

## Oxidative stress induced lung cancer and COPD: opportunities for epigenetic therapy

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- Oxidative stress, epigenetics, NSCLC and COPD
  - Cigarette smoke mediated cellular proliferation
  - Cigarette smoke mediated lung destruction
  - DNA methylation and lung cancer
  - Histone post-translational modifications and lung cancer
- Linking epigenetics and oxidative stress to the development of COPD and NSCLC
  - HATs and KATs in the developing lung
  - HDACs and COPD
  - Aberrant KAT/HDAC activity in NSCLC
  - ATP-dependent SWI/SNF chromatin remodelling complexes and NSCLC
  - KATs/HDACs and oxidative stress pathways
  - KEAP1-NRF2-ARE signalling pathway
- Epigenetics, COPD, NSCLC and oxidative stress pathways/genes
  - NRF2
  - Heme oxygenase 1
- Hypoxia-inducible factor-1 $\alpha$
- PGC-1 $\alpha$
- Oxidative stress, epigenetics, glucocorticoids and COPD
- Oxidative stress, acetylation and its role in the regulation of NF $\kappa$ B
  - Targeting epigenetic mechanisms in COPD and NSCLC
- Theophylline – a novel agonist of HDACs
  - Current clinical trials involving epigenetic therapies in COPD and NSCLC
- Dietary HDAC inhibitors as therapeutic agents to target
  - COPD or NSCLC?
- Sulforaphane
- Curcumin
- Epigallocatechin 3-gallate (EGCG)
- Can dietary epigenetic inhibitors work in the clinical setting?
- Caveats
- Conclusions

### Abstract

Reactive oxygen species (ROS) form as a natural by-product of the normal metabolism of oxygen and play important roles within the cell. Under normal circumstances the cell is able to maintain an adequate homeostasis between the formation of ROS and its removal through particular enzymatic pathways or *via* antioxidants. If however, this balance is disturbed a situation called oxidative stress occurs. Critically, oxidative stress plays important roles in the pathogenesis of many diseases, including cancer. Epigenetics is a process where gene expression is regulated by heritable mechanisms that do not cause any direct changes to the DNA sequence itself, and disruption of epigenetic mechanisms has important implications in disease. Evidence is emerging that histone deacetylases (HDACs) play decisive roles in regulating important cellular oxidative stress pathways including those involved with sensing oxidative stress and those involved with regulating the cellular response to oxidative stress. In particular aberrant regulation of these pathways by HDACs may play critical roles in cancer progression. In this review we discuss the current evidence linking epigenetics and oxidative stress and cancer, using chronic obstructive pulmonary disease and non-small cell lung cancer to illustrate the importance of epigenetics on these pathways within these disease settings.

**Keywords:** epigenetics • chromatin • oxidative stress • COPD • NSCLC

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## Oxidative stress, epigenetics, NSCLC and COPD

Chronic obstructive pulmonary disease (COPD), is the diagnostic term for a group of disorders that are characterized by respiratory symptoms – dyspnoea, cough and sputum production; airflow limitation and chronic inflammation of the lung [1]. It is currently the fourth leading cause of worldwide mortality, responsible for more than 2.5 million deaths per year and is expected to become the third leading cause by 2020 [2]. In addition individuals suffering from COPD also have an associated increased risk of developing lung cancer [3, 4].

Lung cancer is the leading cause of cancer death worldwide, and is associated with over 1 million deaths annually [5]. Only 13% of lung cancer patients survive more than 5 years. According to the American Cancer Society, approximately 219,440 new cases of lung cancer (both small cell and non-small cell), and 159,390 deaths will occur in the United States during 2009 (source <http://www.cancer.org>). Clinico-pathologically, lung cancer can be subdivided into two broad categories, non-small-cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC can then be further divided into three major types, squamous cell carcinoma (SCC), adenocarcinoma and large cell carcinoma. Lung cancer is often considered to be largely preventable as most cases can be attributed to smoking. However, significant proportions (approximately 25%) of all lung cancers worldwide are not caused by smoking. If considered in a separate category lung cancer in never smokers would rank as the seventh most common cause of cancer death worldwide, before cancers of the cervix, pancreas and prostate [5]. Because of difficulties in its early detection, lung cancer has a high morbidity, and is often associated with resistance to currently available chemotherapy and radiotherapy regimes [6].

Cigarette smoke mediated oxidative stress is well established as being causative for both cellular proliferation in lung cancer and mediating lung destruction in COPD. In the following sections we provide examples of how cigarette smoke may cause both cellular proliferation and destruction, but for extensive reviews on this subject the reader is directed to the following recent reviews [1, 7].

### Cigarette smoke mediated cellular proliferation

For example, in transformed lung epithelial type-II cells cigarette smoke condensate at low concentration (0.1  $\mu\text{g}/\text{ml}$ ) induced cancer cell proliferation, DNA synthesis, reduced glutathione levels and intercellular adhesion molecule-1 expression without any significant change in reactive oxygen species (ROS) and superoxide radicals production [8]. Another recent study has shown that critical miRNAs involved with regulating stress response, apoptosis, proliferation, angiogenesis and expression of genes were found to be down-regulated in the lungs of rats exposed to environmental cigarette smoke for 28 days [9]. Benzo[a]pyrene (B[a]P) is one of the strongest carcinogens present in both the environment and cigarette smoke. B[a]P has now been shown to increase the proliferative potential of

lung adenocarcinoma cells through the epidermal growth factor receptor (EGFR) signalling pathway by overexpression of phosphorylated EGFR protein and inducing expression of the ligands for EGFR such as amphiregulin and epiregulin [10]. Moreover, exposure of human airway epithelial cells to cigarette smoke, results in an increase in EGFR activation over time, leading to prolonged signalling by the EGFR and which may therefore contribute to uncontrolled lung cell growth [11]

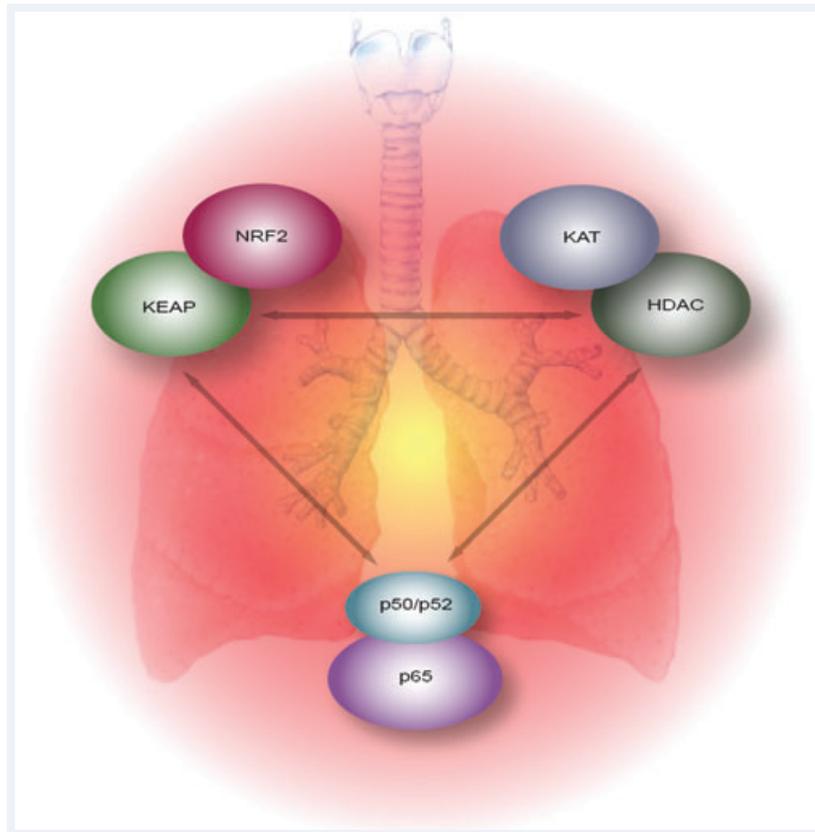
### Cigarette smoke mediated lung destruction

One way by which cigarette smoke extract mediates cellular destruction in the lung has been shown to be *via* disruption of tubulin-microtubules structure and function, and it has been suggested that this results in cellular apoptosis and subsequent tissue damage in smokers [12]. In addition, p21 is overexpressed in alveolar epithelial cells in response to cigarette smoke [13], leading to cellular senescence, and if p21 is disrupted, this response is attenuated, which indicates how critical regulators designed for the protection of the host, can lead to organ damage in the presence of cigarette smoke [14].

