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Animal models and mechanisms of tobacco smoke-induced chronic obstructive pulmonary disease (COPD)

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide, and its global health burden is increasing. COPD is characterized by emphysema, mucus hypersecretion, and persistent lung inflammation, and clinically by chronic airflow obstruction and symptoms of dyspnea, cough, and fatigue in patients. A cluster of pathologies including chronic bronchitis, emphysema, asthma, and cardiovascular disease in the form of hypertension and atherosclerosis variably coexist in COPD patients. Underlying causes for COPD include primarily tobacco use but may also be driven by exposure to air pollutants, biomass burning, and workplace related fumes and chemicals. While no single animal model might mimic all features of human COPD, a wide variety of published models have collectively helped to improve our understanding of disease processes involved in the genesis and persistence of COPD. In this review, the pathogenesis and associated risk factors of COPD are examined in different mammalian models of the disease. Each animal model included in this review is exclusively created by tobacco smoke (TS) exposure. As animal models continue to aid in defining the pathobiological mechanisms of and possible novel therapeutic interventions for COPD, the advantages and disadvantages of each animal model are discussed.

Introduction

Chronic obstructive pulmonary disease (COPD) encompasses a cluster of pathological respiratory conditions that affect approximately 300 million individuals globally. As the third leading cause of death worldwide (WHO 2014), COPD kills approximately three million individuals annually. In the United States (US) alone, an individual dies from COPD every 4 min (CDC 2020a). The global burden of this chronic lung disease is substantial and rising. In 2010, the national annual medical costs for COPD were $32 billion in the US, with a projected increase to $49 billion by 2020 (CDC 2020b).

Those suffering from COPD with chronic airflow obstruction that disrupts normal breathing results in increased hospitalization and mortality rates. The prevalence of COPD is rising due to elevated ambient air pollution exposure, biomass burning, dust and fume exposures in the workplace, extracellular vesicles, continued cigarette and e-cigarette smoking, and contribution of individual genetic/epigenetic factors (Benedikter et al. 2018; Duffy and Criner 2019; Easter et al. 2020; Effah et al. 2022; GOLD 2020; Hikichi et al. 2019; Jin et al. 2021; Kamal et al. 2022; O'Farrell et al. 2021; Silverman 2020; Snoderly et al. 2023). Thus, it is imperative to better understand the risk factors that result in COPD among those exposed to environmental contaminants and tobacco smoke (TS), and improve therapeutic strategies.

Through use of animal models and clinical studies, significant insights have been gained into the biological mechanisms underlying COPD development

including varied manifestations of bronchitis and emphysema, as well as associated overlapping asthma, pulmonary hypertension, and lung infections. The goal of this review was to identify the ideal applications of different animal models for developing treatment strategies. Tobacco smoking is a major risk factor for developing COPD and most commonly used to induce COPD in *in vivo* studies (Fricker, Deane, and Hansbro 2014; Ghorani et al. 2017). To better define smoke-induced COPD-like features and pathogenesis, the review includes only models developed using TS exposures. COPD animal models developed through other targeted interventions, with exception of biomass burning, are not included. The review focuses on 1) critical advances regarding the mechanisms underlying COPD-induced pathological alterations and associated disease risk factors; 2) wide variety of animal models of TS exposure that mimic specific pathologies of human COPD (Tables 1) advantages and disadvantages of each TS-exposed animal model (Table 2).

Methods approach to literature review

A systematic literature review was performed according to PRISMA (preferred reporting items for systematic review and meta-analysis) guidelines. PubMed and Web of Science databases were systematically searched, and references of retrieved articles were examined. The search was not limited to a year of publication/study or geographical region, and not done in collaboration with a librarian or methodologist. The search occurred 03/2023 for articles published from 1960–12/2022.

Keywords included the following: animal models, COPD, chronic obstructive pulmonary disease, emphysema, chronic bronchitis, cigarette smoke, cigarette exposure, tobacco exposure. A flow chart describing the literature review process is provided in supplemental Figure E1.

In total, 865 articles were identified, out of which 626 articles were excluded based upon criteria such as publication language, duplicates, and relevance determined through title and abstract reviews. To be included in the review, articles needed to meet the following criteria: (1) present distinct animal models of COPD; (2) provide a clear and detailed description of the method used to expose animals to TS, which leads to development of COPD; and (3) evaluate parameters that indicate the successful induction of a COPD animal model. Abstracts or unpublished articles, human studies and non-English language articles were excluded. Finally, 177 articles were found to be eligible and were included in the systematic review.

Results and discussion

COPD pathogenesis

Chronic obstructive pulmonary disease (COPD) patients suffer from chronic airflow obstruction attributed to mucous hypersecretion and persistent inflammation which manifests as chronic bronchitis with or without emphysema (alveolar septal wall destruction). These pathologies lead to wheezing, cough, and dyspnea with acute exacerbations of disease which, if severe, may result in hospitalization and death. COPD also overlaps with asthma in some patients (Postma, Rabe, and Drazen 2015; Zeki et al. 2011), and is associated with pulmonary hypertension and cardiovascular diseases such as systemic hypertension and atherosclerosis (Chaouat, Naeije, and Weitzenblum 2008). Current smokers and those suffering from chronic bronchitis attributed to other environmental exposures exhibit increased mucous (goblet) cell numbers as well as cellular hyperplasia in small and large airways (Mullen et al. 1987; Wright, Ngai, and Churg 1992). In comparison to nonsmokers, smokers display an elevated risk of developing chronic bronchitis and COPD-associated lung ailments, and the extent of symptom exacerbation appears to be associated with host susceptibility (Liu et al. 2010; Raju et al. 2014).

Patients diagnosed with emphysema exhibit mucous cell hyperplasia, resembling manifestations of chronic bronchitis. These individuals exhibit destruction of lung parenchymal tissues and alveolar septal walls, which result in airspace enlargement. Destruction of alveolar walls includes loss of the alveolar capillary bed and alveolar surface area, which impairs gas exchange. One potential mechanism underlying alveolar loss involves proteases released by the sustained recruitment, retention, and activation of inflammatory cells, or by alveolar and bronchial epithelial cells following exposure to toxic irritants, allergens, or TS

Table 1. A catalog of the animal models of COPD, and summary of their exposure conditions and observational changes.

Species	Exposure condition	Observation	Possible Mechanism	Ref.
Mice (Neutrophil elastase++ wild type littermates, C57Bl/6 Neutrophil Elastase KO)	2 cig/day, 6 day/week for 6 months (no CO toxicity).	Airspace enlargement determined by chord length was less by 59% in NE KO mice in comparison to mice with NE++.	NE plays a role in development of emphysema.	(Shapiro et al. 2003)
Mice (C57bl/129 TNF-αR KO, C57Bl/129)	4 cig/day, 5 day/week for 6 months. 2R1 reference cig University of Kentucky.	11% increase in Lm in TNFRKO mice and 38% in WT mice. Increased matrix metalloproteinase in WT mice.	TNF-α mediates airspace enlargement, neutrophil influx and matrix breakdown.	(Churg et al. 2004)
Mice (WT Balb/c mice and immunodeficient Scid mice)	5 cig 4 times a d with 30 ms smoke free interval for 5 week & 24 week. 2R4F reference cig University of Kentucky.	Increase in neutrophils, macrophages and dendritic cells in WT and Scid mice after 5 weeks. Increase in neutrophils, macrophages, dendritic cells, CD4+ and CD8+ cells in WT mice after 24 weeks. Pulmonary emphysema demonstrated by increased Lm and associated with increased MMP-12 in lungs and TNF-α in BALF in WT mice and Scid mice after 24 weeks of cig smoke exposure.	Pulmonary Emphysema can occur in the absence of adaptive immunity after chronic smoke exposure.	(D'Hulst et al. 2005)
Mice (C57Bl/6 (C57) and ICR)	2 hr/d 5 day/week for 6 months. 2s, 35-ml puff, 60s interval between puffs. 75, 250 or 600 μg/l of total PM concentrations. 2R4F reference cig University of Kentucky.	Mild emphysema demonstrated by increased Lm in ICR mice after 6 m of exposure to 600 μg/l total particulate matter. Macrophages and inflammatory cells infiltration around alveolar ducts.	There is a role of chronic inflammation in the pathogenesis of emphysema but the inflammation depends on the concentration and duration of exposure.	(Hodge-Bell et al. 2007)
Mice (C57Bl/6 and IL-18 Rα$^{-/-}$)	2 cig twice a day, 5 day/weeks for 6 months. 2R4 reference cig University of Kentucky.	Emphysema in WT mice demonstrated by increased Lm and alveolar destruction.	IL-18 Rα plays a role in cig smoke induced pulmonary emphysema.	(Kang et al. 2007)
Mice (C57Bl/6 WT; C57Bl/6 CD8+ KO)	2 cig/day, 6 day/week for 6 months. Research cig from University of Kentucky.	WT showed macrophage, neutrophil and lymphocyte recruitment to the lung. Emphysema demonstrated by increased Lm in WT. No changes seen in C57Bl/6 CD8+ KO.	CD8+ plays a role in inflammation and alveolar destruction in cig smoke induced pulmonary emphysema.	(Maeno et al. 2007)
Mice (C57Bl/6 WT; C57Bl/6 IL-1RKO; TNFR KO)	Acute exposure: 4 2R1 cig/day for a single day. Chronic exposure: 3 2R1 cig/day, 5 day/week for 6 month.	Acute exposure: No influx of inflammatory cells and matrix breakdown in IL-1RKO mice. Increased IL-1β, neutrophils, macrophages, desmosin and hydroxyproline in C57Bl/6. Chronic exposure: Increased IL-18, neutrophils, macrophages, desmosin and hydroxyproline in C57Bl/6. Increased neutrophil and macrophages in IL-1RKO. IL-1RKO and TNFRKO were 65% and 83% protected against emphysema respectively. Emphysema was demonstrated by analysis of Lm and matrix breakdown.	There is a role of IL-1 and TNF-α in the development of smoke induced emphysema and small airway remodeling in mice.	(Churg et al. 2009)
Mice (C57Bl/6 clarithromycin p.o.)	2 unfiltered cig/d, 6 day/w for 6 months. Research cig University of Kentucky.	Decreased airspace enlargement, decreased alveolar wall destruction and decreased macrophages in BAL in mice treated with clarithromycin for 6 months.	Clarithromycin treatment prevents smoke induced emphysema and airway inflammation in mice.	(Nakanishi et al. 2009)
Mice (C57Bl/6J curcumin p.o.)	Commercially available filtered cig (Marlboro, 12 mg tar/1 mg nicotine; Philip Morris, Richmond, VA). Short term exposure: 5% CS (12 puffs/min) for 60 ms/day for 10 consecutive days. Long term exposure: 12 puffs/min, 60 ms/day, 5 day/week for 12 weeks. Curcumin was added daily 1 hr before exposure.	Decreased neutrophil and macrophages after short term exposure and decreased airspace enlargement after long term exposure in animals administered with curcumin.	Administration of oral curcumin decreases smoke induced emphysema and pulmonary inflammation.	(Suzuki et al. 2009)

(*Continued*)

Table 1. (Continued).

