Role of histone deacetylase 2 in epigenetics and cellular senescence: implications in lung inflammaging and COPD

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THE INFLAMMAGING REFERS TO chronic low-grade inflammation with aging, which occurs in chronic inflammatory diseases, such as chronic obstructive pulmonary disease (COPD) (34, 74, 124). The inflammation and cellular senescence (a state of permanent growth arrest) are intertwined in the process of accelerated or premature lung aging. However, the mechanism and causal role of inflammation and premature aging in the development of COPD remain unknown.

Histone deacetylase 2 (HDAC2) belongs to class I histone deacetylases, which catalyze the removal of acetyl groups from ε-amino-terminal lysine tails of core histone proteins. It was first discovered as a mammalian homolog of the yeast transcriptional regulator RPD3 responsible for transcriptional repression (138). HDAC2 itself does not possess a DNA-binding domain; therefore, it requires the presence of corepressor complex proteins including mSin3, NuRD/Mi2, and NCoR to target the substrate DNA. It is tightly regulated by complex protein-protein interaction, subcellular localization, and posttranslational modification (25, 111). HDAC2 functions in regulation of various cellular processes, such as cell cycle, proliferation, differentiation, inflammation, development, and glucocorticoid function. Recently, it has been shown that HDAC2 along with HDAC1 regulates DNA damage response (DDR) and cellular senescence via an epigenetic mechanism (82, 130). The level of HDAC2 and activity is decreased in lung parenchyma, bronchial biopsies, alveolar macrophages, and peripheral blood monocytes from patients with COPD, as well as in macrophages and lungs of mice exposed to cigarette smoke (4, 20, 47, 137). Yang et al. (137) have shown that NF-κB-mediated lung inflammatory response was associated with oxidative post-translational modifications of HDAC2 in response to cigarette smoke. These modifications led to the ubiquitination and proteasomal degradation of HDAC2 (3, 4). Other studies also showed that HDAC2 is posttranslationally modified by oxidative/carbonyl stress imposed by cigarette smoke, leading to its degradation (75, 80). Interestingly, these modifications and various cellular processes regulated by HDAC2 are shown to be involved in the pathogenesis of COPD. This implicates a pivotal role of HDAC2 in the development of COPD/emphysema. In this perspective review, we have discussed the regulation of HDAC2 in various cellular functions in translational research, particularly in the progression of COPD, and potential for targeting HDAC2 in the intervention of this disease.

Yao H, Rahman I. Role of histone deacetylase 2 in epigenetics and cellular senescence: implications in lung inflammaging and COPD. Am J Physiol Lung Cell Mol Physiol 303: L557–L566, 2012. First published July 27, 2012; doi:10.1152/ajplung.00175.2012.—Histone deacetylase 2 (HDAC2) is a class I histone deacetylase that regulates various cellular processes, such as cell cycle, senescence, proliferation, differentiation, development, apoptosis, and glucocorticoid function in inhibiting inflammatory response. HDAC2 has been shown to protect against DNA damage response and cellular senescence/premature aging via an epigenetic mechanism in response to oxidative stress. These phenomena are observed in patients with chronic obstructive pulmonary disease (COPD). HDAC2 is posttranslationally modified by oxidative/carbonyl stress imposed by cigarette smoke and oxidants, leading to its reduction via an ubiquitination-proteasome dependent degradation in lungs of patients with COPD. In this perspective, we have discussed the role of HDAC2 posttranslational modifications and its role in regulation of inflammation, histone/DNA epigenetic modifications, DNA damage response, and cellular senescence, particularly in inflammaging, and during the development of COPD. We have also discussed the potential directions for future translational research avenues in modulating lung inflammaging and cellular senescence based on epigenetic chromatin modifications in diseases associated with increased oxidative stress.

cigarette smoke; oxidants; inflammation; DNA damage and repair; premature lung aging; polyphenols

