaling [91]. Sustained TGFβ receptor signaling (caused for example by an autocrine TGFβ loop) can be required for the maintenance of EMT in epithelial cells and for metastasis in several mouse models [91,111].

Matrix components in COPD & lung cancer

In addition to growth factors and transcription factors, it is becoming increasingly apparent that the ECM is integral to cancer-related inflammation. A study from our own group had originally shown the importance of the ECM in protecting SCLC cells from apoptosis [112].

Emphysema arises as a result of an imbalance between antiproteinases and proteinases including elastase and matrix-metalloproteinases (MMPs) from activated inflammatory and epithelial cells in the lung. Structural cells of the lung die when they lose attachments because of defective tissue repair and ECM degradation by MMPs. In addition, ECM fragments are chemotactic for inflammatory cells, recruiting them into the lung with subsequent aggravation of the progression of emphysema in mice [113,114]. As a consequence, antagonism of ECM fragments is likely to ameliorate the progression of COPD. Proteinases have also been shown to induce the release of TGF-β and VEGF, both of which play critical roles in lung tumorigenesis and metastasis. The MMP inhibitors marimastat (BB-2516), batimastat (BB-94), prinomastat (AG-3340), ONO-4817 and BMS-275291 are currently being investigated to evaluate their effectiveness in the maintenance and remission after other therapies, or in combination with standard chemotherapy in NSCLC [115–117].

An elegant study by Kim and colleagues has shown the importance of the ECM in generating an inflammatory microenvironment that favors metastatic growth [118]. After screening several cell lines, the authors discovered that Lewis lung carcinoma cells were the most potent macrophage activators leading to production of interleukin (IL)-6 and tumor-necrosis factor (TNF)-α through activation of the toll-like receptor (TLR) family members TLR2 and TLR6. Purification of media from these cells identified the ECM proteoglycan versican, already known to be upregulated in many human tumors including lung cancer [119]. Versican was found to be a macrophage activator that acts through TLR2 and its co-receptors TLR6 and CD14. These results help to explain how advanced cancer cells generate an inflammatory microenvironment where metastatic cells may flourish.

Hypoxia/angiogenesis in COPD & lung cancer

Hypoxia is known to induce pulmonary inflammation by activation of transcription factors and

by triggering the expression of pro-inflammatory genes. In COPD, progressive airflow obstruction and destruction of the alveolar-capillary unit ultimately lead to decreased oxygen transport with resultant alveolar hypoxia. As a result, hypoxia-inducible factor (HIF) is activated, leading to an increase in VEGF transcription and increased angiogenesis [120]. As a result, oxygen therapy will offer significant short-term benefits in hypoxemic patients with COPD. However, chronic oxygen use causes oxidative cellular injury and aggravation of lung inflammation and cell death, which theoretically may predispose to lung cancer. Surprisingly, levels of VEGF and its receptors are actually reduced in emphysematous lungs. This seems to occur as a result of abnormalities in the induction of HIF and other signaling molecules involved in hypoxia sensing in COPD [121]. It remains to be fully elucidated whether these 'contrasting' findings are related to methodological differences in the different studies or whether they do in fact suggest a paradoxical role of VEGF in the bronchi as compared with the alveoli in patients with COPD/emphysema.

When tumors grow their microenvironment becomes hypoxic, HIF is activated, MMPs and VEGF are induced and there is resultant invasion and metastasis of lung cancer. Bevacizumab is a monoclonal antibody against VEGF that has been shown to be effective in Phase II and III trials in combination with standard first-line chemotherapy for stage IV NSCLC; in fact it became part of standard first-line chemotherapy for stage IV NSCLC as a result of these trials. Furthermore, additional antiangiogenic therapies such as aflibercept are being investigated in the treatment of NSCLC, alone or in combination with other chemotherapies.