Species	Exposure condition	Observation	Possible Mechanism	Ref.
Mice (WT mice; adiponectin deficient mice)	2 cig/day, 5 day/week for 6 months. 2R4F reference cig University of Kentucky (2.45 mg nicotine/cig).	Airspace enlargement (increased Lm), decreased tissue elastance and lung inflammation (increased total BAL cells, neutrophils and macrophages) in wild type mice.	There is a proinflammatory role of adiponectin in development of tobacco smoke induced emphysema.	(Miller et al. 2010)
Mice (C57Bl/6; C57Bl/6 IL-17a KO; C57Bl/6 Cc10-IL17a)	4 cig/day (4-5 min/cig) (1 hr), 5 day/week for 4 month Commercial cig (Marlboro 100s). 5 s of puffing of active smoke followed by 25 s of forced air to prevent CO_2 asphyxiation.	Increased lung inflammatory cells in Cc10-IL17a compared to C57Bl/6 and IL-17a KO mice. Exaggeration of tobacco smoke induced emphysema in Cc10-IL17a mice demonstrated by alveolar destruction under microscope and quantitative microcomputed tomography.	IL-17a plays a role in pathogenesis of tobacco smoke induced emphysema.	(Shan et al. 2012)
Mice (C57Bl/6J; DBA/2; ICR)	3 cig/d, 5 day/w for 7 months. Commercial Virginia cig (12 mg of tar and 0.9 mg of nicotine).	Decreased lung elastin in C57Bl/6 and DBA/2 but not in ICR. Focal areas of emphysema in lung parenchyma in C57Bl/6 and DBA/2 determined by morphometric measurement (Lm, ISA, DI).	In mice, development of tobacco smoke induced emphysema is strain dependent.	(Cavarra et al. 2001)
Mice (ICR Nrf2 KO)	2 cig/day, 7 hr, 7 day/week for 6 month; each cig puffed for 2 sec, 1 puff/min for total of 8 puffs. 2R4F reference cig University of Kentucky (2.45 mg nicotine/cig).	Emphysema demonstrated by histopathological analysis, Lm and alveolar destruction more extensive in comparison to WT mice. Increased endothelial and type II epithelial cells. Increased alveolar expression of 8-oxo-7,8-dihydro-2′-deoxyguanosine.	There is a role of Nrf2 pathway in upregulation of oxidant defense which can prevent tobacco smoke induced lung inflammation and emphysema.	(Rangasamy et al. 2004)
Mice (C57Bl/6J; DBA/2)	3 cig/day, 5 day/week for 1,3,6 and 10 months. Commercial Virginia filter cig (12 mg of tar and 0.9 mg of nicotine).	Patchy emphysema analyzed by inter-alveolar distance and ISA in both strains. DBA/2: uniform parenchymal dilatation with apoptotic bodies. Fibrotic areas in parenchyma. C57Bl/6J: mucous (goblet) cell metaplasia.	Development of emphysema and fibrosis is strain dependent.	(Bartalesi et al. 2005)
Mice (C57Bl/CBA; A/J)	C57Bl/CBA: 6 hr/day, 5 days/week for 1 year A/J: 6 hr/day, 5 day/week for 6 months. PM in the chamber: 250 mg/m³.	17% increase in Lm in C57Bl/CBA and 32% increase in Lm in A/J. Increased neutrophils and lung lavage elastase like activity in CS exposed animals. No changes in lung compliance observed.	There is no correlation between structural emphysema and lung compliance.	(Foronjy et al. 2005)
Mice (C57Bl/6 CCR6 KO)	5 cig, 4 times/day with 30 ms smoke free interval 5 day/week for 4 weeks or 24 weeks. 2R4F without filter University of Kentucky.	Increased dendritic cells, neutrophils and T lymphocytes in WT in comparison to CCR6 KO and control animals. Statistically significant higher Lm and DI in WT mice exposed to TS in comparison to CCR6 KO mice.	There is a role of CCR6 in the development of TS induced pulmonary inflammation and emphysema.	(Bracke et al. 2006)
Mice (C57Bl/6J mice rFGF-2 intranasally administered)	20 filtered commercial cig/day for 1,or 4 days or 6 months (5 days/week). Eighty-Eight Lights, KT&G, Daejeon, Korea (8.5 mg tar and 0.9 mg nicotine/cig).	Decreased FGF-2 production in lung tissues after short-term smoke exposure (1 and 4 days). After 4-day smoke exposure, recruitment of inflammatory cells was increased, macrophages were increased in the BALF, increased MLI, lung resident cell apoptosis and protease activity developed in the destroyed alveoli.	Intranasal use of rFGF-2 reduced macrophage-dominant inflammation and alveolar destruction in the lungs.	(Kim et al. 2018)
Mice (C57Bl/6J mice)	75 min exposure of smoke generated from ~25 cig/session, 2 sessions/day (morning and afternoon, separated by a recovery period), 5 day/week for 12 weeks. Commercial cig Marlboro, Philips Morris, USA (1 mg nicotine and 11 mg tar/cig).	Lung function decline and airflow limitation. Profound peribronchial and/or perivascular inflammatory infiltration, collagen deposition, and mucus secretion. Enlargement of alveolar and profound airway inflammation with significantly increased number of total cell count, neutrophils, macrophages, and lymphocytes.	CXCL5 levels increased and correlated to granulocyte-colony stimulating factor levels. Circulating levels of CXCL5 correlated to lung functions decline.	(Chen et al. 2019)

(*Continued*)

Table 1. (Continued).

Species	Exposure condition	Observation	Possible Mechanism	Ref.
Mice (BALB/c)	Whole-body exposure with 5 cig, 4 times/day with 30 min smoke-free intervals, 5 day/week for 24 weeks. Nanning Jiatianxia unfiltered cig (12 mg of tar and 0.9 mg of nicotine). Total PM concentrations 140–160 mg/m^3.	Elevated IL-27 was accompanied by an exaggerated IFN-γ+CD8+Tc1 response.	Lung dendritic cells were one of the main sources of IL-27. IL-27 negatively regulated the differentiation of IFN-γ +CD8+Tc1 cells in a STAT1- and STAT3-independent manner.	(Qiu et al. 2022)
Mice (C57BL/6, CCL17$^{−/−}$, and CCL17$^{+/+}$ littermate)	Whole body exposure with 5 commercial non-filtered cig (Peace®; Japan Tobacco Inc., Japan) for 30 min, twice/daily for 8 weeks.	Increases CCL17 production in bronchial epithelial cells and accumulation of alveolar macrophages. Alveolar macrophage accumulation significantly reduced in CCL17-deficient mice.	CCL17 involved in CS-induced accumulation of alveolar macrophages possibly through CCL17-induced production of CCL2 by macrophages	(Machida et al. 2022)
Mice (Balb/cByJ)	Whole body exposure to unfiltered cig for 72 days, except for days 42, 52, and 62. 3R4F reference cig University of Kentucky, Lexington, Kentucky (carbon monoxide level 200–400 ppm).	Increased alveolar enlargement and numbers of macrophages and neutrophils in BALF. Lower body weight accompanied by lower serum leptin levels, more time spent in the inner zone of the open field, and decreased claudin-5 and occludin protein expression levels in brain microvessels.	Neuroinflammation is present in the brain area involved in cognitive functioning and that blood-brain barrier integrity is compromised.	(Pelgrim et al. 2022)
Rat (SH with s.c. administration of sEH inhibitor)	35 ml puff, 2 sec duration, 1 puff/m 6 hr/day for 3 days. 2R4F cig University of Kentucky.	Increase in total cells in BAL in animals exposed to tobacco smoke. Decreased total cells in BAL, neutrophils, alveolar macrophages and lymphocytes in tobacco smoke exposed rats treated with sEH inhibitors.	sHE helps in attenuation of inflammation induced by tobacco smoke.	(Smith et al. 2005)
Rat (SH with simvastatin administered i.p.)	35 ml puff, 2 sec duration once each min, 80–90 mg/m^3 total suspended PM 6 hr/day for 3 days. 3R4F cig University of Kentucky.	Decreased influx of leukocytes, macrophages and neutrophils in rats pretreated with simvastatin prior to and throughout the exposure.	Simvastatin attenuates pulmonary inflammation induced by acute TS exposure.	(Davis et al. 2013)
Rat (SH)	35 ml puff, 2 sec duration once each min, 80–90 mg/m^3 total suspended PM 6 hr/day, 3 days/week for 3 days, 4 weeks or 12 weeks. 3R4F cig University of Kentucky.	Correlation between neutrophil accumulation and expression of adhesion molecules and chemokines in bronchial blood vessels and leucocyte accumulation recovered from lungs. There was no correlation between leucocytes recovered from BAL and other vascular beds.	In SH rat model of COPD, leucocytes are recruited through bronchial circulation.	(Davis et al. 2012)
Rat (Sprague-Dawley)	5 cig every 9 ms, 6 hr/day with 2 hr resting period for 2–4 months. Total PM concentrations in the chamber were 100–120 µg/m^3. 1R3F reference cig University of Kentucky.	Increased IL-18 in BAL. Decreased vascular endothelial growth factor (VEGF) in the lung tissue. Progressive alveolar airspace enlargement, cell death, pulmonary vessel loss, vessel muscularization, collagen deposition, and right ventricular hypertrophy.	There is a role of IL-18 in endothelial cell death that can lead to pulmonary vasculature destruction and emphysema.	(Kratzer et al. 2013)
Rat (SH, male; SH, female; WKY, male)	6 hr/day, 3 day/week for 4 or 12 weeks. Total suspended PM in chamber was 90 mg/m^3. 2R4F research cig University of Kentucky.	Increased total leucocytes, macrophages, TNF-α and lactate dehydrogenase in BAL after 4 weeks of exposure and enlarged alveolar air space after 12 weeks of exposure in SH male rats in comparison to SH female, control and WKY.	Cellular, inflammatory and structural changes similar to COPD changes in SH male rats exposed to tobacco smoke.	(Shen et al. 2016)

(*Continued*)

Table 1. (Continued).

Species	Exposure condition	Observation	Possible Mechanism	Ref.
Rat (SH, male)	6 hr/day, 3 day/week for 3 day or 4 weeks or 12 weeks. Total suspended PM in chamber was 90 mg/m³. 2R4F reference cig University of Kentucky.	Epithelial cell injury in the proximal airways, loss of ciliated epithelium and sloughing of airway epithelium after 3 d of TS exposure. Stratified squamous metaplasia in proximal airways and hypertrophic epithelial cells in mid airway level after 4 and 12 weeks TS exposure. Few TGF-β positive epithelial cells in proximal airways after 4 weeks of exposure. In mid and distal airway, epithelial cells were strongly positive for TGF-β after both 4 and 12 weeks of exposure. Increase in TGF-β2 after 4 and 12 weeks of exposure whereas decrease in TGF-β1 at 12 weeks of exposure demonstrated by ELISA of whole lung homogenates.	Airway generation and exposure duration determine changes in airway structure after TS exposure. During airway remodeling due to TS exposure, there is increase in TGF-β in epithelial and inflammatory cells.	(Hoang et al. 2016)
Guinea pig	10 commercial non filter cig/day, 5 day/week for 1,3,6 and 12 months.	Progressive destruction of lungs parenchyma. Alteration of pulmonary function test.	Development of morphological and physiological changes of emphysema in guinea pigs after chronic exposure to TS.	(Wright and Churg 1990)
Guinea pig	10 cig/day, 5 day/week for 4–8 months.	Increased alveolar air space size, decreased alveolar surface area to volume ratio. Changes in pulmonary vasculature. Pulmonary arteriolar muscularization.	Long term exposure of TS to guinea pigs induce emphysematous changes in lung.	(Wright and Sun 1994)
Guinea pig (Hartley with p.o. administration of NE inhibitor)	Acute exposure: 20 ml cig smoke/ 1.5 m for 24 hr. Chronic exposure: 20 ml cig smoke/ 1.5 m 5 day/week for 6 m. 2R1 research cig University of Kentucky.	Increased neutrophils, desmosine, hydroxyproline returned to normal and airspace enlargement reduced by 45% after administration of NE inhibitor.	NE inhibitor attenuates TS induced airway inflammation and destruction.	(Wright, Farmer, and Churg 2002)
Guinea pig (Hartley with administration of simvastatin)	5 cig/day, 5 day/week for 6 months. 2R1/2R4F research cig University of Kentucky.	TS increased pulmonary arterial systolic pressure. Reversal of pulmonary hypertension and emphysema (demonstrated by morphological and physiological measurement) after administration of simvastatin but no improvement in small airway remodeling.	Simvastatin prevents TS induced emphysema and reverses pulmonary hypertension but there was no effect on small airway remodeling.	(Wright et al. 2011)
Guinea pig	20 commercial non filter cig/d, 5 day/week for 1,3,4,6 and 8 weeks.	Interstitial and peribronchiolar inflammation and emphysematous changes (demonstrated by morphological measurement) at 6 and 8 weeks. Increased collagenolytic activity by 6 and 8 weeks.	There is a role of collagen degradation in the development of TS induced emphysema.	(Selman et al. 1996)
Guinea pig (Hartley)	Two 70 ml puffs/min, 4 hr/day 5 day/week for 1,2,4,8 or 12 weeks. Total PM concentration of 250 mg/m³. 2RF research cig University of Kentucky.	Inflammatory changes after 4 weeks and emphysematous changes after 12 weeks of exposure. Decreased elastin and collagen in the alveolar wall. Increased cathepsin K activity. Increased airspace enlargement.	There is a role of cathepsin K in destruction of an extracellular matrix in TS induced emphysema.	(Golovatch et al. 2009)
Dogs (beagle dogs)	Concentration and puffs of smoke increased gradually. Within 10 w of exposure, the exposure was 16 cig/day, 5 day/week for 12 months. Dogs used mouth pipe to inhale diluted smoke (1:4) from the cig. 1R1 research cig University of Kentucky.	Tracheal epithelial basal cell hyperplasia. Peribronchiolar hypercellularity. Increase in number of mucous (goblet) cells in large airways. No significant differences observed in bronchiolar size distribution, volume proportion of parenchymal structure and alveolar surface area.	Prolonged period of smoking is necessary for the development of irreversible damage after TS.	(Park et al. 1977)
Dogs (adult greyhound)	30–45 ms of smoke, twice/daily, 5 day/week for more than a year (mean 14.74 m).	Inflammation of bronchioles and small bronchi. Alveolar inflammation and disruption.	Concentrated TS can cause parenchymal disruption resembling human emphysema.	(Hernandez et al. 1966)

(*Continued*)

Table 1. (Continued).