Vascular endothelial growth factor (VEGF) is fundamental to the development and maintenance of vasculature. Expression of critical members of this signalling pathway has been shown to be reduced in the lungs of both smokers and patients with COPD compared with non-smokers [15]. Cigarette smoke down-regulated vascular endothelial growth factor receptor (VEGFR)-2 expression, endothelial nitric oxide synthase (eNOS) levels and VEGF-induced VEGFR-2 phosphorylation in human lung microvascular endothelial cells, which results in impaired VEGF-induced endothelial cell migration and angiogenesis [16].

In COPD, defective efferocytosis (apoptosis and alveolar macrophage phagocytic function) may lead to secondary necrosis and tissue damage. Cigarette smoke has been shown to significantly decrease alveolar macrophage phagocytic function in both COPD patients and healthy smokers compared with control subjects indicating that smoking-related reduction in alveolar macrophage (AM) phagocytic ability may play important roles in the destruction of lung [17]. These differences (*i.e.* proliferative *versus* destructive effects) may be due to either cigarette smoke targeting different cell types, or may be cell specific effects. Alternatively, these differential effects may be caused by differential regulation of critical pathways *via* aberrant epigenetic regulation.

Molecular genetic studies of lung cancer frequently reveal multiple genetic and epigenetic changes including DNA sequence alterations, copy number changes and aberrant promoter methylation [18, 19]. The role of epigenetics in cancer is increasingly recognized as a major focus for translational research. NSCLC is no exception and DNA CpG methylation is recognized as having both predictive and prognostic significance in NSCLC [20, 21]. The three main, inter-related forms of epigenetic regulation are: DNA methylation, genomic imprinting and histone modification, all of which have important roles in disease [22].



**Fig. 1** The concept. Overview of the balance between oxidative stress response mechanisms, critical regulators such as lysine acetyltransferases and HDACs and the NF $\kappa$ B pathway in the lung. Perturbations to any of these could lead to aberrant epigenetic regulation of pathways critical to the cells response to oxidative stress, and may lead to COPD or NSCLC.

## DNA methylation and lung cancer

The importance of DNA methylation in the development of lung cancer was recently demonstrated when it was shown that the transformation efficiency for immortalization of normal bronchial epithelial cells could be enhanced by low dose exposure to carcinogens. The mechanism underpinning this involved hypermethylation of 5–10 genes due to elevated expression of DNA methyltransferase 1 (DNMT1). Ablation of DNMT1 was shown to reverse this process. Moreover, stable ‘knockdown’ of DNMT1 prior to carcinogen exposure was sufficient to prevent cellular transformation [23].

## Histone post-translational modifications and lung cancer

Aberrant histone post-translational modifications have also been shown to have both predictive and prognostic significance in NSCLC [24, 25], and deregulation of some of these enzymes in a bronchial epithelial cell transformation model suggest that they play important roles in the transformation process [26]. In addition, strong evidence links aberrant expression of epigenetic regulators, in particular histone deacetylases (HDACs) to COPD [27]. There is therefore an urgent need to improve our understanding of the molecular basis of epigenetic mechanisms in NSCLC and COPD.

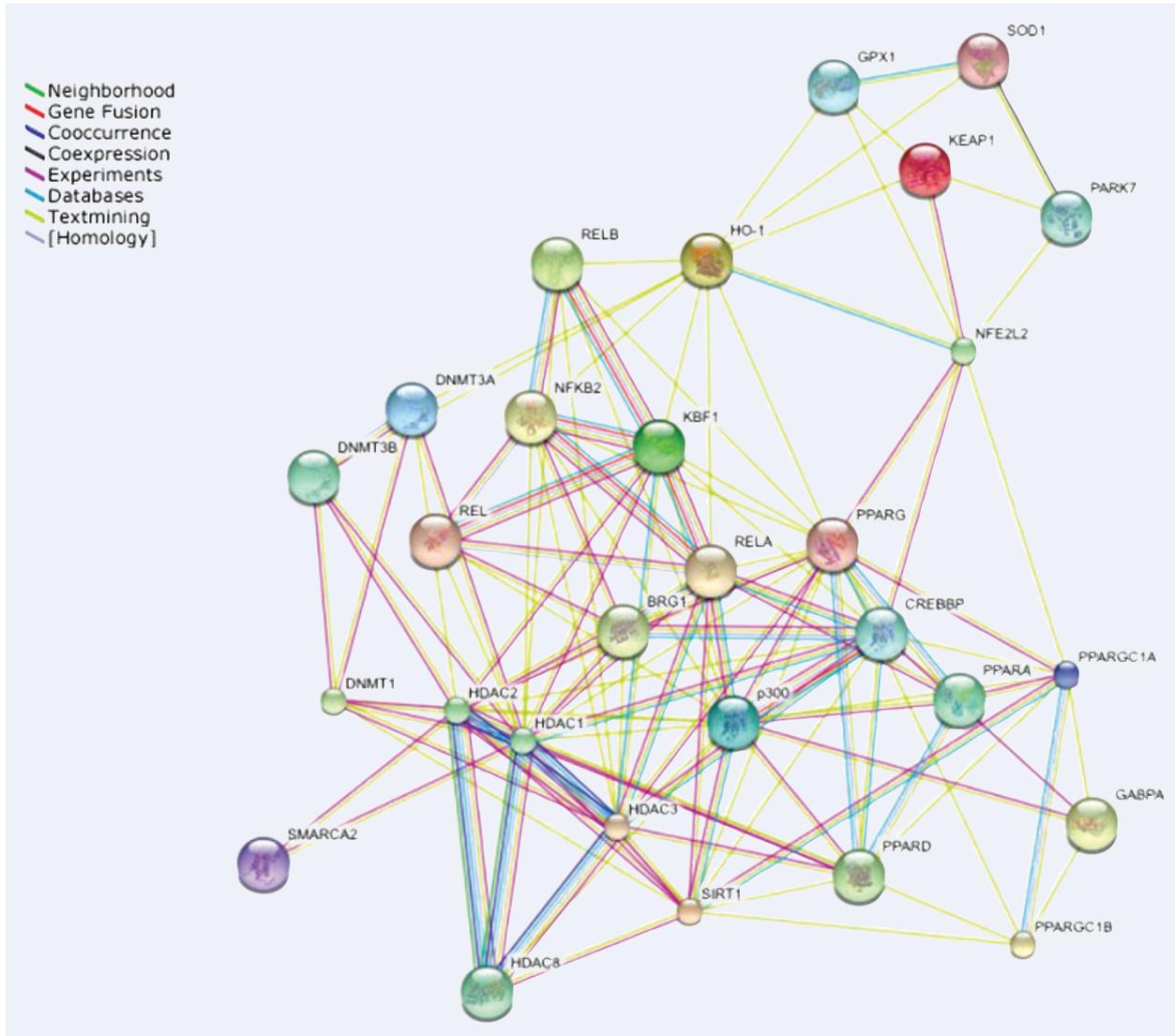
Oxidative stress has been clearly linked to COPD and NSCLC [28] (Figs 1 and 2), and there are many pathways/genes associated with the cellular response to oxidative stress. Among these pathways include genes such as superoxide dismutases (SODs), glutathione peroxidases (GPXs), glucocorticoid receptors (GRs), heme oxygenases (HOs) and hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ).

