Introduction

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable condition characterised by persistent respiratory symptoms and enhanced inflammatory response due to noxious particles and gases (1). While COPD is often associated with other chronic conditions including emphysema and bronchitis, a substantial number of patients suffer from exacerbations which often require hospitalisation. Increased frequency of severe exacerbations of COPD is linked to significantly worsened survival outcomes (2).

COPD is a major public health concern affecting approximately 251 million people globally (1,3). In Australia, a reported $929 million per annum is spent on direct health expenditure attributed to COPD, significantly burdening the health care system (4). COPD currently affects 8–10% of adult populations in the developed world and 15–20% of smokers. The World Health Organisation has projected that COPD will become the 3rd leading cause of death globally by 2030 unless vital action is taken to reduce underlying risk factors, principally tobacco smoking and passive exposure to environmental pollutants (5).

Lung cancer is the leading cause of cancer-related mortality, accounting for almost a fifth of all cancer deaths worldwide (6). By 2030, it is estimated that the number of lung cancer deaths will rise to 10 million per year (7). Despite advancements in survival outcomes for patients with other cancer types, the current overall 5-year survival rate of patients with lung cancer of 16% has remained

Abstract: Chronic obstructive pulmonary disease (COPD) and lung cancer comprise the leading causes of lung disease-related mortality worldwide. Exposure to tobacco smoke is a mutual aetiology underlying the two diseases, accounting for almost 90% of cases. There is accumulating evidence supporting the role of immune dysfunction, the lung microbiome, extracellular vesicles and underlying genetic susceptibility in the development of COPD and lung cancer. Further, epigenetic factors, involving DNA methylation and microRNA expression, have been implicated in both diseases. Chronic inflammation is a key feature of COPD and could be a potential driver of lung cancer development. Using next generation technologies, further studies investigating the genomics, epigenetics and gene-environment interaction in key molecular pathways will continue to elucidate the pathogenic mechanisms underlying the development of COPD and lung cancer, and contribute to the development of novel diagnostic and prognostic tools for early intervention and personalised therapeutic strategies.

Keywords: Pulmonary disease; chronic obstructive; lung neoplasms

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relatively unchanged in the past three decades (8).

Lung cancer is a genetically complex and heterogeneous disease, caused by mutations in oncogenes resulting in the progressive transformation of normal cells to malignant derivatives under the influence of genetic and epigenetic alterations (9). This is further perpetuated by the evasion of normal regulatory systems in the immune system and defective mechanisms that govern normal cellular activity and homeostasis (10). Lung cancer comprises two major subtypes—non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC); which are differentiated by their cellular origin and phenotype. NSCLC comprises the majority (85%) of cases and arises from epithelial cells of the lung. NSCLC is further divided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. SCLC has distinct histological features of neuroendocrine differentiation and small cells with scant cytoplasm and accounts for approximately 15% of cases (11).

This review will discuss common pathogenic pathways contributing to the COPD and lung cancer, and how certain molecular and cellular mechanisms differ. Further understanding of the shared molecular pathways between these two lung diseases will highlight the direction of future studies required for the development of earlier diagnosis and therapeutic strategies.

Epidemiological evidence linking COPD and lung cancer

Tobacco smoking

Exposure to tobacco smoke is the leading cause of preventable disease and the most common risk factor for COPD and lung cancer, accounting for almost 90% of cases. People with COPD carry a greater risk of lung cancer (12) which suggests that shared pathological mechanisms such as chronic inflammation, epigenetic changes, and impaired DNA repair processes as a result of oxidative stress (13). Oxidative stress from exogenous and endogenous factors, autoantibody expression, protease activity and the release of pro-inflammatory cytokines are all known to cause airway destruction, lung hyperinflation and air trapping, damaging the lungs in people with COPD (Figure 1) (14). This repetitive lung injury eventually causes airflow limitation, further contributing to impaired clearance of inhaled toxic particles and increasing exposure at the epithelial level. As a consequence, the chronic inflammation, immune cell infiltration and oxidative stress persists long after inhalation has ceased (15,16).

Among smokers, COPD is an important risk factor for developing lung cancer, and predates lung cancer in up to 70–80% of cases (17). A seminal cohort study by Mannino et al. revealed a distinct linear dose-response relationship between increasing COPD severity and risk of lung cancer (18). A large retrospective study of patients with lung cancer showed that the coexistence of COPD predicted a significantly worse survival outcome (12,19,20). The presence of emphysema has been shown to confer a significant risk of developing lung cancer, particularly squamous cell carcinoma and SCLC (20). Overall, people with COPD have an increased risk (4- to 6-fold) of developing lung cancer, independent of smoking history, age and sex (12,19).