Species	Exposure condition	Observation	Possible Mechanism	Ref.
Dogs (beagle dogs)	2–7 cig/day, 7 day/week for 2–4 months. 1 cig at a time, 35 ml puff volume in 2 sec, puff interval every 20 sec via tracheostomy tube. Code 26 research cig National Cancer Institute (nonfiltered, 27 mg of tar and 3.25 mg of nicotine).	Enlargement of alveolar ducts and alveolar spaces. Destruction of alveolar walls. Interstitial fibrosis of the interalveolar septa.	Short term TS exposure to dogs can cause early emphysematous and fibrotic changes in the lungs.	(Frasca et al. 1983)
Monkeys (cynomolgus macaque)	6 hr/day, 5 day/week for 12 weeks (250 mg/m^3 Total suspended PM).	Airway inflammation. Mucus metaplasia. Submucosal gland hypertrophy and hyperplasia. Peribronchial fibrosis.	No emphysematous changes in a given exposure setting.	(Polverino et al. 2015)
Ferrets	60 ms twice daily for 6 months. 3R4F research cig (200 μg/l total particulate matter).	Airway obstruction. Mucous (goblet) cells metaplasia/hyperplasia. Increased mucus expression in small airways. Early morning spontaneous cough.	Ferrets after TS exposure can develop clinical and pathological features of chronic bronchitis.	(Raju, Kim, et al. 2016)

Abbreviations: cig – cigarette(s); d – day(s); hr – hour(s); ms – minute(s); sec – second(s); WT – wild type; NE – neutrophil elastase; MMP – matrix metalloproteinase; TNFR – TNF-α receptor; ISA – internal surface area of the lungs; DI – destructive index; Lm – mean linear intercept; KO – knock out; p. o. – peroral; TS – tobacco smoke; BAL – bronchoalveolar lavage; i.p. – intraperitoneal; sEH – soluble epoxide hydrolase; s.c. – subcutaneous; rFGF – recombinant fibroblast growth factor; CXCL5 – C-X-C motif chemokine 5; IFN – interferon; CS – cigarette smoke; TS – tobacco smoke.
* Significant different from control at $p < .05$.

(Churg, Zhou, and Wright 2012; Fischer, Voynow, and Ghio 2015). Loss of alveolar septa reduces lung function, increases compliance (reduces elastance), and induces breathlessness and hyperventilation (Boutou et al. 2015; Suki et al. 2013). In smokers, the loss contributes to persistent airway inflammation (Barnes 2016b; Gamble et al. 2007) and airflow obstruction. Host genetic predisposition also induces COPD pathogenesis and symptoms (Alam et al. 2014; Silverman 2020; Sorroche et al. 2015).

Inflammatory cells in COPD

Long-term TS exposure and systemic inflammation that results from chronic airway inflammation also play significant roles in lung tissue damage, adverse remodeling, and vascular dysfunction when mediators are released by inflammatory cells, epithelial cells, and fibroblasts (Figure 1). Inhaled TS in the lungs stimulates activation of macrophages and airway epithelial cells to release chemotactic factors, including interferon-γ-induced protein 10 (IP10; CXCL10), monokine induced by interferon-γ (MIG; CXCL9), interferon-induced T cell α-chemoattractant (I-TAC; CXCL11), interleukin (IL)-6, IL-8, and leukotriene B$_4$, which results in accumulation of neutrophils and CD8+ T cells within the airways (Barnes et al. 2015; Mabley, Gordon, and Pacher 2011; Tanner and Single 2020). The protease, Granzyme B, excreted by CD8+ T cells, was implicated in degradation of extracellular matrix (ECM) leading to tissue remodeling associated with emphysema (Tanner and Single 2020). Proteases released by neutrophils and alveolar macrophages break down connective tissue and elastin in the alveoli contributing to localized (centrilobular) and generalized (panlobular) emphysema (Barnes et al. 2015; Tanner and Single 2020). TS-induced protease/antiprotease imbalance and oxidative stress are key inflammatory processes that promote COPD pathogenesis (Barnes et al. 2015; Fischer, Voynow, and Ghio 2015; Fricker, Deane, and Hansbro 2014). Neutrophil elastase, matrix metalloproteinases (MMPs), and MMP inhibitors play critical roles in development and progression of emphysema and COPD (Gharib, Manicone, and Parks 2018; Ghosh et al. 2019; Vlaykova and Dimov 2014; Zhou et al. 2020). Accumulation and hyperactivation of neutrophils produces mucous (goblet) cell metaplasia and mucus hypersecretion (Shaykhiev 2019). Macrophages and epithelial cells also secrete transforming growth factor (TGF)-β to stimulate proliferation of the epithelium, smooth muscle, and fibroblasts, resulting in fibrosis, airway

Table 2. Advantages and disadvantages of animal models.

Animal Models	Advantages	Disadvantages	Ref.
Mice	Low cost Easy handling Ease of genetic manipulation Rapid breeding rate Availability of antibodies and molecular probe Numerous strains	Takes longer time to develop features like human COPD No bronchial glands Strain dependent Inconsistent reproducibility of studies Difficult to perform pulmonary function test and telemetry	(Groneberg and Chung 2004) (Wright, Cosio, and Churg 2008) (Wright and Churg 2008a) (Churg, Cosio, and Wright 2008) (Kodavanti, Costa, and Bromberg 1998)
Rats	Mimics human chronic bronchitis and airway hyperreactivity Develops similar drug resistance as humans Useful in studying comorbidities of COPD	Few knockout strains Lacks submucosal glands	(Groneberg and Chung 2004) (Wright, Cosio, and Churg 2008) (Wright and Churg 2008a) (Churg, Cosio, and Wright 2008) (Cavailles et al. 2013) (Kodavanti et al. 2000) (Bolton et al. 2009)
Guinea Pigs	Useful in studying comorbidities of COPD Develops emphysema, marked airway remodeling and mucous (goblet) cell metaplasia following smoke exposure. Cough reflex present.	Limited availability and costlier than mice and rats Insufficient availability of immunologic reagents Less genetic information Sparse distribution or absence of bronchial glands	(Groneberg and Chung 2004) (Padilla-Carlin, McMurray, and Hickey 2008)
Dogs	Short term and long-term cigarette exposures produce changes similar to human chronic bronchitis and emphysema respectively Easy to measure pulmonary mechanics and ventilation, bronchoscopy Cough reflex can be measured Relatively large size and easy handling	Costly Lack of genetic manipulation	(Chapman 2008)
Ferrets	Similar to humans with respect to pulmonary physiology and submucosal gland distribution Smoke exposure causes impaired mucus transport	Less studies done in comparison to other animal model	(Raju, Kim, et al. 2016)
Monkey	Pulmonary anatomy and immune system similar to humans Symptoms that develop after exposure are reproducible and comparable to human patients Similar molecular reagents can be used in human and primates Easy to perform pulmonary measurements and bronchoscopy	Expensive Ethical issues Difficulty in purchasing, housing and exposing Takes longer time to develop emphysema (>12 weeks)	(Plopper and Hyde 2008) (Polverino et al. 2015)

remodeling, and chronic bronchitis (Barnes et al. 2015; Herfs et al. 2012; Tanner and Single 2020).

Inflammatory, epithelial, fibroblast, and smooth muscle cells also alter the function of vascular endothelial cells [Figure 2; (Barberà and Blanco 2009; Maiellaro and Taylor 2007; Wright and Churg 2008a)]. TS-induced endothelial injury results in endothelial cell apoptosis, which was shown in animal studies to induce emphysema by blocking vascular endothelial growth factor [VEGF; (Kasahara et al. 2000; Polverino, Celli, and Owen 2018)]. Elevated VEGF concentrations enhance bronchial vascularity and leakage of plasma proteins resulting in airway narrowing in chronic bronchitis (Green and Turner 2017).

Macrophages

Macrophages are key players in the genesis and persistence of COPD. These cells may either mount an inflammatory response directly, or participate in modulating the immune response by promoting healing and repair of damaged airways and parenchyma. Macrophages might acquire different functions as M1- or M2-polarized phenotypes. M1, known as the "classical macrophage," releases cytokines that induce the expression of