Inflammation & cancer: the key signaling molecules

With the increasing interest in the relationship between inflammation and cancer, a number of key signaling molecules have been implicated as key players in orchestrating the inflammation and cancer link (FIGURE 2). Several of these molecules are now discussed.

Integrins & TGFβ

Integrins are heterodimeric transmembrane receptors that are involved in lung inflammation and a variety of cellular functions. The integrin αvβ6 is located on epithelial cells, and its expression is increased during lung inflammation. It has been shown to play a pivotal role in the maintenance of normal lung homeostasis and in preventing lung destruction, as its ablation leads to airspace enlargement in mice by regulation of the TGF-β/MMP-12 pathway [122]. αvβ6 inhibits MMP-12 via a mechanism that requires it to bind to and activate latent TGF-β [122]. Interestingly, the levels of *TGF*-β1 mRNA and protein are upregulated in the airway and in alveolar epithelial cells in patients with COPD, and the levels of *TGF*-β1 mRNA are positively associated with smoking history and degree of small airways obstruction, suggesting a profibrogenic and pro-remodeling role of TGF-β in COPD [123]. Human lung cancer cells may escape the autocrine growth-inhibitory effect of TGF-β because of the loss of TGFβRII. The majority of NSCLC and SCLC show minimal or no expression of TGFβRII, and as a result restoration of TGF-β signaling through expression of TGFβRII may be a potential strategy for chemotherapeutic intervention in lung cancer.

NF-κB

Nuclear factor (NF)-κB induces the expression of inflammatory cytokines, adhesion molecules, angiogenic factors and key enzymes involved in arachidonic acid metabolism, such as cyclo-oxygenase (COX)-2. Several studies have provided good evidence that NF-κB is involved in tumor initiation and progression in the gastrointestinal tract and in the liver, two of the prototypical organs in which cancer-related inflammation seems to occur [124,125]. In the mouse model of colitis-associated cancer (CAC), Greten and colleagues used a strategy targeting IκB-kinase β, and showed that specific inactivation of NF-κB in tumor-infiltrating leukocytes resulted in an 80% decrease in CAC tumor multiplicity [126]. Another mouse model of inflammation-driven cancer is the multidrug resistance 2 (*Mdr2*)-knockout mouse, in which the absence of the MDR2 transporter leads to accumulation of bile acids and phospholipids within hepatocytes, resulting in low-grade liver inflammation, which eventually gives rise to hepatocellular carcinoma at 8–10 months of age [127]. As in the CAC model, inhibition of NF-κB through expression of a IκB superrepressor under the control of a promoter that is highly active in hepatocytes blocked tumor development [128]. Accumulating evidence is pointing to the importance of NF-κB during lung cancer development [128–132], but it is clear that more studies are required to fully dissect its role.

STAT3

Another molecule of particular interest is signal transducer and activator of transcription

(STAT)-3. Recent studies have identified STAT3 as an important mediator of tumor-induced immunosuppression. STAT3 transduces signals from numerous oncogenic proteins and pathways [133–135]. It is not only a potent negative regulator of T helper 1 (TH_1)-cell-mediated inflammation, but is also an important activator of many genes that are crucial for immunosuppression [136–138]. STAT3 has been shown to be constitutively active in a growing number of diverse human cancer cell lines and tumor tissues [139–141]. The first evidence that STAT3 played a role in oncogenesis came from a study that found that STAT3 was constitutively activated in cells transformed by an oncoprotein, SRC, which is a nonreceptor tyrosine kinase [133]. This was followed by studies that showed that STAT3 signaling blocks the transformation of fibroblasts by SRC oncoprotein [142,143], thus providing definitive evidence that STAT3 contributes to oncogenesis. Evidence has subsequently emerged for a role of STAT3 in preventing the apoptosis of human tumor cells [144], and it is important for the development of angiogenesis [145,146]. The ability of STAT3 signaling to facilitate immune evasion by the tumor through inhibiting the expression of pro-inflammatory cytokines and chemokines has been shown for rhabdomyosarcoma and melanoma [147,148]. STAT3 has been shown to regulate the expression of *MUC1*, a gene known to be important in mediating lung cancer cell survival and metastasis *in vitro* and *in vivo* [149].