In the following sections we shall discuss the role that epigenetics plays in the regulation of these genes/pathways using COPD and NSCLC to illustrate the epigenetic dysregulation associated with these diseases and discuss the potential for epigenetic targeting strategies as a therapeutic intervention for the treatment of both.

## Linking epigenetics and oxidative stress to the development of COPD and NSCLC

### HATs and KATs in the developing lung

Evidence is also emerging that HDACs and lysine acetyltransferases play important roles in the developing lung (Table 1). The importance of lysine acetyltransferase (KAT) activity in lung



**Fig. 2** Graphical demonstration linking epigenetic regulators, the NFκB and the oxidative stress response pathways. This figure was generated using STRING 8.0 <http://string.embl.de/>.

development has been shown in mouse studies, where KATs were shown to be highly expressed in the developing lung [29–34], and confirmation of their importance in lung development came from KAT3B knockout studies [35]. Recently a homeodomain protein (HOP), which in the cardiac system associates with HDAC2 to repress cardiac specific genes, was observed to be present in airway epithelium along with HDAC2. HOP was shown to represses lung-specific gene expression in an HDAC-dependent manner, and loss of HOP expression *in vivo* resulted in defective type 2 pneumocyte development [36].

## HDACs and COPD

A link between HDAC activities and COPD was identified when studies revealed that in lung tissue of patients with increasing clinical stages of COPD, graded reductions in HDAC activity, reduced levels of HDAC2 protein and lower mRNA levels for HDACs 2, 5 and 8 were observed [37]. The decreased levels of HDACs have also been observed in separate studies of the effects of cigarette smoking in patients with COPD [38–41], and oxidative stress has also been shown to decrease HDAC2 levels in a bronchial epithelial

**Table 1** KATs, KMTs and HDACs discussed in this review

Gene	Activity	Comments	Reference
KAT2A formerly (GCN5)	Acetyltransferase	Critical for lung development	[30, 31, 34]
KAT2B (formerly PCAF)	Acetyltransferase	Critical for lung development	[30]
KAT3A (formerly CBP)	Acetyltransferase	Critical for lung development Mutated in lung cancer	[29, 32, 33] [72]
KAT3B (formerly p300)	Acetyltransferase	Critical for lung development	[31–33, 35]
KAT13A (formerly SRC1)	Acetyltransferase	Critical for lung development	[32]
HDAC1	Deacetylase	Elevated mRNA in lung cancer	[50]
		Protein detected in lung cancer	[51]
HDAC2	Deacetylase	Association with HOP involved with pneumocyte development	[36]
		Reduced levels of HDAC2 protein in lung tissue of patients with COPD	[37]
		Protein detected in lung cancer	[51]
HDAC3	Deacetylase	Elevated protein detected in NSCLC SCCs	[52]
HDAC5	Deacetylase	Reduced mRNA in lung tissue of patients with COPD	[37]
HDAC8	Deacetylase	Reduced mRNA in lung tissue of patients with COPD	[37]
Sirt1	Deacetylase	Protein levels are reduced in lung tissue of COPD patients	[43]
Sirt2	Deacetylase	Decreases ROS	[77]
Sirt3	Deacetylase	Prevents oxidative stress induced apoptosis	[78]
KMT1A (formerly SUV39H1)	Methyltransferase	Up-regulated in cancer cell lines and following immortalization and transformation of human bronchoepithelial cells	[26]
KMT1B (formerly SUV39H2)	Methyltransferase	Polymorphisms associated with an increased risk for lung cancer	[73]
KMT1C (formerly G9a)	Methyltransferase	Up-regulated in cancer cell lines and following immortalization and transformation of human bronchoepithelial cells	[26]
KMT1E (formerly ESET or SETDB1)	Methyltransferase	Up-regulated in cancer cell lines and following immortalization and transformation of human bronchoepithelial cells	[26]
KMT4 (formerly DOT1L)	Methyltransferase	Up-regulated in cancer cell lines and following immortalization and transformation of human bronchoepithelial cells	[26]

Continued

**Table 1** Continued

Gene	Activity	Comments	Reference
KMT6 (formerly EZH2)	Methyltransferase	Up-regulated in cancer cell lines and following immortalization and transformation of human bronchoepithelial cells	[26]
KMT8 (formerly RIZ1)	Methyltransferase	Polymorphisms associated with an increased risk for lung cancer	[74]

**Table 2** major ROS pathways discussed, epigenetic modifications or enzymes affected and cell function

Pathway	Epigenetic modifications or enzymes affected	Cell function
KEAP1-NRF2-ARE	DNA methylation	Oxidative stress sensor
SP-A	Histone acetylation	Innate host defence
NFκB	Histone acetylation	Cellular stress responses
	Lysine acetyltransferases	Induction of pro-inflammatory genes
	HDACs	
HO-1	Histone acetylation	Cytoprotective cellular stress responses
	Histone methylation	
	SWI/SNF -like chromatin-remodelling complexes	
	Lysine methyltransferases	
HIF-1α	Histone methylation	Regulates responses to oxidative stress
	Histone acetylation	
	Lysine acetyltransferases	
	HDACs	
PGC-1α	Histone acetylation	Regulator of antioxidant genes
	HDACs	
	Histone acetyltransferases	Protects cells from oxidative stress
Glucocorticoids	HDACs	Regulate expression of protective factors in oxidative stress
	Lysine acetyltransferases	

cell line [42]. In addition levels of the NAD<sup>+</sup>-dependent HDAC sirtuin 1 (SIRT1) have also been shown to be reduced in patients with COPD [43].

Surfactant protein A (SP-A) is a member of the collectin family of innate host defence molecules and is expressed primarily in the respiratory epithelial cells of the lung (Table 2). SP-A has been shown to be a biomarker with elevated expression in patients with COPD and in patients who smoke [44–47]. SP-A is negatively regulated by

the transcription factor GATA-6 [48], in a complex containing HDAC2 and Nkx2.1 [36]. As levels of HDAC2 have been shown to be decreased in the lungs of patients with COPD, this could explain why SP-A is elevated in patients with COPD. Within macrophages in the lung environment SP-A has also been shown to decrease the phosphorylation of I-Kappa-B-Alpha (IKBA), a key regulator of NFκB activity, and nuclear translocation of p65 while additionally decreasing the phosphorylation of Akt, a major cell regulator of NFκB [49].

The role of epigenetics in regulating NF $\kappa$ B mediated transcription is discussed in greater detail in a following section.

### Aberrant KAT/HDAC activity in NSCLC

In contrast to COPD, elevated levels of HDAC1 mRNA have been observed in higher stage (stage III or IV) NSCLC [50], while a more recent study has also observed expression of all class I HDACs in lung cancer specimens [51]. Using antibody arrays, HDAC3 protein was found to be elevated in 92% of SCCs of the lung (Table 1) [52].

It is well established that HDACs form large multi-protein complexes to regulate gene expression [53]. Several members of these complexes have also been shown to be altered in NSCLC. For example, mSin3A a member of a multiple component co-repressor complex that contains HDACs has also been observed to have decreased expression in NSCLC [54].