Occupational and environmental air pollution

Industrial exposure to various metals, dusts and fumes are under-appreciated risk factors for COPD (21,22). Comparatively, the causal link between occupational exposures such as asbestos fibres (23) and radon (24,25) as a primary cause of lung cancer is well established. Exposure to particulate matter in urban air has been shown to present a 14% increase in risk of lung cancer with every 10 g/m² increase in concentration of fine particles (26). A recent cross-sectional analysis by Liu et al. revealed a strong association between COPD prevalence and urban particulate matter (27).

Among low-income countries, indoor air pollution is the leading risk factor for COPD and lung cancer (28). Indoor air pollution from combustion of biomass fuels such as wood and coal increase disease risk due to the increase in toxic particulate matter, polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic compounds (29). This presents a significant public health problem as these fuel sources are used by over 3 billion people for cooking and heating (30).

Asian women have a disproportionately high incidence of lung cancer compared with women of other ethnicities, despite cigarette smoking being uncommon in this demographic (31). Several epidemiological studies have reported cooking oil vapours and inadequate kitchen ventilation as a risk factor for both lung cancer (7,32,33) and COPD (34-36). Further studies of women in China (37) and Taiwan (38,39) have reported a dose-response relationship for lung cancer risk and use of volatilised oils, citing the mutagenic or oxidative effects of various components found
in aerosolised oils (40,41).

**Genetic and epigenetic mechanisms linking COPD and lung cancer**

Phenotypically, COPD and lung cancer present as very dissimilar diseases. COPD has features of chronic lung damage, characterised by irreversible airflow limitation, inflammation of small airways and progressive destruction of distal lung parenchyma resulting in emphysema (42). In comparison, lung cancer is characterised by DNA damage resulting in the overproduction of stimulatory...
proteins, excessive unregulated proliferation and defective cell cycle checkpoint regulation which confers selective biological advantage over normal cells (43). While tobacco smoking is the main risk factor for lung cancer and COPD development, only 10–20% of smokers develop COPD (44) and 10–15% of individuals develop lung cancer despite being never-smokers (45). While not all smokers will develop lung cancer, the environmental exposure to tobacco smoke and volatile agents unmask the fundamental genetic differences and the varying degrees of susceptibility to these lung diseases among smokers.

**COPD**

Early genetic studies of familial linkage in COPD identified that mutations in the SERPIN1A1 gene resulted in severe alpha1-antitrypsin (AAT) deficiency and accounted for ~1% of cases (46). More recently, genome-wide association studies (GWAS) have been performed to identify associations between chromosomal regions and disease phenotypes in order to predict disease risk or susceptibility. A large GWAS and meta-analysis by Cho et al. confirmed three known loci at CHRNA3, CHRNA5, FAM13A, and HHIP loci were associated with increased risk of COPD, as well as MMP12, TGFB2 and the novel RIN3 locus (47).

**Lung cancer**

Large scale genomic profiling studies have revealed the molecular complexity of lung cancer (48,49). On the spectrum of mutational frequencies among cancer types, compared to paediatric cancers (0.1/Mb), the high mutation frequency in lung cancer (100/Mb) is mostly attributable to extensive carcinogenic exposure to tobacco smoke and other known inhaled carcinogens (50). Despite the well-established causality between tobacco smoke and lung cancer, there is accumulating evidence to suggest there is also a hereditary component to lung cancer susceptibility (51). The heritability of lung cancer is estimated to be between 15–25% (52), with risk increasing by approximately 50% for patients who have first degree relatives (52), particularly when the relative was diagnosed at a young age and cases where multiple family members are affected (53,54). Early linkage analyses of 52 lung cancer affected families identified a high penetrance susceptibility locus in the 6q23–25 region (52). Further analyses on 93 high-risk families confirmed this rare genetic variant significantly increases lung cancer risk and ongoing targeted sequencing studies seek to identify a causal variant (55).

Lifelong never-smokers account for 25% of lung cancer cases and harbour specific molecular and pathological features (56). More than half of women with lung cancer have never smoked; however these figures vary between certain geographical locations (Asia > North America > Europe) (57). Studies have suggested that a hormonal element, hereditary risk and certain environmental exposure may influence this unique subset of patients (56). Polymorphisms in CYP1A1 and GTSM1, xenobiotic metabolising enzymes, are associated with lung cancer risk in Caucasian non-smokers (58), and polymorphisms in MLH1, a mismatch pair enzyme, have been cited to have a role in the development of cancer in the absence of tobacco smoke (59). In never smokers, polymorphisms in genes associated with inflammatory pathways, including interleukin (IL)-10, tumour necrosis factor (TNF) (60), IL1-RN and IL-6 (61), are associated with an increased risk of lung cancer.