Chronic Obstructive Pulmonary Disease (COPD)
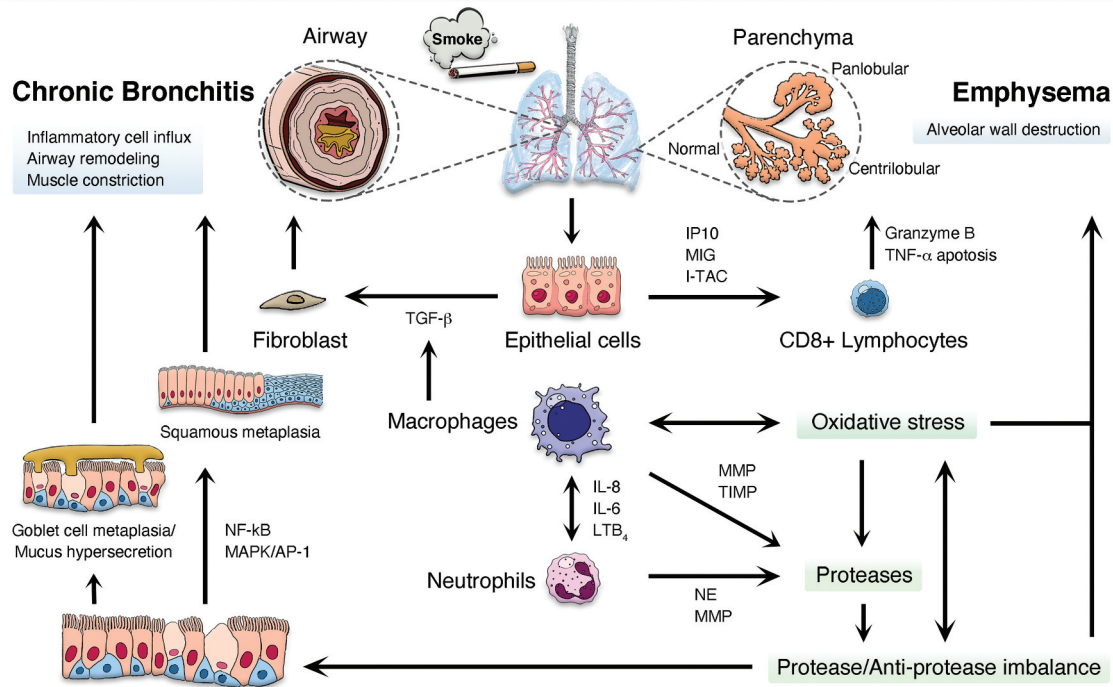
Figure 1. Potential pathways for the development of chronic obstructive pulmonary disease (COPD). IP10, interferon-γ-induced protein 10; MIG, monokine induced by interferon-γ; I-TAC, interferon-inducible T cell α-chemoattractant; IL, interleukin; LTB$_4$, leukotriene B$_4$; NE, neutrophil elastase; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; TGF-β, transforming growth factor beta; NF-κB, nuclear factor-kappa B; MAPK/AP-1, mitogen-activated protein kinase/activator protein-1.
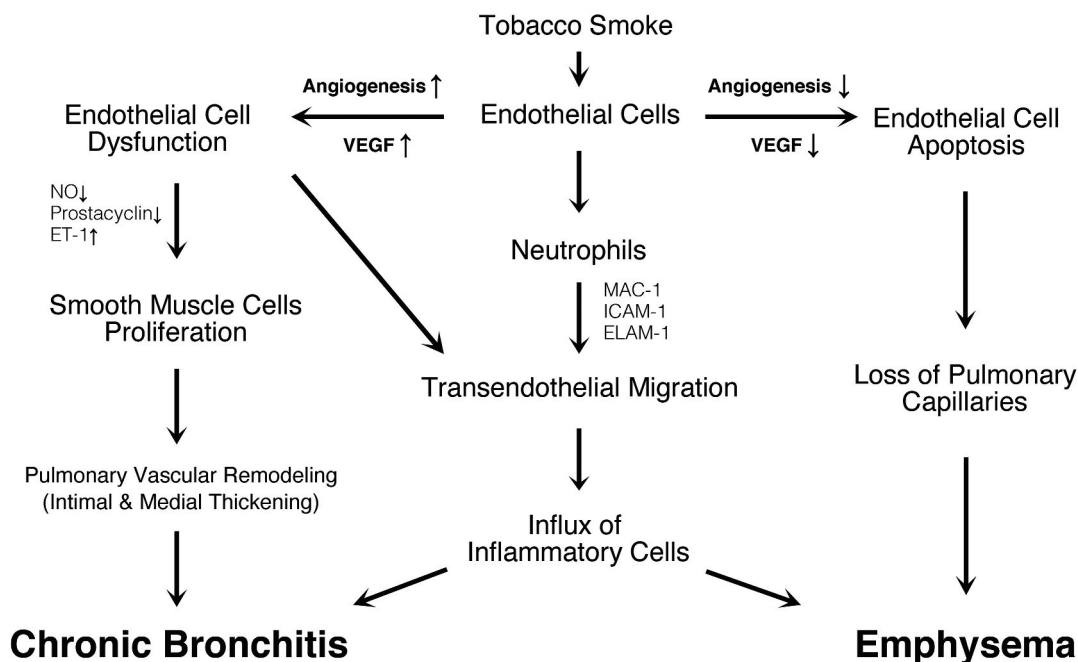
pro-inflammatory genes following exposure to inhaled irritants. In contrast, the M2-polarized macrophage participates in repair and healing mechanisms, and immune-regulation. COPD patients may exhibit phenotypic alterations of M1 and M2 populations (Kaku et al. 2014). Such alterations might persist even after smoking cessation and contribute to chronic airway inflammation seen in former smokers (Harada and Basrur 1998; Hodge et al. 2011). In addition, macrophage phagocytic function which is necessary for the resolution of infections and inflammation is impaired in COPD, a process known as "efferocytosis" (Ito et al. 2020; Liu et al. 2020). Efferocytosis refers to the key role macrophages play in the elimination of apoptotic neutrophils and epithelial cells, which if not cleared might lead to secondary inflammatory cascades which contributes to chronic airway inflammation in COPD. Further, Machida et al. (2022) recently demonstrated that lung epithelial cells produce CC motif chemokine ligand 17 (CCL17), a key mediator of cell accumulation in

type 2 inflammation that binds to CC chemokine receptor (CCR) 4, upon exposure to cigarette smoke. This CCL17 production is associated with accumulation of alveolar macrophages and development of elastase-induced pulmonary emphysema, possibly through CCL17-induced production of CCL2, a chemoattractant, present in macrophages. These findings may shed new light on the pathogenesis of COPD, and further research is warranted to gain a better understanding of the underlying mechanisms and explore potential therapeutic strategies.

Neutrophils

Granulocytic airway inflammation, typically associated with acute inflammatory episodes, is prominent in COPD. Neutrophils, which contain proteases and other cationic peptides, migrate into lung tissue utilizing a variety of chemoattractants including biological molecules and chemokines secreted by

The Role of Endothelium in COPD

Tobacco Smoke

Angiogenesis ↑   Endothelial Cells   Angiogenesis ↓

Endothelial Cell Dysfunction ← VEGF ↑   VEGF ↓ → Endothelial Cell Apoptosis

NO↓
Prostacyclin↓
ET-1↑

Neutrophils

MAC-1
ICAM-1
ELAM-1

Smooth Muscle Cells Proliferation

Transendothelial Migration   Loss of Pulmonary Capillaries

Pulmonary Vascular Remodeling
(Intimal & Medial Thickening)

Influx of Inflammatory Cells

**Chronic Bronchitis**   **Emphysema**

Figure 2. Potential mechanisms of endothelial cell injury in the development of chronic obstructive pulmonary disease (COPD). In the inflammatory response of COPD induced by tobacco smoke, neutrophils migrate through the endothelium by trans-endothelial migration (TEM). Macrophage (MAC)-1 antigen, intracellular adhesion molecule (ICAM)-1 and endothelial-leucocyte adhesion molecule (ELAM)-1 on the surface of endothelial cells involved in TEM are upregulated. The expression of cell adhesion molecules also appears to be induced by endothelial dysfunction in COPD. Vascular endothelial growth factor (VEGF) is a regulator that increases endothelial permeability and leads to endothelial cell growth. By blockade of VEGF, the disappearance of lung tissue in emphysema appears to involve the progressive loss of capillary endothelial and alveolar epithelial cells through the process of apoptosis. On the other hand, VEGF concentration in induced sputum is significantly elevated in chronic bronchitis, which increases bronchial vascularity and leakage of plasma proteins resulting in airway narrowing in chronic bronchitis. In COPD, the synthesis and release of NO (nitric oxide) and prostacyclin are reduced, and the endothelin (ET)-1 is increased in pulmonary arteries. These changes of vasoactive mediators result in endothelial dysfunction with the subsequent proliferation of smooth muscle cell, which may contribute to intimal hyperplasia with the ensuing reduction of arterial lumen and increase pulmonary vascular resistance.

damaged resident macrophages, epithelial cells, and endothelial cells. In the process of killing bacteria and other foreign substances, neutrophils also destroy structural elements of alveolar units and promote emphysema onset. In COPD, neutrophils might produce nearly 25-fold greater quantity of proteases found in healthy individuals (Overbeek et al. 2013). For this reason, neutrophilic inflammation likely contributes to development of emphysema in COPD during acute inflammatory events.

Lymphocytes

CD8+ and CD4+ T lymphocytes play complex, but interrelated roles in COPD pathogenesis (Caramori et al. 2016). CD8+ cytotoxic T cells are thought to

contribute to development of emphysema by releasing perforins and granzymes that initiate tissue destruction and apoptosis (Figure 1). CD8+ cytotoxic T cells also produce inflammatory cytokines such as IFN-γ and tumor necrosis factor-alpha (TNF-α) that provoke emphysema (Fairclough et al. 2008; Freeman et al. 2013; Maeno et al. 2007).T helper 1 (Th1) lymphocytes, a lineage of CD4+ cells, produce cytokines like IFN-γ, which contributes to COPD pathogenesis by promoting autoimmune responses and inflammation (Barnes and Cosio 2004). These and other T cells collectively, through increased enzymatic lytic activity, Fas-ligand mediated-apoptosis (Figure 3), and pro-inflammatory cytokine release, contribute to development of emphysema and other inflammatory responses in COPD (Fairclough et al. 2008; Green and Ferguson 2001; Hotchkiss et al. 2009). The
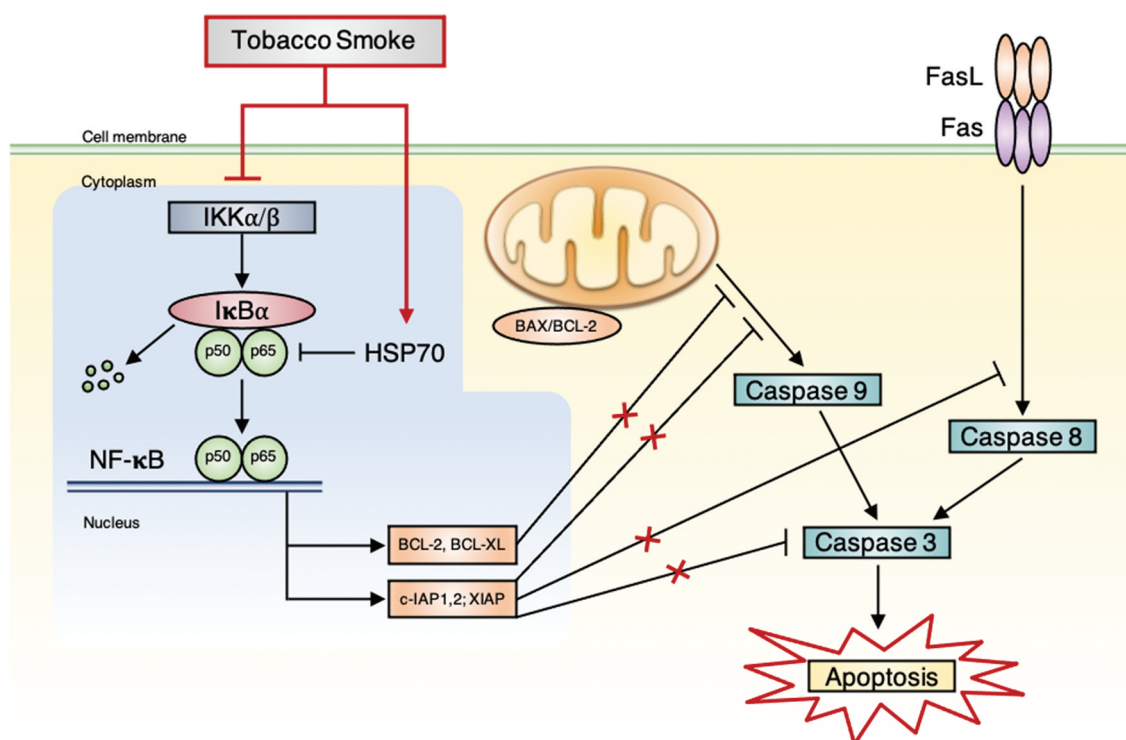
Figure 3. Proposed mechanisms of NF-κB pathway in tobacco smoke (TS)-induced apoptosis in the lungs of rats. Fas-ligand (FasL) mediated-apoptosis involves binding of ligands to the death receptor (Fas), which leads to activation of caspase 8. Both mitochondrial (caspase 9) and death receptor (caspase 8) pathways lead to activation of caspase 3, which ultimately result in cell death by apoptosis. Nuclear factor-kappa B (NF-κB) is the major anti-apoptosis transcription factor, which regulates the expression of anti-apoptotic genes, such as cellular inhibitors of apoptosis (c-IAP) and BCL-2 family members (e.g., BCL-2 and BCL-XL). It exists as a complex consisting of p50 and p65 (RelA) subunits. NF-κB is maintained in the cytoplasm by the inhibitory protein IκB, mainly IκBα, which is rapidly phosphorylated by IκB kinases (IKK) upon stimulation. Through suppression of IKK and induction of heat shock proteins (HSP70), TS inhibits NF-κB activity and downregulates NF-κB-dependent anti-apoptotic proteins, including members of BCL-2 and c-IAPs, leading to caspase activation, eventually inducing apoptosis. Reproduced from Zhong CY, Zhou YM, Pinkerton KE. NF-κB inhibition is involved in TS-induced apoptosis in the lungs of rats. Toxicology and Applied Pharmacology 2008; 230: 150–158.

contribution of T cells to TS-induced COPD is evident from the tissue destruction that ensues in healthy mice injected with T cells obtained from TSexposed mice (Eppert et al. 2013).