### ATP-dependent SWI/SNF chromatin remodelling complexes and NSCLC

Members of the ATP-dependent SWItch/Sucrose (SWI)/NonFermentable (SNF) chromatin remodelling complexes have also been shown to be altered in the lung. In NSCLC cell lines, the SWI/SNF complex has been found to form a larger complex containing neuron-restrictive silencer factor and its co-repressors, mSin3A and CoREST [55], and although this complex has been shown to regulate neuronal specific genes, it was suggested that deregulation of neuron-restrictive silencer factor regulated genes in NSCLC could in fact contribute to enhanced tumourigenicity [55]. Additional evidence now links other members of these SWI/SNF complexes to the pathogenesis of NSCLC adding support to this notion. Expressions of the SWI/SNF ATPase subunits, BRG1 and BRM (BRG1/BRM), have been shown to be either mutated or lost in approximately 30% of human non-small lung cancer cell lines [56–60]. Critically, BRM has been shown to be regulated epigenetically [61], and HDAC inhibitors have been shown to reactivate expression of BRM [62, 63]. When examined in primary tumours, 10% of NSCLC had concomitant loss of both BRG1 and BRM, which correlated with the poorest prognosis [56]. A multiple tissue array approach examining 12 core proteins involved with chromatin remodelling machinery in 300 NSCLC samples (150 adenocarcinomas and 150 SCCs), found two clusters which either contained BRM, Ini-1, retinoblastoma, mSin3A, HDAC1 and HAT1, or which contained BRG1, BAF155, HDAC2, BAF170 and RbAP48 (Table 1) [64]. Positive nuclear BRM (N-BRM) staining correlated with a favourable prognosis in patients with a 5-year survival of 53.5% compared with 32.3% for those patients with tumours that were negative for N-BRM ( $P = 0.015$ ). Co-positivity for both N-BRM and nuclear BRG1 had an increased 5-year survival of 72% compared with 33.6% ( $P = 0.013$ ) for those patients whose tumours were positive for either marker, or

negative for both markers. In contrast, membranous BRM (M-BRM) staining correlated with a poorer prognosis in AD patients with a 5-year survival of 16.7% compared with those without M-BRM staining (38.1%;  $P = 0.016$ ) [64].

The expression of metastasis-associated protein 1 (MTA-1) has been shown to be significantly elevated in NSCLC and was found to be associated with both tumour invasiveness and metastasis [65]. As MTA-1 is known to associate with HDACs [66–71], this would suggest that HDAC activity associated with MTA-1 is involved with NSCLC invasiveness and metastasis.

Mutations within the lysine acetyltransferase KAT3A have also been identified in a small subset of NSCLC [72], and other histone modifying enzymes have been identified for which polymorphisms within these genes are associated with an increased risk for lung cancer including lysine methyltransferase KMT1B (formerly SUV39H2) [73], and KMT8 (formerly RIZ/PRDM2) [74] (Table 1). Polymorphisms in another epigenetic regulator, Methyl-CpG binding domain 1 (MBD-1) have also been shown to be associated with an increased lung cancer risk [75]. Intriguingly, the polymorphism associated with increased risk, also had significantly higher promoter activity. As MBD-1 has been shown to associate with HDACs to repress transcription [76], this polymorphism may therefore lead to elevated MBD-1/HDAC complexes forming, resulting in aberrant gene expression leading to NSCLC development, but this will require functional testing.

### KATs/HDACs and oxidative stress pathways

HDACs have been linked to the regulation of genes important for oxidative stress pathways (Figs 1 and 2). For instance, oxidative stress in adipose and kidney cells increases the levels of the NAD<sup>+</sup> dependent HDAC SIRT2. SIRT2 then binds to FOXO3a and reduces its acetylation level. This results in increased FOXO3a DNA binding which elevates the expression of p27 (Kip1), manganese SOD and Bim. As a consequence, this then decreases cellular levels of ROS in these cells [77]. Similar studies in human cardiomyocytes have shown that SIRT3 can prevent oxidative stress-mediated cell death by deacetylating the Ku70 protein, which results in enhanced binding of Ku70 to the proapoptotic protein Bax, and preventing its translocation to the mitochondria, thus protecting the cardiomyocytes from oxidative stress induced apoptosis [78].

The transcription factor Bach1 (BTB and CNC homology 1, basic leucine zipper transcription factor 1) has been shown to be a repressor of the oxidative stress response in mice [79]. One mechanism by which Bach1 functions involves its role in oxidative stress-induced cellular senescence by a complex that contains p53, HDAC1 and the nuclear co-repressor N-CoR to repress target genes [80].

### KEAP1-NRF2-ARE signalling pathway

One of the most important oxidative stress protective pathways is the Kelch-like ECH-Associated Protein 1 (KEAP1)-NFE2-Related

Factor 2 (NRF2)- antioxidant response element (ARE) or KEAP1-NRF2-ARE signalling pathway (Table 2, Fig. 1) which plays critical roles in protecting cells from endogenous and exogenous stresses [81], and plays important roles within the lung [82]. It has been hypothesized that low expression of the oxidative stress sensor KEAP1 is thought to be involved in carcinogenesis. Evidence supporting this notion in lung cancer has emerged whereby down-regulation of KEAP1 by DNA CpG methylation has been shown to occur in both NSCLC cell lines and tumour tissues [83], and an additional study in NSCLC cell lines showed that loss of KEAP1 function activated NRF2 and provided cellular growth advantages [84]. In COPD, the KEAP1-NRF2-ARE pathway has been shown to be disrupted but in this instance, levels of KEAP1 were found to be unchanged whereas significant decreases in NRF2 and DJ-1 protein were found. (DJ-1 is a protein that stabilizes NRF2 by impairing KEAP1-dependent proteasomal degradation of NRF2). Of these, DJ-1 expression was negatively associated with severity of COPD [85]. Another study confirmed the loss of NRF2 in lung tissues of patients with emphysema but in addition also demonstrated elevated expression of KEAP1 in these patients [86]. Overall, it would appear that dysregulation of this critical oxidative stress responsive pathway occurs in both COPD and NSCLC (Table 2).

## Epigenetics, COPD, NSCLC and oxidative stress pathways/genes

### NRF2

Emerging evidence is beginning to link direct chromatin remodeling to the cellular response to oxidative stress. The KEAP1-NRF2-ARE pathway is strongly implicated as an antioxidative stress pathway in the lung [82]. Under normal conditions NRF2 is found associated with the cytoplasmic inhibitor, KEAP1. Upon oxidative stress, NRF2 becomes phosphorylated and dissociates from KEAP1, whereupon it translocates to the nucleus and binds to an ARE, inducing the expression of genes that encode antioxidant-detoxifying proteins, to rescue cells against oxidative injury [87].

An early indication that NRF2 may utilize chromatin remodeling to regulate the expression of its cognate target genes came from studies of the structural motifs contained within it (Table 2). Two transcription activation domains, Neh4 and Neh5, were found to associate with KAT3B and acted synergistically to obtain maximal activation of gene expression by NRF2 [88]. Other proteins with histone modifying ability which have been shown to associate and regulate NRF2 transactivation domain activity include the lysine acetyltransferases KAT3A/KAT3B/KAT2B (formerly CBP/p300/ pCAF), and arginine methyltransferases CARM1 and PRMT1 [89] (Fig. 2).