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as phosphorylation, carbonylation, nitration, and nitrosylation (22, 88, 95, 120) (Table 1). This is corroborated by the observations that a decrease of HDAC2 level and activity in patients with COPD is associated with its posttranslational modifications, including oxidation/carbonylation, nitrosylation, acetylation, phosphorylation, and subsequent degradation in response to oxidants derived from cigarette smoke (47, 75) (Fig. 1). Tsai and colleagues (120) first identified HDAC2 as a phospho-protein with unique phosphorylation on serine, but not threonine or tyrosine site. We also found that cigarette smoke caused HDAC2 phosphorylation at Ser394, Ser411, Ser422, and Ser424 in macrophages, lung epithelial cells, and mouse lungs (3, 4). Interestingly, HDAC2 phosphorylation is required for its interaction with transcription factors (e.g., p53), cAMP-responsive element-binding protein binding protein (CBP), and corepressor complex formation as well as its acetylation on lysine residues (3). Protein kinase CK2 is responsible for HDAC2 phosphorylation induced by cigarette smoke, which leads to reduction of its deacetylase activity and subsequent loss of HDAC2 deacetylase activity (22). Nott et al. (88) observed that HDAC2 phosphorylation, acetylation, and nitrosylation of HDAC2 have a different effect on its deacetylation activity (95). Endogenous nitric oxide (NO) generation from mice expressing constitutively active endothelial NO synthase (eNOS) leads to S-nitrosylation of HDAC2 at cysteine, and subsequent loss of HDAC2 deacetylation activity (22). Nott et al. (88) observed that HDAC2 S-nitrosylation at cysteine residues 262 and 274 occurs in rat cortical neurons, which prevents its association with gene promoters (i.e., Fos and Egr1) but has no effect on its deacetylation activity (88). Thus the nitration and nitrosylation of HDAC2 have a different effect on its deacetylase and transrepression activity, although tyrosine residue 253

![Diagram](https://example.com/diagram.png)

**Fig. 1.** Role of histone deacetylase 2 (HDAC2) in oxidative/carbonyl stress-induced inflammation and steroid resistance. Oxidative/carbonyl stress imposed by cigarette smoke causes HDAC2 phosphorylation, carbonylation, and nitrosylation, thereby leading to the decrease of its level and activity. HDAC2 reduction increases the acetylation of histone, NF-κB, and glucocorticoid (G) receptor (GR), which results in the augmented transcription of proinflammatory genes and steroid resistance. A variety of pharmacological and dietary compounds, such as theophylline, sulforaphane, curcumin, baicalin, and quercetin are under investigation to restore steroid efficacy via regulation HDAC2 posttranslational modifications and activity. P, phosphorylation; 4-HNE, carbonylation; NO-Cys, nitrosylation; Ac, acetylation.

**Table 1. HDAC2 modifications, causal factors, and consequence**

<table>
<thead>
<tr>
<th>Modification</th>
<th>Residue</th>
<th>Causal Factor</th>
<th>Consequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylation</td>
<td>Ser394, Ser411, Ser422, Ser424, and threonine</td>
<td>CK2</td>
<td>Ubiquination and degradation</td>
<td>(3, 4, 120)</td>
</tr>
<tr>
<td>Carbonylation</td>
<td>Cysteine, histidine, lysine</td>
<td>Aldehyde and 4-HNE</td>
<td>Decreased level and activity</td>
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<td>Nitration</td>
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<td>(3)</td>
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HDAC2, histone deacetylase 2; CBP, cAMP-responsive element-binding protein binding protein.

suggesting the regulation of HDAC2 modification by kinases in steroid resistance. These signaling mechanisms may be merged downstream to regulate HDAC2. Because HDAC2 has no nuclear export signal, it was initially proposed that it would be a predominantly nuclear protein. However, HDAC2 is also present in the cytoplasm, which is reduced in patients with COPD compared with nonsmokers (116, 126). Further study is required to investigate the mechanism for HDAC2 cytoplasmic localization and whether its phosphorylation is involved in lung inflammatory response incited by cigarette smoke.