Epigenetic regulation of gene expression in COPD pathogenesis

As epigenetic modulation of gene expression is increasingly recognized as an important regulator of pathobiological processes, COPD pathogenesis and TS exposure are postulated to be linked to changes in circulating micro-ribonucleic acid (miRNA; non-coding RNA which plays a role in regulating gene expression) profiles in *ex-vivo*, epidemiological, and *in vivo* mouse studies (Gross et al. 2014; Leuenberger et al. 2016; Takahashi et al. 2013; Yu et al. 2019). Although the causal roles of specific

miRNA molecules have not been confirmed in COPD pathogenesis, a number of miRNAs associated with inflammatory processes may be elevated in COPD patients. *In vitro* and human population studies demonstrated pathological indices of COPD including (1) inflammation, tissue remodeling, and emphysema, (2) involve a distinct set of miRNA changes that correspond to alterations in expression of genes associated with each of the listed pathologies (Bracke and Mestdagh 2017). While current therapeutic strategies are not designed to specifically alter the genetic susceptibility or complex pathology associated with COPD, miRNAs were targeted for potential therapeutic intervention (Bracke and Mestdagh 2017; Liao et al. 2017). Although a number of animal investigations involving TS-induced pathology examined the role of specific miRNAs in cell signaling (Halappanavar et al.

2013; Xi et al. 2013; Yu et al. 2019), it needs to be emphasized that the relevance of miRNA changes in smokers and COPD patients as compared to animal models of TS-induced inflammation has not been established.

In addition to RNA interference, other epigenetic modifications might be mediated by different mechanisms, including DNA methylation and histone modification, which might also play essential roles in COPD development. DNA methylation is a chemical modification that involves addition of a methyl group to cytosine residues in CpG dinucleotides, resulting in formation of 5-methylcytosine. DNA methylation might occur in promoter regions of genes, leading to gene silencing or reduced gene expression. In COPD, alterations in DNA methylation patterns were noted in genes involved in inflammation, oxidative stress, and tissue remodeling, which are critical processes in COPD pathogenesis (Alfahad et al. 2021). Previously Zeng et al. (2020) suggested that cigarette-induced oxidative stress plays a role in mediating pulmonary apoptosis and hypermethylation of the B-cell lymphoma/leukemia-2 (Bcl-2) promoter, an apoptosis regulator, in COPD through DNA methyltransferase enzyme 1 (DNMT1), a key DNA methyltransferase enzyme. Similarly, aberrant DNA methylation was reported to be a widespread occurrence in small airways of COPD patients and was associated with altered expression of genes and pathways related to COPD, such as NF-E2-related factor 2 oxidative response pathway (Vucic et al. 2014).

Histone modifications, on the other hand, involve chemical modifications of the proteins called histones that package DNA into a compact structure termed chromatin. These modifications might either activate or repress gene expression by altering accessibility of DNA to transcriptional machinery. Histone modifications including acetylation, methylation, phosphorylation, and ubiquitination, might influence expression of genes involved in inflammation, immune responses, and other processes relevant to COPD (Zhang et al. 2020). It is of interest that Wu et al. (2018) found altered histone deacetylase (HDAC) activity, which leads to dysregulated histone acetylation in COPD patients and was suggested to be implicated in regulation of inflammation in COPD.

Taken together investigations involving miRNA, DNA methylation, and histone modifications in COPD revealed epigenetic changes that contribute to this disease pathogenesis. These modifications might alter gene expression, affecting crucial biological processes involved in COPD development and progression. Understanding these epigenetic mechanisms may lead to potential therapeutic targets and personalized treatment strategies for COPD patients. However, further research is needed to fully comprehend the complex interplay between epigenetic modifications and COPD pathogenesis.

COPD risk factors

Environmental determinants of COPD

Although various environmental stimuli including air pollutants including secondhand smoke, nitrogen dioxide, biomass smoke and allergens as well as infectious diseases attributed to viruses and bacteria may be associated with or possibly exacerbate COPD (Andersen et al. 2011; Bhalla et al. 2009; Cochard, Ledoux, and Landkocz 2020; Eisner 2007; Eisner et al. 2010; Harting et al. 2012; Salvi and Barnes 2010; Sana et al. 2018; Schikowski et al. 2014), the major contributor to the pathogenesis of COPD is TS exposure (Hylkema et al. 2007; Laniado-Laborin 2009; Lugade et al. 2014; Tamimi, Serdarevic, and Hanania 2012). TS contains over 6,000 constituents including nicotine, aldehydes, polycyclic aromatic hydrocarbons, glycoproteins, and metals, which are known to be both antigenic and carcinogenic (Hou et al. 2019; Oldham, Coggins, and McKinney 2013). In humans, exposure to first- and possibly secondhand TS might lead to COPD and cancer (Hou et al. 2019; Song, Chen, and Liu 2021), and potentially lead to development of asthma and cardiovascular diseases (Onor et al. 2017; Saha and Brightling 2006). Even nonsmokers with long-term exposure to secondhand TS or smoke from biomass burning might develop bronchitis leading to COPD (Coultas et al. 2001; Goldklang, Marks, and D'Armiento 2013; Ramirez-Venegas et al. 2019; Whittemore, Perlin, and DiCiccio 1995).

## Physiological determinants of COPD: comorbidities and sex

The severity of the development of COPD is partly dependent upon individual risk factors, such as a history of cardiovascular, metabolic, or autoimmune diseases, which increases susceptibility (Campo et al. 2015; Hizawa 2016). It is known that patients with α1-antitrypsin deficiency, as well as polymorphisms and mutations in genes associated with cancer and cardiovascular diseases, exhibit an elevated risk of developing COPD (Castaldi et al. 2014; Kaur-Knudsen et al. 2011; Liu et al. 2010).

Although gender may also influence COPD development, this continues to be an active area of research. While previous investigators noted that COPD prevalence was higher among men than women (de Torres et al. 2006; Lindberg et al. 2005a, 2005b). Evidence suggests it may now be greater in women (Barnes 2016a; Pinkerton et al. 2015; Ramirez-Venegas et al. 2019). These findings might be attributed to changing gender patterns of smoking (GOLD 2020), but several investigators suggested women are at greater risk to the adverse effects of cigarettes leading to COPD and carcinogenesis (Han et al. 2007; Langhammer et al. 2003; Stabile and Siegfried 2003; Viegi et al. 2001).

In addition to respiratory decrements, COPD patients often suffer from cardiovascular pathologies including atherosclerosis, hypertension, and heart failure, as well as metabolic diseases such as diabetes and osteoporosis (Agusti et al. 2010). Significant clinical evidence demonstrates a positive association between COPD and enhanced risk of developing other diseases including coronary artery, cerebrovascular, and peripheral arterial diseases, independent of TS exposure (Finkelstein, Cha, and Scharf 2009; Sin and Man 2005).

At this time, smoking cessation is the most effective means of reducing the progression of cellular and tissue-based injury, and the exacerbation of COPD symptoms produced by years of smoking (Groneberg and Chung 2004; Tashkin 2015). It is well-established that in those who quit smoking, the rate of lung function decline lessens compared to active smokers but remains higher than healthy nonsmokers over time (Jimenez-Ruiz et al. 2015; Wu and Sin 2011). Although smoking cessation might improve quality of life and attenuate some features of COPD, the effects of prolonged TS exposure are often irreversible and difficult to treat with pharmacological intervention alone (Chang and Rivera 2013; Hawkins, Virani, and Ceconi 2013; Hersh 2010). Recently, Wu et al. (2020) demonstrated the inflammatory and morphological changes induced by long-term TS exposure in lungs of rats became irreversible even after prolonged smoking cessation. Further, intermittent exposure to TS may contribute to persistent chronic inflammation, increased severity of emphysema, and a higher incidence of tumorigenesis in lungs compared with continuous TS exposure as illustrated in Figure 4 (Kameyama et al. 2018; Pinkerton and Poindexter 2018).
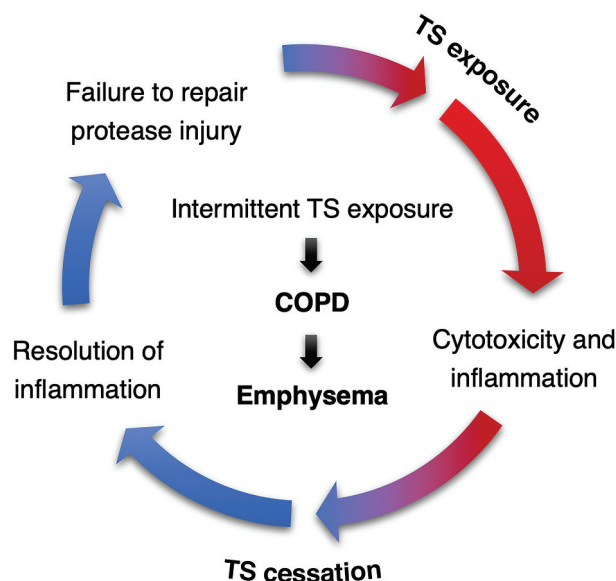
Figure 4. Possible mechanisms for the genesis of tobacco smoke (TS)-induced lung disease. During smoking exposure inflammation, injury and remodeling are highly activated, while smoking cessation leads to a resolution in inflammation. Repeated TS exposure and cessation creates a renewed insult to the lungs, resulting in renewed inflammation that repeats and amplifies the injury process. Release of proteolytic enzymes during this injury further damages and remodels the lungs through a loss of elastic fibers, thus causing airspace enlargement of the alveoli. Intermittent exposure to TS promulgates these effects, leading to enhanced emphysema, which is one of the most common conditions that contributes to chronic obstructive pulmonary disease (COPD). Reproduced from Pinkerton KE, Poindexter ME. Harmful interruptions: Impact of smoking patterns on tumorigenesis and emphysema. Am J Respir Cell Mol Biol 2018;59:133–134.

TS exposure in COPD animal models mimicking real-life human exposure

The generation and exposure conditions of tobacco smoke (TS) are crucial factors in interpreting animal studies investigating the development of chronic obstructive pulmonary disease (COPD). The variability in TS exposure conditions, such as yield measurement methods, smoking regimes, smoke characteristics, and dosage, significantly impact the consistency and reproducibility of study results.

An excessively high dose of TS in animals may be employed to achieve the desired effects of COPD development, whereas this approach may be criticized for its relevance to real-life human exposure. On the other hand, some investigators attempted to model COPD using lower doses of TS that more closely resemble human exposure levels. However, lower doses of TS in animal models may fail to induce definitive phenotypes of COPD, as seen in the study by Hodge-Bell et al. (2007), where only mild emphysema was observed in a minimal number of mice exposed to mainstream cigarette smoke (MS) with varying total particulate matter (PM) concentrations, suggesting that MS exposure in this study may not have been sufficient to induce a definitive COPD phenotype. This highlights the importance of carefully selecting appropriate dosages of TS in animal models for both better mimicking real-life exposure in humans and understanding the pathogenesis of the disease.

To better mimic human exposure, dosages of TS in animal models might include measurements of PM concentration, nicotine levels, smoke volume, and other relevant factors recalculated per airway square footage or lung volume/respiratory volume to provide a more accurate assessment of the dose-response relationship. In addition, standardized methods for measuring the yield of tar, nicotine, and carbon monoxide (CO) from cigarettes (Chae, Walters, and Holman 2017), such as the Federal Trade Commission (FTC) or International Organization of Standardization (ISO) methods, require consideration of animal studies. Further, smoking regimes, including number of cigarettes smoked per day, duration of smoking, and depth of inhalation, as well as type of cigarette (filtered, non-filtered or e-cigarette) and characteristics of the smoke such as particle size distribution or concentration of toxic constituents also needs to be considered with caution and reported in animal studies.