Under conditions of oxidative stress the SWI/SNF complex component BRG1 interacts directly with NRF2 to selectively

mediate induction of responsive genes [90]. More recently, the NF $\kappa$ B p65 subunit was shown to repress the NRF2-antioxidant response element (ARE) pathway at the transcriptional level (Fig. 2). In this regard, in cells where NF $\kappa$ B and NRF2 were simultaneously activated, p65 unidirectionally antagonized the transcriptional activity of NRF2, and ARE-dependent expression of haemoxygenase-1 (HO-1) was strongly suppressed. The mechanism found to cause this effect involved the selective prevention of the lysine acetyltransferase KAT3A association with p65 resulting in the inactivation of p65. This was followed by the recruitment by p65 of HDAC3 to ARE-dependent promoters to repress transcription [91].

### Heme oxygenase 1

HO-1 is a cellular stress response gene which has been shown to protect the lung from oxidative stress based insults such as cigarette smoke and COPD *via* degradation of heme to biliverdin-IX $\alpha$ , carbon monoxide and iron, each with candidate roles in cytoprotection. At low concentrations, carbon monoxide can confer similar cyto- and tissue-protective effects as endogenous HO-1 expression, involving antioxidative, anti-inflammatory, anti-proliferative and anti-apoptotic effects. [92]. Regulation of HO-1 has been shown to involve chromatin remodelling (Fig. 2), and exposure of cells to heme results in *de novo* hyperacetylation and hypermethylation of histone H3 at the HO-1 enhancers resulting in its enhanced transcription [93]. The BRG1 catalytic subunit of SWI2/SNF2-like chromatin-remodelling complexes has been shown to utilize NRF2 to regulate expression of HO-1 in a colon cell line in response to oxidative stress [90]. Deletion of a particular region in NRF2 (the Neh5 domain) resulted in a reduction in expression of HO-1. Loss of this domain reduced the ability of KAT3B and BRG1 (Brahma-related gene 1) to associating with NRF2, thereby reducing their co-operative enhancement of HO-1 promoter activity [94].

### Hypoxia-inducible factor-1 $\alpha$

Hypoxia is a common event in solid tumours [95], but is also strongly associated with NSCLC [96] and COPD [97]. Similarly, levels of HIF-1 $\alpha$  or other markers of hypoxia such as Carbonic Anhydrase IX have also been linked to NSCLC [98–101]. Knockdown siRNA experiments targeting HIF-1 $\alpha$  in a Lewis mouse model of lung cancer have been shown to prolong survival by reducing cancer cell proliferation, angiogenesis and apoptosis, and other cellular responses in the lung tumour tissue [102].

In a study examining the progressive structural changes in the bronchial epithelium with subepithelial fibrous remodelling, it was shown that HIF-1 $\alpha$  was expressed in epithelial cells associated with increased progression of structural changes, increased reticular basement membrane (RBM) thickness, and with a reduction in the number of blood vessels in the subepithelium of COPD patients [103], while in a clinical study of healthy human volunteers,

when placed under conditions of hypoxia, it was found that hypoxia induced oxidative stress *via* an overgeneration of ROS, which was positively correlated with an up-regulation of HIF-1 $\alpha$ , VEGF and DNA oxidation [104].

HIF-1 $\alpha$  has recently been shown to regulate epigenetic regulators including lysine demethylases [105–107].

Epigenetic mechanisms have been shown to affect HIF-1 $\alpha$  mainly through stabilization of the protein itself, by HDACs [108–111]. In addition, the MTA-1 has also been shown to both stabilize HIF-1 $\alpha$  and enhance tumour metastasis *via* angiogenesis through direct associations with HDACs [112, 113]. Indeed, in many cell culture models inhibition of HDACs has been shown to both destabilize HIF-1 $\alpha$  and reduce angiogenesis [108, 114–118].

HIF-1 $\alpha$  functionally associates with lysine acetyltransferases (KATs and HDACs) to regulate gene expression in response to hypoxia [111, 119–122]. These associations are known to be essential to drive hypoxia-responsive transcription, from studies in mice which were engineered to carry deletions in the domains which associate with these proteins. The domains necessary for KAT3A and KAT3B interactions with HIF-1 $\alpha$  were genetically non-redundant and indispensable for HIF-1 $\alpha$  transactivation function, while a similar yet larger effect was observed for the domain required for HIF-1 $\alpha$  association with HDACs (35–50% compared to 70%) [123]. Indeed for some target HIF-1 $\alpha$  responsive genes both lysine acetyltransferases and HDACs were required for greater than 90% of the genes response, suggesting that a functional interaction between lysine acetylases and HDACs are essential for nearly all HIF-1 $\alpha$  responsive transcription [123].

## PGC-1 $\alpha$

Peroxisome proliferator-activated receptor- $\gamma$  co-activator 1 $\alpha$  (PGC-1 $\alpha$ ) was originally isolated from a brown fat cDNA library linked to adaptive thermogenesis [124]. Since then it has been associated with various other important pathways including mitochondrial oxidative metabolism, adipocyte cell fate decision, glucose homeostasis and hepatic gluconeogenesis [125]. It is well established that PGC-1 $\alpha$  acts as a transcriptional co-activator associating with both HDACs [126] and lysine acetyltransferases [127] to regulate expression of genes (Table 2). Under various conditions of stress PGC-1 $\alpha$  has been shown to increase the expression of major antioxidant enzymes including copper/zinc SOD (SOD1), manganese SOD (SOD2), catalase and GPx1 [128–131]. PGC-1 $\alpha$  can regulate the expression of uncoupling protein 2 and uncoupling protein 3, both of which are now understood to be able to mitigate ROS formation [132, 133]. Nitric oxide, a critical regulator of oxidative stress has also been shown to regulate the protection of mitochondria by modulating PGC-1 $\alpha$  expression, resulting in changes to the expression of mitochondrial ROS detoxification system members [134].

The PGC-1 transcriptional co-activators are major regulators of several crucial aspects of energy metabolism, and NRF1 and NRF2 are key targets of the PGC-1s in mitochondrial biogenesis [82]. In

a neural cell model studying the metabolism of ROS, levels of PGC-1 $\alpha$  were induced in cells given an oxidative stressor, H<sub>2</sub>O<sub>2</sub>, resulting in greatly enhance protection from oxidative-stressor-mediated death [82, 128].

## Oxidative stress, epigenetics, glucocorticoids and COPD

Glucocorticoids are a standard treatment option for patients with COPD. In particular they are frequently used for the treatment of patients with acute exacerbations of their disease [135]. However, many COPD patients develop resistance or have a reduced sensitivity to glucocorticoids [136]. Glucocorticoids act by binding to the GR, normally found in the cytoplasm. This binding results in translocation of the receptor to the nucleus, where it can suppress the expression of responsive genes in particular pro-inflammatory gene expression.

One of the genes negatively regulated by glucocorticoids is SP-A which, as described, has a role in regulating NF $\kappa$ B in airway epithelial cells [137]. GR regulates the expression of the SP-A gene *via* chromatin remodelling and assembly of complexes containing either HDACs or KATs to functionally regulate transcription of this gene [138]. It has been suggested that IKBA, a key regulator of NF $\kappa$ B activity is a major contributor to the anti-inflammatory effects of SP-A [137].

As levels of HDAC2 have been shown to be decreased in the lungs of patients with COPD, this would indicate the reason why SP-A is elevated in patients with COPD. It has been suggested that accumulation of IKBA, a key regulator of NF $\kappa$ B activity is a major contributor to the anti-inflammatory effects of SP-A [137]. Indeed, within macrophages isolated from the lung environment, SP-A has in fact been shown to decrease the phosphorylation of IKBA, resulting in decreases of nuclear translocation of p65 and phosphorylation of Akt, a major cell regulator of NF $\kappa$ B, and resulting in a decreased inflammatory response [49].