Apart from phosphorylation, HDAC2 can be modified by carbonyl stress, such as 4-HNE and reactive aldehydes, which are increased in lungs of patients with COPD (79, 84, 101, 137). Nitration of HDAC2 at tyrosine 253, present in the catalytic domain, has been shown to inactivate its deacetylase activity (95). Endogenous nitric oxide (NO) generation from mice expressing constitutively active endothelial NO synthase (eNOS) leads to S-nitrosylation of HDAC2 at cysteine, and subsequent loss of HDAC2 deacetylation activity (22). Nott et al. (88) observed that HDAC2 S-nitrosylation at cysteine residues 262 and 274 occurs in rat cortical neurons, which prevents its association with gene promoters (i.e., Fos and Egr1) but has no effect on its deacetylation activity (88). Thus the nitration and nitrosylation of HDAC2 have a different effect on its deacetylase and transrepression activity, although tyrosine residue 253

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as well as cysteine residues 262 and 274 are at the COOH-terminal end of HDAC2 catalytic domain (57). Indeed, cigarette smoke caused upregulation of inducible NOS and eNOS in mouse lung, despite that the latter effect is transient (110). This may increase NO generation and subsequent HDAC2 nitration and nitrosylation in COPD (75). Because HDAC2 itself does not possess a DNA-binding domain but requires the presence of corepressor complex proteins to target it to substrate DNA (25), it is possible that S-nitrosylation at Cys 262 and Cys 274, as well as nitration on tyrosine 253, affects the ability of HDAC2 to interact with required DNA-binding proteins present in the corepressor complex. HDAC2 nitrosylation occurs at Cys 262 and Cys 274, which is increased in alveolar macrophages from patients with COPD. This leads to inhibition of glucocorticoid receptor (GR)-transrepression activity (75). Interestingly, treatment with Nrf2 activator sulforaphane significantly reduced HDAC2 nitrosylation and degradation, which is in agreement with the findings that reduced HDAC2 abundance is observed in lungs of Nrf2-deficient mice in response to cigarette smoke exposure (2, 75). Thus Nrf2 is an important antioxidant transcription factor that targets HDAC2 via phase II gene induction, thereby reversing corticosteroid resistance in COPD and other corticosteroid-resistant inflammatory diseases. HDAC2 is also regulated by cellular redox GSH status. Intracellular levels of GSH retain HDAC2 in a reducing environment because an increase in intracellular GSH levels can inhibit the oxidatively posttranslational modifications of HDAC2 (4, 75, 137). HDAC2 forms a complex with HDAC1 and HDAC3. Treatment of cigarette smoke extract decreases the protein levels of HDAC1, HDAC2, and HDAC3 in human monocyte/macrophage in cell culture experiments (68, 134, 137), although there is no report regarding the reduction of HDAC1 or HDAC3 in lungs of patients with COPD. Further study is required to determine whether HDAC2 reduction disrupts its complex with HDAC1 and HDAC3, leading to the change of their abundance and activity. Furthermore, it remains unknown whether other antioxidants, such as N-acetyl-L-cysteine, superoxide dismutase, and peroxynitrite scavengers, also can protect against HDAC2 oxidative modifications and nitrosylation.