EGF receptor (EGFR) targeting in NSCLC is an established treatment modality. In NSCLC cell lines that have constitutively active mutant EGFR, STAT3 is phosphorylated and is necessary for the proliferative effects associated with mutant EGFR [150]. Furthermore, inhibiting STAT3 activity abrogates the transforming effects of EGFR-activating mutations [151]. *In vitro* data show that EGFR blockade decreases STAT3 activation. Similarly, cell lines resistant to EGFR inhibitors demonstrate persistent activation of STAT3 [152]. Thus, STAT3 is a key molecule in maintaining a transformed phenotype, and inhibition of STAT3 has become a potential target for drug development in lung cancer [153]. Indeed, blockade of STAT3 results in extensive apoptosis of NSCLC cells [152]. It has previously been demonstrated that combined inhibition of EGFR and STAT3 using small molecules has synergistic antiproliferative effects in a variety of NSCLC cell lines [154,155]. Given the importance of the STAT3 signaling pathway, it is clear that the development of new drug targets are of particular interest in the treatment of lung cancer [153]. Moreover, the effects of STAT3 on both inflammation and carcinogenesis further highlight the links that may exist between inflammatory conditions such as COPD and lung cancer.

TNF

TNF, originally named owing to its ability to induce hemorrhagic necrosis of tumors [156], is another pro-inflammatory cytokine that plays an important role in cancer-related inflammation. This first became apparent when it was discovered that TNF-deficient mice are protected from skin carcinogenesis produced by a combination of the carcinogen 7, 12-dimethylbenz[α] anthracene (DMBA) and the tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA; DMBA-TPA) [157]. It has since been shown in a series of papers that TNF receptor-1-deficient mice are also resistant to skin carcinogenesis induced by DMBA-TPA [158], and have attenuated lung and liver metastases compared with controls [159,160].

COX-2

Chronic inflammation is often accompanied by the excessive formation of ROS and RNS that are potentially damaging to DNA, lipoproteins and cell membranes. Inflammatory cells also release metabolites of arachidonic acid, or eicosanoids, including prostaglandins and leukotrienes. The cyclo-oxygenases are key enzymes that control rate-limiting steps in prostaglandin synthesis. The expression of the isoform COX-2 is induced by inflammatory and neoplastic cells, and metabolites produced by the action of COX-2 on arachidonic acid have been shown to impact various carcinogenic pathways. High constitutive expression of COX-2 has been documented in humans in precursor lesions of adenocarcinoma of the lung [161] and in more established lung cancer [162]. Staining for COX-2 in tumor cells has been found for adenocarcinomas and squamous cell carcinoma. More recently, COX-2 expression has been shown to be greater in lymph node metastases than in the primary tumor itself [162]. COX-2 expression has been found to be a poor prognostic indicator in both adenocarcinoma [163] and NSCLC [164]. Furthermore, a study has shown there to be an association between a common polymorphism in the *COX2* gene and the risk of NSCLC. Interestingly, there has been shown to be a decreased risk of lung, colorectal and breast cancer in patients who regularly use the COX-2 antagonist aspirin [165].

Conclusion

There have been dramatic advances in our understanding of the links between COPD and lung cancer. What started as a case report in the 1960s has progressed to a realization of the importance of inflammation to lung cancer genesis and perpetuation. We have gone from bedside to bench during this period, but it is likely that in order to see benefits from what we have already discovered, we must see an 180° shift back from bench to bedside. In spite of the advances in lung cancer treatment in the last 5–10 years, it is clear that other approaches to management are required. There needs to be a shift to education and prevention, early recognition and individually tailored therapies. We believe that screening for lung cancer will progress dramatically in the future. We have begun to define high-risk cohorts of patients such as smokers with COPD and a family history of lung cancer, and it is imperative that those at greatest risk be screened early and at regular intervals. Screening trials are already underway, and we need to establish protocols and algorithms that are cost-effective and efficacious. Through a greater understanding of the molecular and cellular mechanisms that drive airway destruction and chronic inflammation in COPD that predisposes to lung cancer, we may be able to identify biomarkers that predict a high risk for lung cancer.