Further research and discussion on appropriate dosages and TS characteristics in animal models are needed to improve the translatability of findings to humans and enhance our understanding of the pathogenesis of COPD. Careful consideration and standardization of TS generation and exposure conditions in animal studies are critical to ensure the robustness and relevance of research observations in advancing our understanding of COPD and developing effective interventions for this complex disease.

Measured parameters to define COPD in animal models

Lung function measurement

Pulmonary function testing used to define COPD in animals involves measuring various parameters related to lung function, including forced expiratory volume (FEV1), forced vital capacity (FVC), peak expiratory flow (PEF), lung compliance, resistance, functional residual capacity (FRC), and total lung capacity (TLC) (Bailey 2012). These parameters are measured using a variety of techniques, including plethysmography, spirometry, and forced oscillation techniques. By measuring these parameters, investigators are able to assess the extent of airway obstruction, inflammation, and other features associated with COPD in animals.

Cell counting in bronchoalveolar lavage fluid (BALF)

The BALF is collected from the lungs through lavage, followed by analyzing the cell population within the fluid (Lofdahl et al. 2005). Cell counting is performed using a hemocytometer or an automated cell counter to quantify total number of cells present in the BALF. The cell population is typically divided into different types of cells, such as macrophages, neutrophils, lymphocytes, and eosinophils. The relative proportions of these different cell types provide important information regarding the inflammatory response in lungs and severity of COPD.

Histological examination

Pathological changes in animal models are analyzed using lung tissue samples under a microscope, involving collecting lung tissue samples and processing by standard histological techniques (Shu et al. 2017). The most frequently used stains for histological examination of lung tissue are hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) stains for identifying cell types and structural components of the lung, and mucus substances in the airways, respectively. Histological examination provides information regarding the morphological alterations that occur in lungs following TS exposure. The morphological alterations detected include inflammation, fibrosis, destruction of alveolar walls, and mucus accumulation in the airways.

Biomedical assays

Biomedical assays are used to examine biological fluids and tissues for various biomarkers of inflammation, oxidative stress, and lung function which provide a sensitive approach for measuring progression of COPD in animal models, and are often employed in combination with other techniques such as pulmonary function testing and histological examination (Barnes et al. 2015; Churg et al. 2009). Some frequently utilized biomedical assays include (1) enzyme-linked immunosorbent assay (ELISA) to measure levels of specific proteins, such as cytokines and chemokines, in biological fluids in blood, BALF, and lung tissue homogenates; (2) western blotting, for detecting changes in protein expression levels; (3) immunohistochemistry, for determining specific proteins in lung tissue sections using specific antibodies and visualizing the spatial distribution of specific proteins within lung tissue; and (4) oxidative stress assays, used to measure levels of reactive oxygen species (ROS), which might result in oxidative damage in cells and tissues, and postulated to play a key role in development of COPD.

In addition to the above commonly employed methods, several other techniques may also be utilized to define COPD in animal models including (1) gene expression analysis, measurement of alterations in gene expression levels, which are performed using microarray or RNA sequencing technologies, and provide insights into the molecular mechanisms underlying COPD development; (2) proteomics, a large-scale analysis of proteins in biological samples to identify proteins that are differentially expressed in response to TS exposure; (3) metabolomics, an analysis of metabolites in biological samples to provide information on the metabolic pathways that are dysregulated in COPD; and (4) imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), which are used to assess lung structure and function in animal models of COPD (Al Faraj et al. 2014; Kim et al. 2022). By using a combination of different methods, investigators might gain a more comprehensive understanding of the mechanisms underlying COPD pathogenesis.

Animal models of COPD

While no animal model might truly recapitulate the decades-long TS exposures that humans endure, numerous animal models were developed to examine the cellular and molecular mechanisms underlying COPD pathogenesis (Table 1). Regardless of the animal model, the fundamental features include airspace enlargement, mucus hypersecretion, chronic inflammation, and some form of airway remodeling. Each of these conditions exist to varying degrees of severity in COPD patients. In fact, disease heterogeneity may explain the lack of a universally reliable therapeutic approach for COPD, and require a regime of personalized medicine (Franssen et al. 2019). Unfortunately, the best available animal models display only limited aspects of COPD pathologies, thus reducing their ability to fully recapitulate the entire spectrum of clinical COPD. It is of interest most animal models of emphysema do not truly mimic the human disease, but rather reflect airspace enlargement due to either loss of collagen/elastin or TS-induced insults and incomplete alveolarization of the lungs during developmental windows of morphogenesis (Wright, Cosio, and Churg 2008). Ideally, an animal model of COPD needs to recapitulate the aforementioned morphological and biological changes in a manner similar to human disease. Therefore, it is critical to acknowledge the limits of animal models during any experimental process

to reliably interpret and clinically contextualize data relevant to human disease.

Several animal models clearly replicate one or more key pathologic features of COPD such as emphysema and bronchitis. However, when selecting a COPD model, several factors need to be considered. These include but are not limited to inter-species (animal-to-human) differences in genetics, lung development, and susceptibility to a given pulmonary insult.

Since humans and rodents possess a number of genetic homologies, mice and rats were widely used to study human respiratory diseases. For measurement of TS-related lung responses, mice and rats are generally exposed via a whole-body or nose-only exposure systems (Perez-Rial, Giron-Martinez, and Peces-Barba 2015). Depending upon the objective of the investigation, the exposure regimen might range from days to months. In general, to induce emphysema-like changes and airway remodeling, animals are exposed to TS for at least 4–6 months. However, exposure duration might vary with model species, exposure methods, and exposure concentrations/internalized doses (Wright and Churg 2002).

There are a number of structural and immunological differences to consider when extrapolating findings from animal models to humans. Although humans and rodents mount similar host responses to exogenous stimuli, there are inter-species differences in the anatomy of the tracheobronchial tree. These include (1) dichotomous symmetrical versus asymmetrical airway branching in humans versus rodents, respectively; (2) absence of respiratory bronchioles in mice and rats compared to humans; and (3) lack of a bronchial circulation in mice but not rats or humans (Kotoulas et al. 2014; Mitzner et al. 2000). Indeed, while humans, dogs, and ferrets exhibit several generations of membranous and respiratory bronchioles (Churg, Cosio, and Wright 2008; Wright and Churg 2008a), there exists in rats and mice an abrupt transition from the conducting airways to the alveolar duct. Despite inter-species structural variability in the respiratory system, animal models provide approximations of human disease pathology that continue to serve a critical role in the study underlying COPD pathogenesis.

Mice

Mice are commonly used to model lung disease. Advantages include low cost, easy handling, rapid reproductive turnover with large litters, and a potential for genetic manipulation that fosters their use over other animal species for lab research. This is because the mouse genome is similar to humans, diverse inbred strains are well-characterized, and generation of transgenic mice provides a rapid and relatively inexpensive approach to determine the exact function of particular genes (Gurumurthy and Lloyd 2019; Vandamme 2014). The wide availability of mouse antibodies and molecular probes are additional advantages. Mouse models of COPD include emphysema-like phenotypes induced by administration of elastase (Jo et al. 2022; Mano et al. 2022) or an extract solution of cigarette smoke (Yang et al. 2022), bronchitis and emphysema phenotypes induced by whole body or nose-only TS exposure (Pelgrim et al. 2022; Shapiro et al. 2003), and other COPD phenotypes initiated chemically by nitrogen dioxide or sulfur dioxide (Groneberg and Chung 2004).

Disadvantages of COPD mouse models include the time it takes to develop morphological changes consistent with human COPD, and the reversibility of these alterations, the latter being highly inconsistent with progressive and irreversible nature of human COPD. Mice are also poor models of chronic bronchitis due to their lack of bronchial submucosal glands, and minimal development of mucous (goblet) cell metaplasia in the smaller airways (Churg, Cosio, and Wright 2008). Further, development of emphysema in mice is strain-dependent and cannot always be consistently reproduced (Guerassimov et al. 2004; Nadziejko et al. 2007). Finally, the lengthy process required to produce disease in the mouse further limits the utility of telemetry and pulmonary function testing. These tests are often used in studies of cardiovascular disease, a common comorbidity of COPD (Kodavanti, Costa, and Bromberg 1998; Wright, Cosio, and Churg 2008).

Rats

Rats are frequently employed to study respiratory, cardiovascular, metabolic, and autoimmune diseases, all of which were reported to be associated with TS-induced COPD (Cavailles et al. 2013). One of the advantages to using rats is the ability to produce emphysema-like injury within 2 to 6 months of TS exposure. Rats also mimic human chronic bronchitis and airway hyperreactivity (Shore et al. 1995). With prolonged exposure to TS, rats demonstrate mucous metaplasia in the larger and smaller airways (Wright, Cosio, and Churg 2008), a common feature of human COPD. Similar to humans, rats display drug resistance, a critical factor when considering therapeutic glucocorticoid and antibiotic treatments in human COPD (Cowan et al. 1986; Fedosenko et al. 2015; Kabir and Morshed 2015; Maslarova et al. 2011).

Limitations to using rats compared to mice in COPD investigations include greater expense and fewer knockout strains available. Rats also lack submucosal glands; although, mucous (goblet) cells might increase and proliferate from a small baseline population (Kodavanti, Costa, and Bromberg 1998).

Given the comorbidities of diabetes, atherosclerosis, and hypertension commonly reported in COPD patients, rats were used to study such comorbidities following TS exposure. TS-exposed rats develop elevated pulmonary arterial pressure, suggesting development of pulmonary hypertension (Lee et al. 2005; Wright, Cosio, and Churg 2008). Spontaneously hypertensive rats (SHRs), established during the 1960s by selective breeding of Wistar-Kyoto (WKY) rats for high blood pressure traits, were found to be useful in studies of respiratory and cardiovascular diseases.

Spontaneously hypertensive rats (SHR) model of human COPD

Spontaneously hypertensive rats (SHR) demonstrate COPD-like phenotypes similar to humans following inhalation of PM (Kodavanti et al. 2000) and TS (Bolton et al. 2009; Shen et al. 2016; Yu et al. 2008), as well as intratracheal instillation of lipopolysaccharide (LPS), also known as endotoxin present in the outer cell membrane of gram negative bacteria (Wang et al. 2013). Since SHRs naturally develop elevated blood pressure, these animals have been widely utilized as genetic models of essential hypertension, arise in blood pressure without any secondary cause (Doggrell and Brown 1998; Zhou and Frohlich 2007). In SHRs, systemic blood pressure begins to rise by 5 to 6 weeks of age, and plateaus at values ranging from 180–200 mm mercury (Hg) by adulthood (12–13 weeks). In contrast, normotensive WKY rat strains display a systemic blood pressure ranging from 110–150 mm Hg (Buttner et al. 1984). Given the variety of more than 22 different SHR strains ranging from stroke-prone to Dahl/Rapp salt-sensitive rats and also exhibiting an array of different cardiovascular and metabolic pathologies, the SHR model may be ideal for study of COPD (Bolton et al. 2009; Chen et al. 2015; Shen et al. 2016; Wu et al. 2020; Yu et al. 2008).

SHRs afford us the opportunity to investigate mechanisms underlying COPD pathogenesis and enable testing of novel drug therapies (Davis et al. 2013). Previously investigators s demonstrated that SHRs manifest neutrophilic airway inflammation and nuclear factor-kappa B (NF-κB) and mitogen activated protein kinase-/activator protein-1 (MAPK/AP-1) activation which likely play a role in TS-induced pathogenesis (Zhong et al. 2005; Zhong, Zhou, and Pinkerton 2008). NF-κB and AP-1 regulate the expression of the inflammatory genes, IL-17, IL-8, and TNF-α, which are produced in COPD (Schuliga 2015). These TS-induced pro-inflammatory cytokines upregulate airway epithelial hyperplasia and squamous metaplasia through NF-κB and MAPK/AP-1 signaling [Figure 1; (Zhong et al. 2005)]. In addition, TS inhibits NF-κB activity and downregulates NF-κB-dependent anti-apoptotic proteins (BCL-2 and c-IAPs), leading to activation of caspases 3, 8, and 9, to induce programmed cell death by apoptosis as illustrated in Figure 3 (Zhong, Zhou, and Pinkerton 2008)]. Similarly, TS produces pulmonary toxicity via MAPK signaling and AP-1 transactivation (Mossman, Lounsbury, and Reddy 2006). SHRs also demonstrate increased expression of TGFβ in epithelial and inflammatory cells, and leukocyte recruitment in response to TS seems to occur mainly through bronchial circulation (Davis et al. 2012; Hoang et al. 2016). Overall, these studies

demonstrate that SHRs provide new and unique insights into the molecular mechanisms underlying TS-associated lung diseases that cannot be attained with other COPD models.