**HDAC2 in Inflammation and Steroid Resistance**

Corticosteroids suppress inflammation by recruiting HDAC2 to NF-κB-driven proinflammatory gene promoters, thereby inhibiting the transcription of these genes. Furthermore, HDAC2 is required for GR deacetylation, which enables GR binding to the NF-κB complex, leading to the inhibition of NF-κB-dependent proinflammatory gene transcription (38, 48). NF-κB (RelA/p65) itself can also be acetylated and activated by cigarette smoke, which is attenuated by HDAC2 (21, 137, 141). However, HDAC2 level and activity are decreased in lungs of patients with COPD (47). This renders corticosteroids unable to inhibit proinflammatory gene transcription, leading to steroid resistance in COPD (Fig. 1). This is corroborated by the findings that hypoxia-inducible factor-1α activation by hypoxia, which occurs in lung microenvironment of patients with COPD, decreases HDAC2 level, resulting in augmented inflammation and steroid resistance (18). Furthermore, HDAC2-deficient mice are not responsive to budesonide in inhibiting LPS-induced lung inflammation (LPS itself has no effect on HDAC2 level or activity) (2). Hence, the attenuation of HDAC2 posttranslational modification and reduction would restore the effectiveness of steroid in inhibiting inflammation. A variety of pharmacological and dietary compounds can influence HDAC2 activity and level (Fig. 1 and Table 2). For example, treatment of monocytes/macrophages with curcumin (active ingredient in dietary spice turmeric or *Curcuma longa*), a polyphenolic compound, attenuated cigarette smoke extract-induced carboxylation, phosphorylation, and proteosomal degradation of HDAC2, which was associated with the restoration of steroid effectiveness in vitro (80). Nrf2 activator sulforaphane (present in broccoli and cruciferous vegetables) restored dexamethasone sensitivity in alveolar macrophages obtained from patients with COPD and treated with cigarette smoke extract ex vivo (75). Nortriptyline, a tricyclic antidepressant, has been shown to restore corticosteroid sensitivity induced by cigarette smoke-mediated oxidative stress, which is associated with increased HDAC activity (81). Low concentration of theophylline restores corticosteroid responsiveness in rats with smoke-induced airway inflammation and increases HDAC2 activity in alveolar macrophages from patients with COPD (23, 114). A recent clinical trial has demonstrated that a combination therapy with an inhaled corticosteroid and low-dose of theophylline attenuated inflammatory inflammation in patients with COPD (32). Theophylline also suppressed peroxynitrite-induced tissue remodeling via pathways involving NF-κB/TGF-β and/or HDAC in the human fibroblast HFL-1 cell line (113). Erythromycin, a macrolide, restored the effect of cigarette smoke-induced decline in HDAC1, HDAC2, and HDAC3 levels associated with inhibition of NF-κB activation (68). Baicalin, a flavonoid compound isolated from the root of Scutellaria baicalensis Georgi, can influence cigarette smoke-induced decline in HDAC1, HDAC2, and HDAC3 levels associated with inhibition of NF-κB (80). Oxidative stress, which occurs in lung microenvironment of patients with COPD, decreases HDAC2 level, resulting in augmented inflammation and steroid resistance (18). Furthermore, HDAC2-deficient mice are not responsive to budesonide in inhibiting LPS-induced lung inflammation (LPS itself has no effect on HDAC2 level or activity) (2). Hence, the attenuation of HDAC2 posttranslational modification and reduction would restore the effectiveness of steroid in inhibiting inflammation. A variety of pharmacological and dietary compounds can influence HDAC2 activity and level (Fig. 1 and Table 2). 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### Table 2. Regulation of small molecules in HDAC activity and steroid resistance

<table>
<thead>
<tr>
<th>Molecule</th>
<th>HDAC Activity</th>
<th>Steroid Effectiveness and Inflammation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>Inhibition of HDAC2 phosphorylation and degradation</td>
<td>Restores steroid effectiveness</td>
<td>(80)</td>
</tr>
<tr>
<td>Sulforaphane</td>
<td>Inhibition of HDAC2 nitrosylation and degradation</td>
<td>Restores dexamethasone sensitivity</td>
<td>(75)</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>Increases HDAC activity</td>
<td>Restores corticosteroid sensitivity</td>
<td>(81)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Increases HDAC2 activity</td>
<td>Increases corticosteroid effectiveness in COPD</td>
<td>(23, 32)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Inhibits the reduction of HDAC1, HDAC2 and HDAC3</td>
<td>Inhibits NF-κB activation</td>
<td>(68)</td>
</tr>
<tr>
<td>Baicalin</td>
<td>Inhibits HDAC2 phosphorylation</td>
<td>Overcomes steroid resistance</td>
<td>(67)</td>
</tr>
<tr>
<td>Salmeterol or formoterol</td>
<td>Restores HDAC activity</td>
<td>Inhibits inflammatory response</td>
<td>(99)</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease.
specific HDAC2 modulators in attenuating its posttranslational modifications and degradation so as to enhance steroid efficacy in management of COPD. However, HDAC2 is covalently modified in carboxyl/oxidative and inflammatory conditions. A simple activation of HDAC2 may not be effective in reversing steroid resistance unless its covalent posttranslational modifications are reversed.