The association between a family history of, and the development, of lung cancer is becoming increasingly clear. Interestingly, the genetic susceptibility that exists for developing lung cancer is similar to the hereditary risk for developing breast cancer. As we better define the genetic alterations that increase susceptibility to lung cancer, we will be able to screen first-degree relatives for genetic abnormalities. Those at highest risk may then be placed into the more clinically orientated algorithms mentioned above.

Recent research has identified that cancers may have unique gene and molecular signatures. It is likely that these signatures will define the nature of the lung cancer, its chemotherapeutic sensitivities, and the clinical course that it may follow. As our understanding increases, we will be able to specifically tailor therapies to individuals based on the gene and molecular signature of their lung cancer. Furthermore, the importance of stem cells and their relation to cancer development is only in its infancy at present. We believe that cellular manipulation will one day become possible, and that a thorough understanding of stem cell biology will be critical and necessary for this development to be taken further.

Studies on ligand and signaling pathways that mediate communication between lung cancer and inflammatory cells may provide therapeutic strategies for blocking the tumor-permissive environment (which promotes lung cancer growth and resistance to therapy) and stimulate anticancer immune responses.

Downregulation of biochemical molecules that are common to the pathogenesis of both COPD and lung cancer, such as NF-κB, may improve treatment of both diseases.

Future perspective

COPD and lung cancer share many common biochemical and cellular mechanisms that may provide novel therapeutic targets for both diseases. In addition, the chronic inflammation associated with COPD is an important risk factor for developing lung cancer. Understanding how the chronic inflammation in COPD predisposes to lung cancer may provide key biomarkers and preventative strategies in smokers/ex-smokers most at risk of developing lung cancer.

Screening for lung cancer has been intensely investigated in the last decade, and it is likely that many more studies will be published focusing on the cost–effectiveness and efficacy of screening in relation to defined cohorts of patients. This will be a costly exercise, and it is likely that only limited advances will be made in the next 5 years. However, we believe that with better rational biomarkers (which define how lung cancer is initiated), screening will become more cost-effective and predictive of disease. Once this has happened these biomarkers will be of fundamental importance in the management of lung cancer in the future.

The biology associated with inflammation, COPD and lung cancer is complex. In the next 5 years there will be a rapid expansion in knowledge, with new and exciting research and biological principles being elucidated. In particular, the role of stem cells in lung cancer initiation and progression, and their interaction with inflammatory cells, will be intensely studied and provide new targets and strategies for intervention.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

- Lung cancer is the leading cause of cancer deaths in the world.
- Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality.
- Cancer-related inflammation is the 'seventh hallmark of cancer'. The importance of inflammation to cancer development and progression has been clearly highlighted.
- The role of chronic infections in predisposing to lung cancer development is becoming increasingly clear, particularly in relation to TB and HIV.
- We have defined 'master' molecules, such as NF-κB, STAT3, COX-2 and TNF-α, that have a critical role in tumorigenesis
- The habitat in which a tumor finds itself is an important determinant of tumor survival and progression. Of particular importance is the matrix upon which the tumor resides and the infiltrating cells that mediate cross-talk and interaction, such as the tumor-associated macrophages.
- The link between COPD and lung cancer has been firmly established. It is clear that COPD increases the risk of lung cancer even in the absence of smoking history. A family history of lung cancer further increases this risk.
- Screening algorithms and individually tailored treatments are likely to become the mainstay of lung cancer management of the future.

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