The UPC3 gene, implicated as a protective factor in oxidative stress has also been shown to be regulated *via* glucocorticoid mediated chromatin remodelling. In muscle cells, glucocorticoids activate UPC3 transcription *via* histone acetylation through the activity of KAT3B, and repress its transcription through the activity of the HDAC SIRT1 [139].

Indeed acetylation is an important regulator of GR at many levels. Intriguingly, GR activation requires HDAC1 to act as a co-activator (Table 2), in that HDAC1 is required for the induction of some genes by the GR [140], while HDAC6 has been shown to be important in the maturation of GR *via* heat shock protein 90 (Hsp90). In cells deficient for HDAC6, Hsp90-dependent maturation of the GR is compromised, resulting in reduced in ligand binding, altered nuclear translocation and aberrant transcriptional activation [141].

Using a synthetic glucocorticoid (dexamethasone), investigators have demonstrated that GR mediated repression of genes regulated by the pro-inflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ )

utilize the activities of lysine acetyltransferases and HDACs. Initial studies found that dexamethasone inhibits IL-1 $\beta$ -induced acetylation at histone H4 lysine 8 at a target promoter in a concentration-dependent manner. Two mechanisms for this were identified. In one, GR acts as a direct inhibitor of KAT3A lysine acetyltransferase activity. In the other mechanism, GR recruited HDAC2 to the p65-KAT3A complex [142]. Additional studies determined that the major mechanism required the recruitment of HDAC2 to this p65-KAT3A complex [143].

Oxidative stress has been implicated in affecting glucocorticoid sensitivity in patients with COPD. One potential mechanism to explain this may be that levels of specific HDACs (in particular HDAC2) are decreased in the lungs of COPD patients (Table 2). Critically as a consequence, loss of GR acetylation induces glucocorticoid insensitivity towards NF $\kappa$ B-mediated gene expression [144], and restoration of HDAC2 by overexpression in glucocorticoid-insensitive alveolar macrophages from patients with COPD is able to restore glucocorticoid sensitivity [144].

### Oxidative stress, acetylation and its role in the regulation of NF $\kappa$ B

Chronic inflammation is a pervasive element strongly associated with cancer [145]. Indeed, pro-inflammatory cascades are important elements in oxidative stress mediated responses in COPD [146] and NSCLC [147]. One of the critical mediators of pro-inflammatory cascade signalling pathways involves the activation or regulation of NF $\kappa$ B [148]. The NF $\kappa$ B/Rel family consists of five subunits, but NF $\kappa$ B typically consists of a heterodimeric protein comprised a p50 and a p65 (RelA) subunit.

One of the first indicators that oxidative stress affects NF $\kappa$ B activation within the lung came from studies in cells which showed that oxidative stress and TNF- $\alpha$  activated NF $\kappa$ B/AP-1 in a lung cancer cell line model [149], which resulted in induced histone acetylation and enhanced pro-inflammatory IL-8 release from these cells [149]. The activation of NF $\kappa$ B itself relies heavily on the activities of lysine acetyltransferases and HDACs (Fig. 1). Initial studies found that the lysine acetyltransferases KAT3B and KAT3A acted as key co-activators in regulating NF $\kappa$ B driven gene expression [150–152], through associations with the RelA/p65 subunit, whereas another lysine acetyltransferase KAT13A was found to also potentiate NF $\kappa$ B transactivation through association with the other subunit p50 [153]. Indeed within the setting of the lung, oxidative stress induces the expression of pro-inflammatory genes such as IL-6 and IL-8 *via* NF $\kappa$ B recruitment of lysine acetyltransferase activity of KAT3A or KAT3B to stimulate the transcription of these genes (Fig. 2) [154–156]. Once lysine acetyltransferases were found to associate with NF $\kappa$ B, interactions between HDACs and NF $\kappa$ B were quickly found to be necessary to occur in order to repress expression of NF $\kappa$ B regulated genes or to control the levels of expression of NF $\kappa$ B induced genes (Fig. 2) [157–159].

One of the critical regulators of NF $\kappa$ B transcriptional activation is I-Kappa-B Kinase-Alpha (IKKA) [160]. Cytoplasmic sequestra-

tion of p65 by IKBA is sufficient to translocate nuclear co-repressors silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) to the cytoplasm [161]. As SMRT normally acts to repress transcription through association with HDACs [162], this results in derepressed transcription of its target genes. The association between p65 and IKKA's ability to directly bind SMRT could be inhibited in a dose-dependent manner by the KAT3A lysine acetyltransferase further implicating a role for lysine acetylation in this process [163]. It was subsequently shown that IKKA activates NF $\kappa$ B mediated transcription by phosphorylating (SMRT), resulting in the exchange of transcriptional co-repressor (HDAC3) for co-activator (KAT3B) complexes, allowing KAT3B to acetylate RelA/p65 (Fig. 2) [164, 165].

Inhibition of NF $\kappa$ B activation has been shown whereby the protein Daxx binds to the region of RelA/p65 that contains the main sites of acetylation mediated by KAT3B/KAT3A [166]. Presumably, this may prevent acetylation of RelA/p65 by direct interference, but it must also be noted that Daxx itself has been shown to directly associate with HDAC2 [167], and so may represent a mechanism by which KATs and HDACs compete for critical lysines on NF $\kappa$ B subunits. In this regard, small ubiquitin-like modifier modification of KAT3A has been shown to negatively modulate its transcriptional potential by recruiting a Daxx complex containing HDAC2 [168].

Finally, cigarette smoke, a critical element in airway induced oxidative stress has been linked to NF $\kappa$ B activation in part through post-translational modification of HDAC1, HDAC2, and HDAC3 protein by nitrotyrosine and aldehyde-adduct formation [169]. SIRT1 has also been shown to regulate NF $\kappa$ B transactivation by physically associating with one of its subunits RelA/p65, and by removing acetylation at a critical lysine on RelA/p65 prevents NF $\kappa$ B dependent transcription [170]. Cigarette smoke has also been shown to down-regulate SIRT1 in the lung, resulting in increased acetylation of RelA/p65 with concomitant activation of NF $\kappa$ B mediated pro-inflammatory cytokine release [171].

A large literature now exists concerning the role of lysine acetylation in the regulation of NF $\kappa$ B, and for a comprehensive review of the role of KATs and HDACs in the regulation of NF $\kappa$ B the reader is directed to following review by Calao *et al.*, [172].

### Targeting epigenetic mechanisms in COPD and NSCLC

Epigenetic targeting remains an attractive target for pharmacological intervention in disease. Two main epigenetic therapeutic avenues have been extensively explored for the treatment of NSCLC. These are aimed at targeting DNA methylation *via* DNMT inhibitors (DNMTi), and at targeting histone acetylation *via* HDAC inhibitors. As DNA methylation is a common event in lung cancer [18–21], many *in vitro* studies involving DNMTi have been carried out demonstrating the loss of expression of critical genes in NSCLC and showing that such genes can be reactivated in response to DNMTi treatment [173–183]. Intriguingly it has

recently been shown that DNA CpG hypermethylation and down-regulation of the oxidative stress sensor KEAP1 is common in NSCLC cell lines and tumour tissues, and can be reactivated following treatments with the DNMTi 5-aza-2'-deoxycytidine (Decitabine) [83].

We have recently extensively reviewed the potential for therapeutic targeting of HDACs in NSCLC and other disease conditions involving chronic inflammation in great detail and the reader is directed to the following reviews [184, 185]. Several of these epigenetic inhibitors have received FDA approval for the treatment of various diseases including Vorinostat (suberoylanilide hydroxamic acid, a HDi) and Dacogen (5-aza-2'-deoxycytidine, a DNMTi) and Vidaza (5-Azacytidine, a DNMTi) [186–188].