HDAC2 Regulation in Histone Acetylation and Methylation As Well as in DNA Damage Response

Cigarette smoke has been shown to cause DNA damage and to impair double-strand break (DSB) repair, which is aggravated in patients with COPD/emphysema (8, 15, 16, 27, 28, 97). A recent study has demonstrated that HDAC2 functions in the DNA damage response (DDR) along with HDAC1 to promote DNA nonhomologous end joining (NHEJ) repair (82). Furthermore, HDAC2 regulates chromatin plasticity and enhances DNA vulnerability, suggesting that HDAC2 inhibition can lead to DNA damage (76). This may be due to HDAC2-mediated transcriptional repression, which prevents transcription from interfering with the repair process. Another possibility is that HDAC2 recruitment influences the ability of DNA repair factors (e.g., Ku70/Ku80) to bind the damaged DNA or to function effectively (82) (Fig. 2).

Histone modifications play an important role in regulating DNA damage/repair, proinflammatory gene transcription, genomic instability, and premature aging (59, 93, 107, 128, 144). Apart from direct histone deacetylation (e.g., H3K56, H4K5, H4K12 and H4K16), HDAC2 can also interact with protein methyltransferases/demethylases to form a large and multiple-protein complex(es), thereby regulating histone methylation (e.g., H3K4 and H3K9 methylation) (66, 72, 127, 136) (Table 3). Histone methylation at H3K79 and H4K20 has been shown to affect DDR after DSB induction by recruiting 53BP1 and Crb2 to DNA damage foci (12, 46, 106, 131). Furthermore, H3K36 dimethylation increases the rate of the association of DNA repair proteins Ku70 and NBS1 near DSB (31, 129). In addition, H3K79 dimethylation is required for ionizing radiation-induced 53BP1 foci formation when H4K20 dimethylation is low (63, 131), suggesting a cross-talk of histone modifications to coregulate DDR. Opening, repairing, and closing of chromatin occur in response to DDR to repair the damaged DNA (96). Hence, this histone acetylation and methylation after DNA damage affect chromatin status between euchromatin and heterochromatin so as to recruit DNA repair factors and cofactors.

Table 3. HDAC2 regulation in histone acetylation and methylation in DDR and cellular senescence

<table>
<thead>
<tr>
<th>Histone Modification</th>
<th>Specific Residue</th>
<th>Effect of HDAC2</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>H3K56</td>
<td>Reduce its acetylation</td>
<td>Involved in DDR and DNA repair</td>
<td>(82)</td>
</tr>
<tr>
<td></td>
<td>H4K5</td>
<td>Reduce its acetylation</td>
<td>Involved in DNA repair</td>
<td>(41, 105)</td>
</tr>
<tr>
<td></td>
<td>H4K12</td>
<td>Reduce its acetylation</td>
<td>Regulate telomere function and premature aging</td>
<td>(41, 50, 105, 145)</td>
</tr>
<tr>
<td></td>
<td>H4K16</td>
<td>Reduce its acetylation</td>
<td>Involved in DNA repair and cellular senescence</td>
<td>(59, 82)</td>
</tr>
<tr>
<td>Methylolation</td>
<td>H3K4</td>
<td>HDACs inhibit H3K4 methylation via LSD1</td>
<td>Involved in gene activation and DDR</td>
<td>(30, 45, 77, 132)</td>
</tr>
<tr>
<td></td>
<td>H3K9</td>
<td>HDACs increase its methylation via SUV39H1</td>
<td>Involved in replication checkpoint and cellular senescence</td>
<td>(13, 87, 102, 127)</td>
</tr>
<tr>
<td></td>
<td>H4K20</td>
<td>Trichostatin A reduces its methylation</td>
<td>Influence DDR after DSB</td>
<td>(12, 36, 106)</td>
</tr>
</tbody>
</table>

DDR, DNA damage response; DSB, double-strand break.
(e.g., HP1) to damaged sites. We and others have shown that cigarette smoke alters the profile of histone acetylation and methylation in lung epithelial cells, which is associated with HDAC2 reduction (3, 4, 70, 115, 140). However, it remains to be known whether cigarette smoke-induced HDAC2 reduction alters histone acetylation and methylation as well as their cross talk, thereby regulating DNA damage and repair during cellular senescence and premature aging of the lung in COPD.