**Lung cancer and COPD genetic overlap**

The COPD and lung cancer overlap may exist beyond their smoking-induced pathogenic processes through shared genetic susceptibility (62). GWAS investigating lung function and lung cancer have identified associations with several overlapping loci, supporting the notion that some of the loci that determine COPD susceptibility are also important for lung cancer susceptibility in smokers (63–65). Epithelial-mesenchymal transition (EMT) is a pathogenic feature of COPD and lung cancer as well as inflammation, and the rs7326277TT genotype in VEGFR1 promotes these processes as well as tumour growth and is a susceptible locus in both diseases (Figure 1) (66). Additionally, an increased risk of COPD and lung cancer has also been demonstrated in polymorphisms in the anti-inflammatory gene IL10 (67,68).

An early GWAS using sub-phenotyped smoker control groups identified overlapping loci at chromosome 15q25 and 4q31 in both COPD and lung cancer (69,70). Chromosome 15q25 locus has been shown to be independently associated with COPD susceptibility (16,36,58,69), while chromosome 4q31 locus associated with a reduced risk of COPD, as well as lung cancer independent of COPD (64,70,71). More recently, a large GWAS of over 250,000 individuals identified 35 new genetic risk loci for COPD, of which 13 of these 35 were associated with lung function, supporting the genetic susceptibility component of COPD development. Bioinformatics analysis of this
data further elucidated the biological pathways, genes and cells were implicated and potential therapeutic targets (72). However it has been suggested that some GWAS studies may have underestimated the contribution of COPD and its underlying genetic determinants of lung cancer, since spirometry is not routinely performed for lung cancer studies (73). Further, a comprehensive understanding of how these genes are implicated in the molecular pathogenesis of COPD and lung cancer will require further studies integrating next-generation technologies and omics data.

**Somatic mutations**

Somatically acquired loss-of-function mutations in *PTEN* and *TP53* and gain-of-function mutations in *EGFR* and *Ras* are frequently persistent in epithelium of smokers and patients with lung cancer (74). *EGFR* and *KRAS* are two of the most commonly mutated oncogenes in adenocarcinoma, with *EGFR* being found to be over-expressed in 40–80% of NSCLC cases and associated with higher metastatic and tumour proliferation rate, advanced staging and poorer prognosis (75,76). Further mutant forms of *EGFR* (deletion in exon 19 and L858R in exon 21) with tyrosine kinase activity have been identified to play a role in lung carcinogenesis and further have been considered targets for treatment (77,78). This led to the development of specific *EGFR* tyrosine kinase inhibitors, such as gefitinib and erlotinib which were approved by the FDA as therapeutics for advanced and metastatic NSCLC (79,80). These drugs act by inhibiting receptor phosphorylation in the intracellular domain by competing with ATP for the for the ATP binding site (80). It has been proposed that the incidence of *EGFR* mutations is due to genetic instability causing gene amplification (81).

Due to the proven mutagenic effect of cigarette smoke, it is thought that acquired somatic mutations, rather than germline polymorphisms may contribute to the molecular pathogenesis of COPD (82). Early evidence suggests that there is a link between somatic mutations and COPD pathogenesis, however this mutated gene clustering has not been shown to definitely contribute to COPD. Nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) signalling is upregulated in epithelium of smokers and COPD patients. Consequently, amplification of NF-κB and AP-1 signalling is mediates the expression of inflammatory cytokines and proteinases in response to oxidative stress (83). Further, the presence of these somatic mutations and their intermediate molecules converge with transcription factors causing an altered bronchial epithelium phenotype in the form of squamous metaplasia (84). This impaired phenotype contributes to COPD through compromised immune defence contributing to persistent inflammation, recurrent infections and insensitivity to inhaled corticosteroids in some patients (82).

**Epigenetics**

Distinct from somatic mutations and underlying genetic predisposition, epigenetic alterations are modifications that influence gene expression without changes to the nucleotide sequence. Tobacco smoking-induced modification to the airways and lungs of patients with lung cancer and COPD has been shown to influence epigenetic alterations including DNA methylation, microRNA expression and histone acetylation (85).