Limited data are available on the combined effects of enhanced susceptibility to cardiopulmonary complications and long-term TS exposure on pulmonary structural and cellular alterations frequently detected in COPD patients. SHRs exhibit risk factors similar to those found in COPD patients making this rat strain a promising human-relevant animal model of lung disease induced by inhaled irritants (Bolton et al. 2009; Kodavanti et al. 2006; Yu et al. 2008). Recently Pham et al. (2020) used SHR and WKY rats to examine the effects of long-term TS exposure on lung injury and airway inflammation, and elucidate the inherent risk factors and pathophysiology of COPD. The degree and severity of TS-induced pathological changes noted in SHRs were significantly different from WKY rats with long-term progressive exposure to TS up to 12 weeks. Data suggest a genetic contribution, which plays an important role for predicting TS-induced COPD onset in humans. Previous investigations with prolonged TS exposure and subsequent cessation demonstrated that SHRs responded similarly to humans. Such findings encourage further exploration of the SHR model in future studies of smoking cessation and COPD treatments (Pham et al. 2020; Wu et al. 2020).

It is also important to note that while genetically modified animal models may provide useful insights into the biological mechanisms underlying COPD, there may be limitations in extrapolating these findings to humans. The use of these models may be criticized as not necessarily representative of normal genetic diversity as found in humans. Therefore, it is important to consider the advantages and limitations of different animal models and their relevance to human disease when interpreting findings from such studies.

Guinea pigs

Guinea pigs are also widely used to model COPD. TS inhalation studies in guinea pigs ranged from 1 to 12 months to produce progressive pulmonary function abnormalities and emphysema-like lesions (Groneberg and Chung 2004) that are easily recognizable and more prominent than those observed in mice (Wright and Churg 2008b).

In guinea pigs, long-term TS exposure produced a rise of number of polymorphonuclear leukocytes (PMNs) and accumulation of macrophages and CD4+ T-cells (Meshi et al. 2002). Pulmonary inflammation and emphysematous destruction induced by chronic TS exposure were amplified by the presence of latent adenoviral infection (Meshi et al. 2002). Long-term TS exposure also induced selective endothelial dysfunction in pulmonary arteries of guinea pigs confirming that smoking exerts deleterious effects on pulmonary vessels and may contribute to development of pulmonary hypertension in COPD (Ferrer et al. 2009). Advantages of using guinea pigs include the ability to model common COPD comorbidities including pulmonary hypertension, autoimmune disease, and viral infection (Dominguez-Fandos et al. 2015; Ferrer et al. 2009; Groneberg and Chung 2004; Gu et al. 2012; Meshi et al. 2002).

Shortcomings associated with the guinea pig COPD model include irregularly distributed or absent bronchial glands, which make it a poor model for chronic bronchitis (Goco, Kress, and Brantigan 1963), and increased cost relative to mice and rats. Substantial molecular studies using guinea pigs, and commercially available antibodies and probes that cross react with guinea pig proteins are also lacking (Padilla-Carlin, McMurray, and Hickey 2008; Perez-Rial, Giron-Martinez, and Peces-Barba 2015).

Dogs

In general, COPD-related studies in dogs have been beset by inconsistent findings, confounding factors, incomplete reporting, and/or lack of controls. This may be attributed to the timeframe in which experiments were conducted and published. Some of the earliest studies examining the effects of cigarette smoking were in dogs. Repeated chamber exposures over the course of approximately one year yielded epithelial hyperplasia (Reece and Ball 1972), peribronchiolar hypercellularity with basal and mucous (goblet) cell hyperplasia (Park et al. 1977), and inflammatory cell influx in peribronchiolar regions (Hernandez et al. 1966; Park

et al. 1977). When exercise stress was combined with TS exposure for 11 months, signs of hypoxemia manifested (Reece and Ball 1972).

In some early investigations, expected changes in pulmonary function were mostly absent despite indications that TS inhalation might lead to chronic bronchitis and emphysema. For example Park et al. (1977) and Wanner et al. (1973), demonstrated that TS inhalation using mask or "mouthpipe" exposures for 6 or 12 months yielded decreased tracheal mucous velocity, while alterations in pulmonary function parameters, including pulmonary resistance and dynamic compliance, lung diffusing capacity of carbon monoxide, arterial blood gases, or lung volumes were not apparent. Similar experiments using tracheostomy exposures for 10 months yielded mucus hypersecretion without a change in mucous velocity (King et al. 1989). No changes in ciliary beat frequency were noted (Park et al. 1977) suggesting TS-induced alterations in mucus viscosity played a role in protecting airway epithelium and parenchyma (King et al. 1989; Park et al. 1977). However, at least one study suggested that TS-induced mucus hypersecretion protected against nonspecific airway reactivity to inhaled methacholine (Desanctis et al. 1987).

Emphysema induced by TS was reported by several investigators (Auerbach et al. 1967; Frasca et al. 1983; Hernandez et al. 1966; Zwicker et al. 1978). However, these studies were criticized for confounding factors, such as pulmonary infections, and lack of sham controls (Thomas and Vigerstad 1989).

Interestingly, most investigations utilized young adult dogs even though COPD was proposed to be a disease of accelerated lung aging (Ito and Barnes 2009). Given that dog lungs undergo restructuring, "senile emphysema," as in humans later in life (Hyde et al. 1977; Mauderly 2000), there may still be an opportunity to better define this aspect of COPD with this model.

Ferrets

One of the significant components of COPD pathogenesis is bronchitis with mucus hypersecretion and airway inflammation. Bronchitis pathogenesis is associated with chronic impairment in epithelial sodium channels and hindrance of mucociliary clearance through inhibition of the cystic fibrosis transmembrane conductance regulator (CFTR) protein (Cantin 2016; Mall 2016; Rab et al. 2013; Raju et al. 2016). Ferret and human CFTRs function in similar ways to produce changes in epithelial permeability (Fisher et al. 2012). Distribution of submucosal mucus glands and airway physiology are similar in ferrets and humans enabling the bronchitis component of TS-induced human COPD to be mimicked (Raju et al.). Long-term TS exposure in ferrets induces clinical features of human bronchitis such as cough, airway mucous (goblet) cell hyperplasia, and mucus hypersecretion with associated bronchiolitis (Raju et al.). Considering the molecular and pathological similarities between human and ferret TS-induced bronchitis, this animal model has the potential to enhance our understanding of the pathogenesis of human bronchitis, and enable subsequent testing of novel therapeutics (Solomon et al. 2016).

Nonhuman primates (Monkeys)

As nonhuman primates (NHPs) exhibit anatomical, physiological, and immunological similarities to human lungs, this species might serve as reliable models for COPD (Miller et al. 2017). However, there are several limitations to their use in research. First is the cost of purchase, per diem, and exposure conditions required to begin to simulate a COPD-like condition. Exposures require up to several years to recapitulate human COPD, although shorter term exposure may elicit inflammation (bronchitis), mucous metaplasia, and airspace enlargement when exposure begins in infancy. NHP models also present challenges in genetic manipulation techniques, while specialized facilities required to house monkeys demand high associated costs. Thus, only a few studies have attempted to model COPD through exposure of monkeys to TS.

Over 35 years ago, baboons were trained to smoke and assessed after 6 pack-years of smoking (over the course of three years). Interestingly, bronchial reactivity was blunted, residual lung volume was preserved following methacholine challenge, and no other changes in lung volumes or flow-volume measures were observed in

smoking monkeys (Roehrs, Rogers, and Johanson 1981). While inhalation of nicotine was found to acutely blunt the methacholine response without affecting baseline lung function, the response to histamine was unaffected (Wallis, Rogers, and Johanson 1982). Notwithstanding, nicotine also produces bronchoconstriction by decreasing guanylate cyclase activity in male Sprague Dawley rats (Vesely and Levey 1978). Aspects of lung barrier function, such as mucus secretion and mucociliary function were not assessed in these studies.

In a more recent examination of heavy smoking approximating 4 packs per day, female macaques (average age = 11 years) were exposed in chambers over the course of 4 or 12 weeks to determine TS-induced pathological processes in the model (Polverino et al. 2015). Inflammation, mucus metaplasia, glandular hypertrophy/hyperplasia, and peribronchial fibrosis were evident. A "pre-emphysematous" profile was indicated by elevated MMP, apoptosis of alveolar septal cells, and oxidative stress in lung tissue. After 12 weeks, pulmonary function changes were minimal relative to sham exposure. These included a trend toward reduced forced expiratory volume in one second, and an approximately 20% increase in quasistatic compliance versus a 7% decrease in sham-exposed controls. Further studies in this model showed that a combination of TS and influenza infection enhanced connective tissue growth factor expression in bronchial epithelial cells which might accelerate cell senescence (Jang et al. 2017).

COPD animal models in clinical studies

Animal models were instrumental in advancing our understanding of COPD and its potential treatments. Studies utilizing animal models provided insights into mechanisms underlying COPD pathophysiology, determined novel interventions, explored regenerative potential, and identified potential biomarkers. Qiu et al. (2022) observed that elevated levels of IL-27, a member of IL-12 family cytokines, was accompanied by an exaggerated IFN-γ+CD8+Tc1 response in a smoking mouse model of emphysema. These observations suggested that IL-27 negatively regulated IFN-γ +CD8+Tc1 response in the late stage of chronic cigarette smoke exposure, which may provide

a new strategy for the anti-inflammatory treatment of smoking-related COPD/emphysema. Kim et al. (2018) demonstrated that fibroblast growth factor-2 (FGF2) was decreased in lungs of cigarette smoke-exposed mice and intranasal recombinant FGF-2 (rFGF-2) significantly reduced macrophage-dominant inflammation and alveolar destruction in lungs, suggesting that inhaling rFGF-2 may be a new therapeutic option for patients with COPD. Chen et al. (2019) established a mouse model of COPD utilizing cigarette smoke exposure and found that C-X-C motif chemokine 5 levels correlated with granulocyte-colony stimulating factor levels in both plasma/serum and BALF obtained from patients and the mouse model. Chen et al. (2019) suggested that the elevated circulating C-X-C motif chemokine 5 are associated with lung function decline and might serve as a blood-based biomarker for preliminary screening and diagnosis of COPD. In a study using an animal model of hypoxia-induced pulmonary hypertension (PH), Jiang et al. (2018) showed that circulating levels of miR-190a-5p correlated with the severity of PH secondary to COPD and suggested that miR-190a-5p may serve as a biomarker for diagnosis and prognosis of COPD-PH. Turino et al. (2018) noted that hyaluronan aerosol reduced the severity of elastase-induced emphysema in animal models and indicating promising results in preliminary human studies by decreasing levels of desmosine and isodesmosine (DI), which might serve as biomarkers of elastin degradation.

Previously Baker, Donnelly, and Barnes (2020) reported that senolytic therapies induce apoptosis and clearance of senescent cells, as well as diminish senescence-associated secretory phenotype response in animal models of aging and other age-related diseases. Data suggested that the combination of senolytics and senostatics, which are drugs that inhibit cellular senescence, may hold promise as a novel approach for COPD treatment, and need to be evaluated in animal models of COPD. Further, Ng-Blichfeldt et al. (2019) found that numerous preclinical studies showed induction of regeneration in animal models of COPD/emphysema, proposing an important role for COPD animal studies to be considered in clinical trials. Overall, these studies highlight the invaluable role of animal models in developing potential

therapeutic strategies for COPD treatment in clinical studies.