Histone acetyltransferase CBP/p300 has shown to acetylate histone H3 and H4 at the sites of DSB, thereby regulating DNA damage and repair (93, 128). CBP/p300 is also recruited to the promoter of proinflammatory gene IL-6, leading to its transcription via acetylating histone H3K9 in mouse lung exposed to cigarette smoke (142). Therefore, CBP/p300 may be recruited on the sites of DSB in response to cigarette smoke exposure in regulating DNA damage. We have recently found that CBP interacted with HDAC2, leading to HDAC2 acetylation in response to cigarette smoke (3). Thus HDAC2 may mediate the regulation of CBP in DNA damage and repair as well as proinflammatory gene transcription. Histone acetyltransferase (CBP/p300) inhibitors may attenuate HDAC2 acetylation in lung cells and hence overcome steroid resistance in patients with COPD.

Recent studies have shown that HDAC inhibitors, not only impair NHEJ and homologous recombination, but also induce acetylation of the key components, such as Ku70, during DNA repair (5, 19, 56). The increased acetylation of Ku70 reduces its DNA-binding affinity but does not affect Ku70/Ku80 heterodimer formation. Further study is required to determine whether Ku70 and other DNA repair proteins undergo acetylation/deacetylation under oxidative stress imposed by cigarette smoke and whether HDAC2 has any effect on their posttranslational modification status for DNA repair.

**HDAC2 Regulation in Cellular Senescence**

Recent studies have shown that persistent DNA damage causes stress-induced premature senescence (SIPS) and senescence-associated secretory phenotype (SASP) (103, 104). SASP has been introduced to describe the senescent cells that secrete proinflammatory mediators, such as IL-6, IL-8, and matrix metalloproteinases. This is partially due to NF-κB activation by DDR. Interestingly, NF-κB inhibition in turn delays the DNA damage-induced senescence and aging in mice (118), suggesting the reinforcement of cellular senescence by SASP as well as positive feedback between SIPS and SASP. Replicative senescence occurs as a result of exhaustion of proliferative ability of the cells in concomitance with shortening of telomeres and alteration in telomerase due to organisms/cellular aging independent of environmental stress. Thus cigarette smoke-mediated DNA damage may be an important contributing factor in initiating and maintaining SIPS and SASP. Indeed, a link between cellular senescence and premature lung aging has been proposed in the pathogenesis of COPD (7, 11, 37, 52, 65, 73, 124). Cigarette smoke induces senescence in lung epithelial cells and fibroblasts in vitro (86, 91, 92, 121–123). Senescence of fibroblasts and myofibroblasts may result in fibrosis in smokers and patients with COPD. It is also likely that carbonyls/oxidants generated or present in cigarette smoke cause immunosenescence of T- and B-cells, leading to abnormal recognition of self-antigens, which is important for the development of COPD (14, 54, 62, 90, 109). The involvement of cellular senescence in the pathogenesis of emphysema is also shown in various studies using genetically altered mouse strains (55, 64, 108, 112, 139). However, the molecular mechanisms that underlie cigarette smoke-induced cellular senescence in vitro in lung cells and in vivo in mouse lung and the precise role of SIPS in the development of COPD/emphysema are unclear. HDAC2 has been shown to protect against cellular senescence via regulating prosenescence genes (i.e., p21 and p16) (69, 130, 133, 146, 147). In addition, deletion of prosenescence gene p21 protects against cigarette smoke-induced cellular senescence in lung cells and subsequent emphysema (139). Hence, reduction of HDAC2 may lead to cellular senescence and pulmonary emphysema (83). Hyperoxia occurs during oxygen therapy for patients with COPD with severe resting hypoxemia. However, hyperoxia causes DNA damage and cellular senescence as well as impairs lung development in neonatal rodents via decreasing HDAC2 level and activity (71, 148). This provides a possibility that HDAC2 is an essential factor in regulating premature cellular senescence and the susceptibility to develop COPD. Further study using HDAC2 global and cell-specific knockout mice as well as transgenic animals would identify the role of HDAC2 in cellular senescence during cigarette smoke-induced airspace enlargement/lung destruction (emphysema).