**DNA methylation**

DNA methylation is the reversible modification of DNA structure by the addition of a methyl group to the 5' position of a cytosine residue (86). This process can be a part of a CpG island or cluster of tumour suppressor genes, which increase proliferation (87). In relation to COPD, DNA methylation is most likely attributed to hypomethylation of immune-modulatory genes such as *SERPINA1* encoding alpha1-antitrypsin, leading to gene overexpression (88).

In lung cancer, both promoter and tumour suppressor genes are observed to be hypermethylated. The reversibility of methylation makes it an attractive target for cancer therapy (89). DNA methylation profiles may be able to distinguish between smokers and COPD, as well as different subtypes of NSCLC (85) and therefore characterising methylation signatures in peripheral blood may have a potentially important role as a biomarker for diagnosis and disease monitoring (88).

In an epigenome wide association study, DNA methylation and the repression of genes *CCDC37* and *MAP1B* were identified to be significantly more prevalent in patients with both lung cancer and COPD, compared to non-COPD cases. Lung cancer patients showed a higher degree of methylation than COPD patients (90).

**MicroRNA (miRNA) regulation**

miRNAs are small non-coding RNA molecules and primary
epigenetic mediators involved in critical posttranscriptional regulation, through either translational repression or degradation of target mRNA (91). More than 60% of protein-coding genes are subjected to regulation by miRNAs (92). Owing to their broad range of target genes, they are involved in regulating many key physiological processes such as cell metabolism, apoptosis, tissue differentiation and DNA repair (91), as well as the initiation and progression of pathogenic processes leading to chronic infection, inflammation (93) and tumorigenesis (44).

A recent study highlighted the significant downregulation of miR-218-5p in the lung tissue of COPD patients compared with never smokers, and was strongly correlated with severity of airway obstruction (94). Clinical and preclinical validation further showed that the highest expression of miR-218-5p was localised at the bronchial airway epithelium. Further perturbation experiments suggest that miR-218-5p may have a role in defence against cigarette smoke-induced inflammation and COPD pathogenesis (94).

Disease-specific miRNA expression patterns are promising diagnostic and prognostic biomarkers in NSCLC and COPD (95). Recently, Keller et al. profiled miRNAs in the blood of COPD patients and identified nine miRNAs significantly downregulated in patients who developed lung cancer. Candidate miRNAs were implicated in the regulation of cancer-related pathways including MAP kinase, integrin signalling and focal adhesion (96). Further, using a three-miRNA classifier (miR-450a-5p, miR-4677-3p, and miR-9-3p), an area under the curve (AUC) of 0.87 predicted the development of lung cancer (vs. no cancer) among the COPD patients.

Recently, Mateu-Jimenez and colleagues revealed that the expression of a four-miRNA signature (miR-21, miR-200b, miR-210, miR-let7c) and total DNA methylation was increased in lung cancer patients with COPD compared to those without (97). Further, the tumours of the COPD-lung cancer patients harboured decreased expression of the miRNA target genes including PTEN, MARCKs, and KRAS compared to patients without COPD. This study highlighted the significant role of epigenetics in regulating cellular pathways that increase risk of tumorigenesis in COPD and how epigenetics profiles may influence the efficacy of cancer treatments among patients with underlying respiratory disease (97).

**Gene expression in COPD and lung cancer**

Large gene expression profiling studies have been performed to better understand the pathogenesis of COPD and lung cancer through quantification of the transcriptome (72,98). The advancements in omics technologies has seen a shift towards analysis and publication of multi-omics datasets, whereby the use of transcriptome, translatome and proteome data is used to enhance the accuracy and validity of molecular studies (99). The lung stroma is a composition of fibroblasts and mesenchymal stromal cells that form a structural and functional support network for tissues (100). Recently, a multi-omics approach was used by Sandri and colleagues to characterise two distinct stromal gene expression patterns in lung cancer that diverged on the basis of lung function (FEV₁) (101). Patients with normal to mild impairment demonstrated mammalian target of rapamycin (mTOR)-predominant pathway activation to drive cancer development. Conversely, the stroma of patients with severe airflow obstruction coordinated cancer-associated signalling via the fibrotic extracellular matrix (ECM). It has been suggested that further investigation into the role of the stroma in lung cancer development may benefit from the use of a lung cancer and control group, as well as direct assessment of airflow obstruction based on CT imaging rather than the use of FEV₁ as a surrogate for impaired lung function (102).