## Theophylline – a novel agonist of HDACs

One of the most interesting recent new developments in relation to epigenetic targeting of COPD has been the discovery that theophylline an agent developed for the treatment of COPD can act as an agonist of HDACs [40]. As decreased HDAC activity has been observed in patients suffering from COPD [37], several clinical trials are in process to assess the clinical efficacy of this drug in both COPD and NSCLC (Table 3).

### Current clinical trials involving epigenetic therapies in COPD and NSCLC

A large number of clinical trials in patients with COPD or NSCLC involving epigenetic therapies have been either completed or are ongoing (Table 3), and as these and other trials proceed, greater understanding of the potential side effects and dose-limiting toxicities of these drugs begins to emerge, and for the most part these first generation inhibitors have shown well-tolerated safety profiles [184].

## Dietary HDAC inhibitors as therapeutic agents to target COPD or NSCLC?

Dietary interventions with cruciferous vegetables (and in particular Broccoli) may prove to have a critical role in the treatment of COPD or in preventing the development of lung cancer.

## Sulforaphane

A compound found in broccoli, L-sulforaphane (SFN) was originally identified as a potential chemopreventative compound, which could protect against oxidative stress by activating phase II antiox-

idant enzymes [189]. Recent work has however found that this compound also acts as a HDAC inhibitor [190].

Evidence is now emerging that this compound may have efficacy in treating both COPD and NSCLC. In patients with COPD, loss of the DJ-1 protein has been shown to result in a decrease of NRF2 protein levels [85], and *in vitro* cell culture models where DJ-1 was either silenced by siRNA, or DJ-1 deficient mouse embryonic fibroblasts were employed found that treatments with sulforaphane up-regulated expression of NRF2 mediated antioxidant enzymes [85]. In NSCLC cell line models, sulforaphane has been shown to cause apoptosis by various mechanisms including binding to tubulin and disrupting microtubule polymerization [191–193], causing cell cycle arrest [194] and by sensitizing cells to tumours necrosis factor-related apoptosis-inducing ligand (TRAIL) [195]. Sulforaphane has also been shown to have efficacy in a mouse model. In this model, the A/J mouse model, following administration of tobacco carcinogens, lung adenomas appear during weeks 16 to 19 after administration, and adenocarcinomas occur generally during weeks 28 to 36. When the effects of sulforaphane on this model were tested, no effects on adenoma formation were observed but significant inhibition in the progression to adenocarcinomas was observed in the group fed sulforaphane, (1.5  $\mu\text{mol/g}$  diet), compared to the control [196].

## Curcumin

Another compound that has been shown to target epigenetic mechanisms may also play a role in the treatment of COPD is curcumin (diferuloylmethane). This is a compound derived from turmeric and has been shown to have potent anti-inflammatory properties and antioxidant properties [197, 198], and has been suggested to be a dietary intervention option in the treatment of inflammatory lung disease [199]. Curcumin has received a lot of attention as an 'anti-cancer' agent including studies on its potential effectiveness in lung cancer [200–203], and clinical trials have shown that it is well tolerated and lacks significant toxicity [204]. One of the mechanisms by which curcumin appears to act in its capacity as an anti-cancerous agent involves its ability to inhibit both lysine acetyltransferases [205–208], and HDACs [209, 210], although other studies have found that curcumin has no effect on HDACs [208].

Curcumin inhibits NF $\kappa$ B mediated responses either by either preventing the phosphorylation of IKBA [211], or by preventing its degradation [210], thereby reducing nuclear translocation NF $\kappa$ B.

It has also been suggested that curcumin may also prevent NF $\kappa$ B mediated chromatin remodelling through its ability to inhibit lysine acetyltransferases. The inhibition of NF $\kappa$ B in turn leads to the down-regulation of pro-inflammatory cytokines such as Chemokine, CXC Motif, Ligand CXCL1 and CXCL2 [212], or up-regulation of genes that control pro-inflammatory genes such as mitogen-activated protein kinase phosphatase-5 [213].

Curcumin and similar dietary compounds have been shown to induce HO-1 and NRF2 in breast cells [214] (Fig. 1), and so may

**Table 3** Clinical Trials involving epigenetic therapies in COPD and NSCLC

Company	Intervention	Phase	Clinical trial identifier*	Published*
Ottawa Health Research Institute	Theophylline	III	NCT00299858	
Ontario Thoracic Society				
University of Glasgow	Theophylline	III	NCT00119496	[265]
	<i>versus</i>			
GlaxoSmithKline	Rosiglitazone			
Chest, Heart & Stroke Scotland				
Chief Scientists Office				
Hospital Universitari Son Dureta	Theophylline	I?	NCT00671151	[266, 267]
Fondo de Investigación Sanitaria (FIS)				
Sociedad Española de Neumología y Cirugía Torácica				
National Cancer Institute of Canada	Theophylline	III	NCT00003684	
Imperial College London	Theophylline	II	NCT00241631	[37]
Mitsubishi Tanabe Pharma Corporation				
Medical Research Council				
Assaf-Harofeh Medical Center	Theophylline	?	NCT00893009	
National Cancer Institute of Canada	Theophylline	III	NCT00003684	
Argenta Discovery Ltd	Theophylline and Budesonide	II	NCT00634413	
NCI – Center for Cancer Research-Medical Oncology	Decitabine and FR901228	I	NCT00041158	
National Cancer Institute (NCI)				
Arthur G. James Cancer Hospital and Richard J. Solove Research Institute	Decitabine and valproic acid	I	NCT00084981	
NCI				
NCI – Center for Cancer Research-Medical Oncology	Decitabine	I	NCT00019825	
NCI				
Sidney Kimmel Comprehensive Cancer Center	Azacitidine and MS-275	I/II	NCT00387465	
NCI				
Memorial Sloan-Kettering Cancer Center	Phenylbutyrate and Azacitidine	II	NCT00006019	
NCI				
H. Lee Moffitt Cancer Center and Research Institute	LBH589 and Erlotinib	I/II	NCT00738751	

Continued

**Table 3** Continued

Company	Intervention	Phase	Clinical trial identifier*	Published*
Genentech				
Novartis				
NCI – Center for Cancer Research-Medical Oncology	MS-275	I	NCT00020579	
NCI				
Titan Pharmaceuticals	Pivanex	II	NCT00073385	
Dartmouth-Hitchcock Medical Center	Vorinostat	?	NCT00735826	
Merck				
Fred Hutchinson Cancer Research Center	Vorinostat, Paclitaxel and radiation therapy	I/II	NCT00662311	
NCI				
Nereus Pharmaceuticals, Inc.	NPI-0052 and Vorinostat	I	NCT00667082	
Penn State University	Carboplatin, Paclitaxel, Bevacizumab and Vorinostat	I	NCT00702572	
California Cancer Consortium	Carboplatin and Paclitaxel with or without Vorinostat	II	NCT00481078	
NCI				

\*As found on clinicaltrials.gov (<http://clinicaltrials.gov>).

also induce related antioxidant defences in COPD and NSCLC [215, 216]. Furthermore, curcumin has been shown to restore HDAC2 levels, and restore steroid sensitivity in monocytes exposed to cigarette smoke raising the possibility that this compound may have efficacy in the treatment of COPD [217]. Intriguingly, curcumin and L-sulphoraphane have been shown to act synergistically to down-regulate pro-inflammatory markers raising the possibility that these compounds could be combined in therapies targeting chronic inflammation such as COPD or NSCLC [218].

One reason for the differences observed in the mechanism of action of curcumin may relate to either the concentrations/doses used and or the cells targeted. Obviously, this may have profound effects on the potential clinical utility of this compound, and will require further evaluation.