HDAC2 interacts with poly(ADP-ribose) polymerase-1 (PARP-1) and reduces its acetylation that is required for full NF-κB-dependent transcriptional activity (44). PARP-1 is an important molecule that activates DNA repair programs, and PARP-1/NF-κB signaling cascade is activated during cellular senescence (94, 135). Therefore, it is possible that HDAC2 regulates SIPS and SASP via PARP-1-dependent mechanisms on DDR and telomere function in response to cigarette smoking. This contention requires further studies to translate the findings of HDAC2 regulation in replicative senescence in pathogenesis of COPD.

**HDAC2 Regulation in SASP**

The senescent cells are prone to secrete proinflammatory cytokines, which may in turn reinforce cellular senescence, as the deficiency of IL-6, IL-6R, or CXCR2 prevents SIPS (1, 35, 60, 103). Indeed, the percentage of proinflammatory senescent type II cells expressing both p16 and phosphorylated NF-κB (i.e., SASP) was increased in lungs of patients with COPD compared with smokers and nonsmokers (121). Increased proinflammatory gene transcription in senesced cells may be due to abnormal histone acetylation and methylation on these gene promoters. In general, histone acetylation is associated with gene activation. Unlike histone acetylation, histone H3K4 and H3K36 trimethylation activates chromatin (euchromatin), whereas histone H3K9 and H3K27 trimethylation silences gene transcription (heterochromatin) (26, 29, 33, 89). This has been shown to occur by cigarette smoke extract treatment, which increases histone H3K4 and H3K36 methylation but reduces H3K9 and H3K27 methylation in lung epithelial cells (140). It is proposed that histone deacetylation of H3K9 by HDAC2 is a prerequisite for its methylation (87, 102). Moreover, histone H3K4 methylation limits the accumulation of histone H3K4 acetylation at the gene promoter (42). These findings suggest the cross-regulation of histone methylation
and acetylation, even at the same residue. Hence, the study on the pattern and cross-talk of histone acetylation and methylation at these residues on the promoters of SASP genes will unravel an epigenetic chromatin mechanism underlying abnormal inflammatory response observed in COPD. It also remains to be studied whether HDAC2 regulates histone methylation on the promoters of SASP genes in a residue-specific manner (Fig. 2). Glucocorticoids are shown to suppress selected components of the SASP (61). However, HDAC2-dependent steroid resistance occurs in patients with COPD. Hence, it is possible that steroids may not be able to suppress SASP and thereby SIPS in patients with COPD.

Administration of CXCR2 inhibitor reduces lung mucus production and goblet cell hyperplasia in animal models of pulmonary inflammation (17, 117). Genetic ablation of HDAC2 renders these mice more susceptible to cigarette smoke-induced lung inflammation (2). However, it is unknown whether CXCR2 antagonist can prevent cigarette smoke-induced lung cellular senescence and subsequently the development of emphysema, particularly in HDAC2 knockout-susceptible mice, although NF-κB inhibitor has no effect on premature senescence and airspace enlargement in mice (139).