**Neoplasia and tumour microenvironment**

Tobacco smoking negatively affects the airway epithelium through both inflammatory and immunosuppressive effects. A recent study explored effects of tobacco smoking on the immune microenvironment in normal human airway epithelium by measuring the levels of T cell activation and infiltration through gene expression and reported the level of immunosuppression had a stepwise correlation with increased smoking exposure (103). In COPD, epithelial hyperplasia and metaplasia is a result of cigarette smoke and/or infections causing repeated respiratory tract epithelial mucosal damage (104).

Lung cancer has also been demonstrated to develop within metaplastic microenvironments (105). Inflammation can induce hyperplastic lesions under non-neoplastic conditions, however if these lesions undergo uncontrolled proliferation they can transform and adapt a neoplastic and atypical adenomatous appearance, acquiring invasive, angiogenic and metastatic characteristics (10).

It is reported that tumour growth and progression is influenced by bidirectional crosstalk between tumour...
cells and their surrounding stroma, with extracellular cues in the tumour microenvironment also being able to regulate cancer progression and metastasis (106,107). It is therefore important to understand how and when the lung microenvironment is implicated and cells start to promote tumour associated angiogenesis and inflammation (108). Previous studies have been able to identify potential biomarkers for lung cancer diagnosis by analysing and comparing genetic and epigenetic mutations from FFPE histologically normal tissue to matched tumour tissue (109). A study by Keohavong et al. found that KRAS mutations were present in histologically normal tissue surrounding lung tumour in 16% of patients with lung adenocarcinoma (110). More information is needed to determine when and how these molecular changes occur and what influences these changes, such as inflammation or infection. Understanding the early cellular events that have govern the eventual development of COPD and lung cancer could be guided by studies investigating the gradient from tumour to non-tumour tissue and emphysematous tissue, to help determine at which stage tissue displays neoplastic changes.

**Field cancerisation**

Field cancerisation is a theory describing the broad indiscriminate molecular and cellular changes to the surface epithelium that occur in a given anatomical region upon exposure to carcinogens. Early studies by Slaughter and colleagues describes the priming effect of carcinogens and the “field cancerization” of epithelial tissues leading to tumour development (111). The concept suggests that histological abnormalities are shared between neoplasm and adjacent normal appearing tissue, including epithelial hyperplasia and hyperkeratinisation (111,112). These studies hypothesise that somatic alterations can occur naturally or as a result of a mutagenic exposure and drive the formation of a premalignant field which may result in neoplastic growth following clonal expansion. The premalignant fields often occur without any histopathological change and despite having growth and survival advantage, may or may not result in a tumour (113-116). This model of tumorigenesis and further study of cancerized fields may improve predicting cancer development or risk of tumour recurrence (117).

**Biological mechanisms shared between COPD and lung cancer**

**Inflammation**

COPD patients exhibit pronounced inflammation which positively correlates with disease severity (118), and as this destructive inflammation is operative in emphysema and found in airways and airspaces, this could contribute to lung cancers being developed in proximal and distance tissue (44). Further studies have identified characteristics of immune cells specific to COPD that differ to lung cancer, such that COPD patients have M1 phenotype skewed alveolar macrophages as shown by cells from BAL fluid polarising towards a T helper 1 (T\_1) phenotype through interferon-γ. Cytokines released include CXCL10, which can induce macrophage elastase from macrophages. The release of IL-8 from alveolar macrophages in conjunction with lung epithelial cells causes the presence of neutrophilic infiltrates in COPD patients. This Th1 cytotoxic profile would seem to be favourable for tumour microenvironment, however does not occur with solid tumours representing a Th2 phenotype activated by M2 macrophages.

Competing disease microenvironments can occur due to the nature of inflammatory cells that surround lung cancers in emphysematous lungs (44). In COPD, the cytotoxic environment has been found to be conducive of tumour initiation due to ROS providing genotoxic stress through macrophages and neutrophils allowing DNA adduct formation to occur leading to genetic mutation. This allows early stage neoplasms in the surrounding microenvironment to release chemokines and cytokines (such as TNFα, IL-1β, IL-6 and NF-κB) that alter immune cell composition and exhibit direct effects on tumour cells (44,119,120). Additionally, inflammation has been linked to many aspects of lung tumour progression, including cell proliferation, metastasis and response to chemotherapy treatments. For example, IL-17C, which promotes neutrophil recruitment, may promote inflammation in the tumour microenvironment and enhance tumour growth (121).