## Epigallocatechin 3-gallate (EGCG)

Another dietary polyphenol, which may prove of use in the treatment of COPD or NSCLC, is the green tea polyphenol EGCG. This compound has been shown to inhibit DNMTs [219, 220], and as such may function to affect epigenetic regulation of genes during oxidative stress. Indeed, EGCG has been shown to have efficacy in enhancing the expression of antioxidant genes in a mouse model

of pulmonary fibrosis but the molecular mechanism underpinning this has yet to be determined [221]. Suggestive evidence has also emerged from a colon cancer study in mice where EGCG was found to up-regulate the expression of NRF2 [222], and gene expression arrays on the effect of EGCG on bronchial epithelial cells has identified genes affected by this compound [223]. In addition, EGCG has been shown to attenuate cigarette smoke induced NF $\kappa$ B activation in normal human bronchial epithelial cells [224], and has also been shown to inhibit IKK activation and IL-8 gene expression in NSCLC cells [225]. Several studies have shown the efficacy of EGCG as a potential chemopreventative treatment for NSCLC [226–240].

Caveats to the potential usefulness of EGCG in the treatment of pulmonary disease are that it has been linked to the development of asthma [241].

## Can dietary epigenetic inhibitors work in the clinical setting?

Initial epidemiological evidence suggested a link between dietary isothiocyanate (of which sulforaphane is a member) intake and a reduced risk of developing lung cancer [242–246]. Strong evidence

is now emerging within the clinical setting that broccoli or cruciferous vegetable consumption has a protective benefit in smokers, specifically in former smokers. In a placebo-controlled dose escalation trial to evaluate the effects of sulforaphane, on the expression of glutathione-s-transferase M1, glutathione-s-transferase P1, nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase and HO-1 in the upper airway of human subjects, using nasal lavage and RT-PCR, significant increases in expression of all the phase II enzymes compared to baseline were observed for the Sulforaphane treated patients, whereas in the control non-sulforaphane containing alfalfa sprouts, no changes to phase II enzymes were observed [247]. In a similar hospital-based, case-controlled study with lung cancer cases and controls matched on the basis of smoking status it was determined that among smokers, the protective effect of cruciferous vegetable intake ranged from a 20% reduction in risk to a 55% reduction in risk depending on the type of vegetable consumed and the duration and intensity of smoking. In particular, it was found that the consumption of raw cruciferous vegetables was associated with a risk reduction of lung cancer for current smokers. Additionally in patients with actual cancer, the strongest reduction was found for patients with either squamous or small-cell carcinoma, the two subtypes most strongly associated with heavy smoking [248]. Finally, a small cross-over study designed to examine the protective effect of broccoli intake in smokers and non-smokers, found that while broccoli intake (200 g/day) provided significant protection against oxidative stress induced DNA damage, no alterations to HDAC activity occurred over the trial period [249].

These results clearly indicate that dietary sulforaphane intake may have an important chemopreventative role in NSCLC, and may also be important in the treatment of COPD.

## Caveats

Despite all the evidence that indicates that epigenetic mechanisms may be important therapeutic targets in the treatment of oxidative stress based conditions within the lung, there are some caveats which require consideration. For instance, in the lungs of rats treated with sulforaphanes glucosinolate precursor (glucoraphanin), levels of phase I carcinogen-activating enzymes, including activators of carcinogenic polycyclic aromatic hydrocarbons have been shown to be strongly induced suggesting that regular administration of glucoraphanin could actually increase rather than decrease lung cancer risk, especially in smokers as these individuals would be exposed to tobacco smoke related mutagens and carcinogens [250].

HDI have been shown to reactivate BRM [62], but inhibit its function, and subsequent removal of the HDi restores functional activity [63]. As such, it may be important to design staggered therapeutic interventions with HDi to allow the critical cellular functions of reactivated genes to take effect.

Issues of bioavailability may also be encountered in relation to efficacy of dietary epigenetic interventions in the treatment of

COPD and NSCLC. For instance it is well established that curcumin is not easily bioavailable [251]. In a recent phase II clinical trial in patients with advanced pancreatic cancer, even though patients received up to 8 g of curcumin by mouth daily until disease progression, with restaging every 2 months, the bioavailable levels of curcumin achieved in plasma were within the range of 22 to 41 ng/ml [252]. However, curcumin bioavailability has been increased through heat treatment [253], or by the synthesis of novel derivatives [254], and should lead to better compounds for use in the clinical setting. In contrast the bioavailability of SFN is increased if cruciferous vegetables are eaten raw rather than heat treated [255–257]. It is clear that further efforts are required to understand the bioavailability and pharmacokinetics of these dietary derived natural compounds. For instance, in a recent study in human volunteers it was found that repeated ingestion of broccoli did not lead to higher plasma levels of SFN [258].

Finally, while HDi may be beneficial for the treatment of disease, they may not be of great use in targeting oxidative stress induced disease. In this regard, they have been shown to induce apoptosis in cancer cells by inducing oxidative stress [259–261].

Lung cancer and COPD are leading causes of morbidity and mortality in the United States and worldwide. They share a common environmental risk factor in cigarette smoke exposure and a genetic predisposition represented by the incidence of these diseases in only a fraction of smokers. The presence of COPD increases the risk of lung cancer up to 4.5-fold. However, in addition one outstanding issue concerns the fact that a small number of patients who develop lung cancer or COPD are non-smokers raising the possibility that they may be (i) completely different diseases which will require different treatments or (ii) there are genetic/epigenetic susceptibilities or environmental exposures that could drive similar changes in ROS stress and epigenetic modifications without the need for cigarette smoke exposure. We feel that currently we are not yet in a position to distinguish between these two possibilities. At a recent workshop held by the National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), it was decided that future studies should have as their main objectives: (1) to clarify common epidemiological characteristics of lung cancer and COPD; (2) to identify shared genetic and epigenetic risk factors; (3) to identify and validate biomarkers, molecular signatures and imaging-derived measurements of each disease and (4) to determine common and disparate pathogenetic mechanisms [262].

Another area of concern pertains to the different effects of cigarette smoke on cells. This may be due to different cell types, different degrees of antioxidant capacity in the different cell types or are other factors such as chemical and environmental carcinogens may also play important roles? Indeed, some chemical carcinogens such as Benzo[a]pyrene (B[a]P) are present in both the environment and cigarette smoke, and this compound has been shown to increase cellular proliferation in lung cancer cells [10]. Small airways in patients with COPD also have evidence for proliferative responses, particularly with respect to smooth muscle and to epithelial cells [263, 264]. Clearly, future studies will be

required to molecularly dissect the genetic epigenetic and molecular alterations underpinning these differential responses.

## Conclusions

It is clearly evident that the hypoxic environment encountered in COPD and NSCLC results in the development of oxidative stress. Throughout this review we have linked epigenetics as a mechanism by which cells regulate oxidative stress responsive genes and pathways. A fine balance between the epigenetic regulatory mechanisms and their intended targets to both dampen pro-inflammatory conditions and control cellular oxidative stress path-

ways exists (summarized in Fig. 1), which if perturbed may lead to aberrant epigenetic control and subsequently to exacerbations to pre-existing conditions (e.g. COPD), and which may in the end result in tumourigenesis.

Throughout this review we have examined how epigenetics may be involved in the regulation of oxidative stress responses on many levels and *via* various pathways. It is clear that they are intimately linked as shown figuratively in Fig. 2. Finally, we have discussed the current potential for targeted epigenetic therapies for the treatment of COPD or NSCLC, either *via* pharmacological intervention or through the use of dietary chemopreventatives. The available data would indicate that there is compelling evidence for further studies into epigenetic intervention strategies to treat diseases associated with oxidative stress.

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