**HDAC Complex in DDR and SIPS of Lung Stem/Progenitor Cells**

The function of HDAC2 relies on the formation of its corepressor complexes along with HDAC1. Deletion of NuRD complexes including HDAC1 is associated with premature aging and DDR, although ablation of mSin3A or mSin3B does not result in cellular senescence (24, 40, 82, 98). In light of the regulation of cigarette smoke on the interaction of HDAC2 with mSin3, NuRD/Mi2, and HDAC1 (3), it is possible that HDAC complexes are also involved in cigarette smoke-mediated SASP gene transcription, DDR, and SIPS.

Recent studies have demonstrated that HDAC/mSin3A complex regulates the developmental genes, such as Sox-2, Wnt/β-catenin, and bone morphogenetic protein, which are important for branching morphogenesis and epithelial cell differentiation in lungs (10, 39, 43, 53, 85). Apart from lung development, Sox-2 also regulates the maintenance and differentiation of lung progenitor cells, such as Basal and Clara cells, which are senesced in lungs of cigarette smoke-exposed mice and patients with COPD (7, 100, 119, 139). Indeed, human lungs contain identifiable stem cells, which are self-renewing, clonogenic, and multipotent (51). Therefore, the study on the regulation of HDAC2 and its complexes in lung development and lung stem cells as well as progenitor cells will unravel the molecular mechanisms and susceptible factors involved in the pathogenesis of COPD. Furthermore, this will provide the potential to repair and regenerate lung tissue in this disease.

**Translational Impact**

HDAC2 is reduced in lungs of patients with COPD. HDAC2 reduction is associated with inflammation, steroid resistance, and DNA damage as well as lung cellular senescence. A variety of pharmacological and dietary compounds/molecules, such as theophylline, sulforaphane nortriptyline, baicalin, quercetin, erythromycin, and curcumin, are shown to target HDAC2 to reverse steroid resistance in COPD. However, all of the above agents have off-target effects. Hence, the development of a specific activator to activate HDAC2 or specific modulator to reduce HDAC2 posttranslational modification and degradation is urgently required to restore steroid efficacy in inhibiting chronic inflammation and premature cellular senescence in patients with COPD.

**Conclusions and Future Directions**

HDAC2 is an important deacetylase in regulating steroid function to inhibit inflammatory response in lungs. Recent studies have demonstrated a novel role of HDAC2 in regulating DNA damage/repair, premature senescence, and SIPS. We and others have shown that cigarette smoke reduces the level and activity of HDAC2 in cells in vitro and in mouse lung in vivo, which may contribute to the sustained inflammation, DNA damage, and subsequently cellular senescence in lungs of patients with COPD. However, the roles of DNA damage and cellular senescence as well as their regulation by HDAC2 in the pathogenesis of chronic pulmonary diseases with lung inflamming are unclear. Specifically, there are obviously multiple questions that remain unanswered. For example, it is unclear whether HDAC2 has a cell-specific effect in terms of steroid resistance (in macrophages, epithelial cells, and fibroblasts) as well as cigarette smoke/oxidants-induced DNA damage and cellular senescence (in lung epithelial cells and fibroblasts). Does HDAC2 protect telomere shortening, which is an important contributing factor in inducing replicative senescence that is observed in COPD (6)? Moreover, it remains to be known whether the clearance of senesced cells (e.g., p16-positive cells) in HDAC2-deficient lungs using recently developed p16-INK strategy delays or halts the progression of emphysema (9, 58, 147). Furthermore, it is not known which cells in the lung are the primary target for reduced HDAC2-mediated lung cellular senescence. Understanding the above aspects would unravel the role of DDR and premature senescence as well as HDAC2 regulation in the pathogenesis of COPD and provide a novel avenue to intervene this disease. COPD has been shown to increase the risk for developing lung cancer (143). Overexpression of HDAC2 confers oncogenic potential to human lung cancer cells (49). Thus further study is required to resolve this controversy by differentiating HDAC2 regulation and its dependent epigenetic changes in senescence during the development of COPD and lung cancer. This will provide translational HDAC2-based therapeutic approaches in lung inflamming and premature senescence.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: H.Y. and I.R. prepared figures; H.Y. and I.R. drafted manuscript; H.Y. and I.R. approved final version of manuscript; I.R. edited and revised manuscript.

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