Independent of the mutagenic effect of tobacco smoke, chronic inflammation observed in COPD is a potential driver of lung cancer development (67,85,122-124). A number of structural and inflammatory cells are involved in the pathogenesis of COPD and lung cancer, particularly neutrophil, macrophages and CD4\(^+\) and CD8\(^+\) lymphocytes (125-127).
**Oxidative stress**

Nearly all cases of cancerous tissue show inflammation, and it is possible that the chronic inflammation observed in COPD can be a potent driver for lung cancer development (67). Inflammation is also a major source of reactive nitrogen and oxygen species (RNOS), which in COPD patients, levels are persistently elevated (85). Mitochondria is a major cellular source of RNOS and is dysfunctional in COPD. Several studies have linked the methylation of transcription factor A in COPD and squamous cell carcinoma histological subtype of lung cancer (128-130).

**DNA damage and repair mechanisms in COPD and lung cancer**

DNA damage caused by reactive oxygen species, single strand breaks and abasic sites is increased in COPD and lung cancer (131-133). Biomass exposure has also been associated with COPD causing an increase in oxidative stress and DNA damage, however levels are more elevated in cigarette smoke induced COPD (134). Studies have shown that the increase in DNA damage is not due to cigarette smoke alone, and COPD patients have increased double strand breaks in their lung compared to smokers and never-smokers without COPD (135). This is potentially due to COPD patients containing higher levels of microsatellite instability and frequency of somatic mutations, as well as a loss of heterozygosity (132). Further increased levels of oxidative DNA damage are found in clusters of functionally significant sequences (VEGF), suggesting that the role oxidative DNA damage plays is not random (136).

**Immune mechanisms underlying COPD and lung cancer**

The role of the immune system in lung disease is currently at the forefront of clinical interest. Among COPD patients, immunological changes occur as a result of both T-cell dysregulation (increased CD8+, decreased CD4+) (137,138) and T-cell exhaustion where effector function is lost as a result of chronic immune checkpoint binding. Further, PD-1 expression in exhausted T-cells has been shown to contribute to altered immune function in COPD patients (139).

In patients with NSCLC, therapeutic inhibition of the immune checkpoint protein PD-1 suppresses T-cell mediated activity, immune evasion and subsequently induces immune-mediated tumour cell death (140). COPD is commonly considered a negative prognostic indicator among lung cancer patients (141), however recent studies have demonstrated an increase in progression-free survival among advanced-stage NSCLC patients with coexisting COPD receiving anti-PD-1 therapy (142,143). Further, the co-expression of PD-1 and T-cell immunoglobulin and mucin domain-containing molecule-3 (TIM-3) on CD8+ T cells increases with COPD severity (143). Increased efficacy of anti-PD-1 therapy among these patients may be explained by the immunological dysregulation seen in COPD, leading to increased expression of immune checkpoints among T-cells (144).

Alterations in immune cell composition may be a useful biomarker or therapeutic target in COPD-lung cancer. A recent study demonstrated the increase in Th1-polarized CD4+ T-cells in lung tissue is associated with COPD severity and may be a useful biomarker for disease progression (142). Recently, the role of Th17 in COPD and lung cancer progression has been investigated in several clinical and preclinical studies. In a murine model, Chang et al. provided strong evidence for the pathogenic role of Th17 cells in mediating inflammation and lung tumorigenesis (145). Additional studies confirmed that Th17 is augmented upon exposure to tobacco smoke and is potential therapeutic target in COPD (142,146). There is strong evidence to support the immunological link between COPD and lung cancer and further investigation in this area will have practical and therapeutic impact to aid patient stratification and care.

There is conflicting evidence surrounding the severity of COPD and lung cancer incidence. De Torres and colleagues found that lung cancer incidence decrease with COPD severity, with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage IV COPD demonstrating less than half of the incidence of lung cancer than stage I (147). This finding may be explained by the notion that the immune system among smokers and mild COPD may be suppressed and less active against tumorigenesis than an active, nontolerant immune system seen in severe COPD cases (147). Conversely, a longitudinal study of 5,402 participants in the first National Health and Nutrition Examination Survey found that lung cancer incidence increased with COPD severity [mild; hazard ratio (HR) 1.4, 95% confidence interval (CI): 0.8–2.6, moderate-severe; HR 2.8, 95% CI: 1.8–4.4] compared to normal lung function (18).
The lung microbiome

The lung microbiome encompasses the community of bacteria, viruses and fungi that resides in the airways and parenchymal tissue. Alterations in the lung microbiome can influence the host’s susceptibility to various respiratory diseases through impairment of immune defence mechanisms (148). The lower airways of the lung are inhabited with multiple microbial species and thrives on diversity. Consequently, if bacterial diversity decreases adversely, likely resulting in the dominance of one bacterial species which leads to disrupted mucosal immunity and airway dysbiosis (149).

The microbiome varies with exposure to different environmental factors, and tobacco smoke inhalation has been shown to induce pathological changes to the integrity of the airway epithelial barrier and cell-cell contact via the degradation of tight junction proteins (150), inducing cilia autophagy (151), goblet cell hyperplasia (152) and an increase in epithelial permeability (153).

COPD

In COPD, the presence of bacteria is associated with mucus hypersecretion, bronchial wall oedema, tertiary lymphoid follicles, and airway and parenchymal inflammation due to defective mucociliary clearance (14,154). Airway structure and integrity is compromised and cellular responses are impacted, which are vital for the control of bacterial growth (155).

COPD patients are predisposed to microbial colonisation and infection of the airways (where pathogenic bacteria, rather than being innocuous, are persistent in the airways causing airway epithelial injury and chronic inflammation (144) and can trigger an exacerbation (1,156).

The identification of predominant bacterial species during stable state and exacerbations in COPD is remains under debate. Erb-Downward et al. found that the Proteobacteria phylum was the predominant bacteria in lung tissue from very severe COPD patients (157). These findings were similar to that of Hilty et al. (158), however, the majority of other studies have concluded that the Firmicutes phylum is the most common in both moderate and severe COPD (159,160). Currently, non-typeable H. influenzae, S. pneumoniae, M. catarrhalis and P. aeruginosa are pathogens that are clearly implicated in COPD (156).

Our knowledge of the role of the lung microbiome in COPD is currently inconsistent and results to date are poorly reproducible across studies. The identification of different microbiota varies depending on population, disease severity and methods used to isolate and characterise the microbiome. The presence of different micro-niches across different lung compartments may also explain these divergent results (161). Early studies have reported bacterial diversity decreases as COPD severity increases (157,158), however this has not been confirmed by subsequent studies (162). The use of metagenomics may provide functional data on the microbial interactions and assist in interventional studies in COPD (163).

Lung cancer

While it is known that the lung microbiome is altered in cases of chronic lung conditions such as COPD compared to healthy controls (164), there is little known about whether there are key microbial species or common lung microbial profiles increasing susceptibility to or enhancing progression of lung cancer. The presence of inflammation in response to Mycobacterium tuberculosis is associated with increased risk of lung cancer (165), and many microbiome studies in colon cancer have demonstrated the carcinogenic influence of bacteria including Bacteroides fragilis and Fusobacterium nucleatum (166). The mechanistic effects of the lung microbiome on tumour development is a rapidly advancing field of study. Recently, Jin and colleagues proposed that commensal lung microbiota promote inflammation and tumour proliferation through the activation of γδ T-cells and production of IL-17 in the lung (167). Another recent study demonstrated the unique bacterial signature in patients with lung tumours harbouring TP53 mutations (168). This suggest that the microbial composition in lung cancer may be mutation specific, however further elucidation of the molecular and microbial interactions is warranted.

Recent demonstration of the relationship between gut microbiome and responsiveness to immunotherapy have shown that the gut microbiome composition is predictive of anti-PD-1 efficacy in melanoma (169,170) and epithelial tumours including NSCLC (171). These studies demonstrate marked influence of the gut microbiome on therapeutic response, which is expected to have a profound effect on diagnosis and therapeutic strategy as this area of research continues to unfold.
Biomarkers for diagnosis, interventions and novel therapeutic strategies

Extracellular vesicles (EVs)

EVs are nano-sized membranous vesicles that are secreted by both normal and diseased cells to their extracellular environment (172,173). EVs play a key role in local and systemic intercellular communication through the exchange of bioactive molecules such as DNA, mRNA, miRNA, proteins and lipids (174). EVs can be broadly classified into three major vesicle subtypes: apoptotic bodies, microvesicles and exosomes, which are differentiated according to their size, intracellular origin and biogenesis (175). There is a growing body of research implicating EVs in various pathological processes by the selective transfer of nucleic acids, proteins and lipids. The molecular cargo contained in EVs is reflective of their cellular origin, protected and stable in circulation, and can be non-invasively accessed via blood sampling, which makes them an attractive biomarker for diseases including COPD and lung cancer (175).

The role of EVs in disease includes involvement immune system modulation, angiogenesis, parenchymal remodelling and pre-metastatic niche formation (176). The uptake of EVs into target cells is a specific interaction, which occurs through direct fusion, endocytosis or receptor binding (177). Many studies have postulated that the horizontal transfer and uptake of nucleic acid from EVs may be a key influence the heterogeneity of cancer (178-181).

In relation to COPD, recent studies have identified new molecular mechanisms underlying the disease that are regulated by EVs and their cargo, highlighting a new paradigm for paracrine signalling (182,183). Reports that various stress triggers promote secretion of circulating EVs from lung endothelial cells and that these may be a useful predictive biomarker for assessing the degree of lung endothelial injury in COPD and the disease progression (184).

It has been suggested that EV miRNAs and proteins may be useful biomarkers for diagnosis of lung cancer (185) as miRNAs have been shown to play an important role in tumorigenesis, metastasis and drug response in lung cancer, with lung cancer derived EV miRNAs having potential as indicators of cancer progression (185) as their contents reflect the molecular dynamics of tumour behaviour both in situ and in metastasis (185).

In addition to the value of EVs as circulating biomarkers, the potential for exosomes to act as vectors for gene therapy is an emerging topic of interest. Cell-to-cell communication via exchange of translatable RNA offers plausibility for engineering of exosomes to contain DNA or RNA for therapeutic intervention. To date, this genetic exchange has been demonstrated in the tumour microenvironment, by means of a mechanism similar to endocrine signalling in the systemic circulation (174). The absence of host immunogenicity to recipient exosomes processed in vitro, carries a large advantage over viral vectors, many of which initiate immune rejection and require complex methods for delivery enhancement (186).

A recent study by Costales et al. was the first to demonstrate the potential for miRNA to be a druggable target in breast cancer. A small non-protein molecule was designed to recruit an RNAse to selectively cleave miR-96, triggering apoptosis in breast tumour, but not normal cells. The clinical utility of miRNA has further been illuminated by a study reporting that the selective cleavage of miR-96 induces apoptosis in breast tumour cells and is a potential therapeutic target (187). Exploiting the unique biological vehicular nature of exosomes for therapeutic delivery requires further elucidation on the exosomal cargo and their biological functions to aid in the development of exosomes with well-defined composition and activity (188).

Volatile organic compounds (VOCs) for the diagnosis of COPD and lung cancer

VOCs are a by-product of cellular processes which can be excreted directly from cells into the circulation and diffused into the exhaled breath via the alveolar membrane. Modifications in cellular metabolism, microenvironment and various pathological changes can create a unique VOC profile (189,190). Analysis of VOCs profiles in exhaled breath is performed using one of two commonly used methods: gas chromatography-mass spectrometry (GC-MS) or artificial intelligence devices such as the electronic nose (or e-nose). VOCs analysis is a promising non-invasive method for the diagnosis of lung cancer and COPD and is currently an area of intensive investigation.

Both COPD and lung cancer are characterised by inflammation and oxidative stress within the lung tissue, consequently producing various VOCs which can be excreted in the exhaled breath (189). Several studies have highlighted the clinical potential of exhaled VOCs as a biomarker in pulmonary diseases (191), and recently a study used VOCs patterns to classify 17 diseases (including lung cancer) among 1,404 subjects with 86% accuracy (192).

Despite being a cost-effective, non-invasive method broad applicability to various diseases, the application of
these technologies is currently challenged by the intrinsic diversity of pathological conditions and confounding variables such as smoking history, diet, pharmaceuticals and age (193). Furthermore, there is currently no standardised method or means to compare the reliability and replicability of the results obtained between studies. This was demonstrated in a review of ten studies profiling VOCs in lung cancer where only 17/170 VOCs detected in total were cited by more than two studies (194).

**Conclusions**

COPD and lung cancer are leading causes of mortality worldwide and share key modifiable and preventable risk factors including tobacco smoking and air pollution exposure. Only a small proportion (10–15%) of smokers will ultimately develop COPD or lung cancer, indicating that the complex interplay between genetics, epigenetics and environmental factors is key to understanding these diseases. The exact mechanisms behind the increased incidence of lung cancer among COPD patients are yet to be defined, however recent studies provide strong evidence supporting the role of immune dysfunction, the lung microbiome, epigenetic regulation and EVs in the development of COPD and lung cancer.

Early detection and effective, feasible interventions are urgently required to reduce the burden of COPD and lung cancer. Further studies investigating the links between key molecular pathways will continue to elucidate the mechanisms underlying the development of COPD and lung cancer, and provide insights into the development of novel diagnostic and prognostic tools for early intervention and personalised therapeutic strategies.

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**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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