Conclusions

The current global burden of COPD is substantial, and prevalence of this devastating disease is rising. Multiple rational models for human COPD were developed (Table 1), and these are essential to the study of TS-induced lung dysfunction, diseases, and pathologies related to COPD (Table 2). Significant progress was made in our understanding of the biological mechanisms underlying COPD, specifically the role of macrophage phenotypes, lymphocyte subpopulations, and protease/antiprotease defense mechanisms. However, key factors contributing to the progression of inflammation and exacerbation of COPD remain unclear.

While there are considerable limitations to the use of rodent models and questions as to whether these accurately reflect human COPD-like features, SHRs seem to best represent the biological mechanisms and features of human COPD including progressive airway inflammation, mucus hypersecretion, and emphysema compared to other lab animal models (Bolton et al. 2009; Chen et al. 2015; Davis et al. 2013; Hoang et al. 2016; Kodavanti et al. 2006; Nadadur, Pinkerton, and Kodavanti 2002; Pham et al. 2020; Shen et al. 2016; Yu et al. 2008; Zhong et al. 2005; Zhong, Zhou, and Pinkerton 2008). SHRs also exhibit genetic risk factors toward hypertension that are found in COPD patients suggesting that SHR may be a more clinically relevant model of TS-induced disease. The application of new animal models might also be necessary to test novel therapeutic interventions and elucidate the risk factors that lead to disease onset, progression, and disease exacerbations.

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Disclosure statement

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PU and CWW wrote the manuscript and performed the literature search, AP contributed to the original writing and ideas for the review, AAZ, CMR, UPK, MT and HB provided data for animal models and mechanisms of COPD, KEP oversaw the organization, writing and final review.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials https://doi.org/10.6084/m9.figshare.21354531.v1.

References

Agusti, A., P. M. Calverley, B. Celli, H. O. Coxson, L. D. Edwards, D. A. Lomas, W. MacNee, B. E. Miller, S. Rennard, E. K. Silverman, et al. 2010. Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir. Res.* 11 (1):122. doi:10.1186/1465-9921-11-122.

Alam, S., Z. Li, C. Atkinson, D. Jonigk, S. Janciauskiene, and R. Mahadeva. 2014. Z alpha1-antitrypsin confers a proinflammatory phenotype that contributes to chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 189:909–31.

Alfahad, A. J., M. M. Alzaydi, A. M. Aldossary, A. A. Alshehri, F. A. Almughem, N. M. Zaidan, and E. A. Tawfik. 2021. Current views in chronic obstructive pulmonary disease pathogenesis and management. *Saudi Pharm. J.* 29 (12):1361–73. doi:10.1016/j.jsps.2021.10.008.

Al Faraj, A., A. Sultana Shaik, M. A. Pureza, M. Alnafea, R. Halwani, and R. E. Morty. 2014. Preferential

macrophage recruitment and polarization in LPS-induced animal model for COPD: Noninvasive tracking using MRI. *PLoS. ONE* 9 (3):e90829. doi:10.1371/journal.pone.0090829.

Andersen, Z. J., M. Hvidberg, S. S. Jensen, M. Ketzel, S. Loft, M. Sorensen, A. Tjonneland, K. Overvad, and O. Raaschou-Nielsen. 2011. Chronic obstructive pulmonary disease and long-term exposure to traffic-related air pollution: A cohort study. *Am. J. Respir. Crit. Care Med.* 183 (4):455–61. doi:10.1164/rccm.201006-0937OC.

Auerbach, O., E. C. Hammond, D. Kirman, and L. Garfinkel. 1967. Emphysema produced in dogs by cigarette smoking. *J. Am. Med. Assoc.* 199 (4):241–46. doi:10.1001/jama.1967.03120040051008.

Bailey, K. L. 2012. The importance of the assessment of pulmonary function in COPD. *Med. Clin. North Am.* 96 (4):745–52. doi:10.1016/j.mcna.2012.04.011.

Baker, J. R., L. E. Donnelly, and P. J. Barnes. 2020. Senotherapy: A new horizon for COPD therapy. *Chest* 158 (2):562–70. doi:10.1016/j.chest.2020.01.027.

Barberà, J. A., and I. Blanco. 2009. Pulmonary hypertension in patients with chronic obstructive pulmonary disease. *Drugs* 69 (9):1153–71. doi:10.2165/00003495-200969090-00002.

Barnes, P. J. 2016a. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J. Allergy Clin. Immunol.* 138 (1):16–27. doi:10.1016/j.jaci.2016.05.011.

Barnes, P. J. 2016b. Sex differences in chronic obstructive pulmonary disease mechanisms. *Am. J. Respir. Crit. Care Med.* 193 (8):813–14. doi:10.1164/rccm.201512-2379ED.

Barnes, P. J., P. G. Burney, E. K. Silverman, B. R. Celli, J. Vestbo, J. A. Wedzicha, and E. F. Wouters. 2015. Chronic obstructive pulmonary disease. *Nat. Rev. Dis. Primers* 1 (1):15076. doi:10.1038/nrdp.2015.76.

Barnes, P. J., and M. G. Cosio. 2004. Characterization of T lymphocytes in chronic obstructive pulmonary disease. *PLoS Med.* 1 (1):e20. doi:10.1371/journal.pmed.0010020.

Bartalesi, B., E. Cavarra, S. Fineschi, M. Lucattelli, B. Lunghi, P. A. Martorana, and G. Lungarella. 2005. Different lung responses to cigarette smoke in two strains of mice sensitive to oxidants. *Eur. Respir. J.* 25 (1):15–22. doi:10.1183/09031936.04.00067204.

Benedikter, B. J., E. F. M. Wouters, P. H. M. Savelkoul, G. G. U. Rohde, and F. R. M. Stassen. 2018. Extracellular vesicles released in response to respiratory exposures: Implications for chronic disease. *J. Toxicol. Environ. Health B* 21 (3):142–60. doi:10.1080/10937404.2018.1466380.

Bhalla, D. K., F. Hirata, A. K. Rishi, and C. G. Gairola. 2009. Cigarette smoke, inflammation, and lung injury: A mechanistic perspective. *J. Toxicol. Environ. Health B* 12 (1):45–64. doi:10.1080/10937400802545094.

Bolton, S. J., K. Pinnion, V. Oreffo, M. Foster, and K. E. Pinkerton. 2009. Characterisation of the proximal airway squamous metaplasia induced by chronic tobacco smoke exposure in spontaneously hypertensive rats. *Respir. Res.* 10 (1):118. doi:10.1186/1465-9921-10-118.

Boutou, A. K., Z. Zoumot, A. Nair, C. Davey, D. M. Hansell, A. Jamurtas, M. I. Polkey, and N. S. Hopkinson. 2015. The impact of homogeneous versus heterogeneous emphysema on dynamic hyperinflation in patients with severe COPD assessed for lung volume reduction. *COPD* 12 (6):598–605. doi:10.3109/15412555.2015.1020149.

Bracke, K. R., A. I. D'hulst, T. Maes, K. B. Moerloose, I. K. Demedts, S. Lebecque, G. F. Joos, and G. G. Brusselle. 2006. Cigarette smoke-induced pulmonary inflammation and emphysema are attenuated in CCR6-deficient mice. *J. Immunol.* 177 (7):4350–59. doi:10.4049/jimmunol.177.7.4350.

Bracke, K. R., and P. Mestdagh. 2017. MicroRNAs as future therapeutic targets in COPD? *Eur. Respir. J.* 49 (5):1700431. doi:10.1183/13993003.00431-2017.

Buttner, D., H. Hackbarth, F. Wollnik, and H. Borggreve. 1984. Blood pressure in rats: A comparison of a multifactorial experimental design to measurements in an outbred stock. *Lab. Animal* 18 (2):110–14. doi:10.1258/002367784780891334.

Campo, G., R. Pavasini, S. Biscaglia, M. Contoli, and C. Ceconi. 2015. Overview of the pharmacological challenges facing physicians in the management of patients with concomitant cardiovascular disease and chronic obstructive pulmonary disease. *Eur. Heart J. Cardiovasc Pharmacother* 1 (3):205–11. doi:10.1093/ehjcvp/pvv019.

Cantin, A. M. 2016. Cystic fibrosis transmembrane conductance regulator. Implications in cystic fibrosis and chronic obstructive pulmonary disease. *Ann. Am. Thorac. Soc.* 13 (Suppl_2):S150–55. doi:10.1513/AnnalsATS.201509-588KV.

Caramori, G., P. Casolari, A. Barczyk, A. L. Durham, A. Di Stefano, and I. Adcock. 2016. COPD immunopathology. *Semin Immunopathol.* 38 (4):497–515. doi:10.1007/s00281-016-0561-5.

Castaldi, P. J., M. H. Cho, R. San Jose Estepar, M. L. McDonald, N. Laird, T. H. Beaty, G. Washko, J. D. Crapo, E. K. Silverman, and C. O. Investigators. 2014. Genome-wide association identifies regulatory Loci associated with distinct local histogram emphysema patterns. *Am. J. Respir. Crit. Care Med.* 190 (4):399–409. doi:10.1164/rccm.201403-0569OC.

Cavailles, A., G. Brinchault-Rabin, A. Dixmier, F. Goupil, C. Gut-Gobert, S. Marchand-Adam, J. C. Meurice, H. Morel, C. Person-Tacnet, C. Leroyer, et al. 2013. Comorbidities of COPD. *Eur Respir. Rev.* 22 (130):454–75. doi:10.1183/09059180.00008612.

Cavarra, E., B. Bartalesi, M. Lucattelli, S. Fineschi, B. Lunghi, F. Gambelli, L. A. Ortiz, P. A. Martorana, and G. Lungarella. 2001. Effects of cigarette smoke in mice with different levels of α1-proteinase inhibitor and sensitivity to oxidants. *Am. J. Respir. Crit. Care Med.* 164 (5):886–90. doi:10.1164/ajrccm.164.5.2010032.

CDC. 2020a. *Disease or condition of the week. COPD.* Centers for Disease Control and Prevention, Nov 5, 2019 Accessed November 10, 2020. https://www.cdc.gov/dotw/copd/index.html.

CDC. 2020b. *Chronic obstructive pulmonary disease (COPD). COPD costs.* National Center for Chronic Disease

Prevention and Health Promotion, Division of Population Health. Feb 21, 2018 Accessed November 10, 2020. https://www.cdc.gov/copd/infographics/copd-costs.html .

Chae, C., M. J. Walters, and M. R. Holman. 2017. International Organization of Standardization (ISO) and Cambridge Filter Test (CFT) smoking regimen data comparisons in tobacco product marketing applications. *Tob Regul. Sci.* 3 (3):258–65. doi:10.18001/TRS.3.3.2.

Chang, L. H., and M. P. Rivera. 2013. Respiratory diseases: Meeting the challenges of screening, prevention, and treatment. *N C Med J.* 74 (5):385–92. doi:10.18043/ncm.74.5.385.

Chaouat, A., R. Naeije, and E. Weitzenblum. 2008. Pulmonary hypertension in COPD. *Eur. Respir. J.* 32 (5):1371–85. doi:10.1183/09031936.00015608.

Chapman, R. W. 2008. Canine models of asthma and COPD. *Pulm Pharmacol. Ther.* 21 (5):731–42. doi:10.1016/j.pupt.2008.01.003.

Chen, J., L. Dai, T. Wang, J. He, Y. Wang, and F. Wen. 2019. The elevated CXCL5 levels in circulation are associated with lung function decline in COPD patients and cigarette smoking-induced mouse model of COPD. *Ann. Med.* 51 (5–6):314–29. doi:10.1080/07853890.2019.1639809.

Chen, Y., C. Yu, M. Pan, T. Li, W. Li, S. Cai, and Y. Meng. 2015. [Comparison of matrix metalloproteinase-9 between Wistar rat and spontaneously hypertensive rat in pulmonary injury model]. *Zhonghua Yi Xue Za Zhi* 95 (18):1415–20.

Churg, A., M. Cosio, and J. L. Wright. 2008. Mechanisms of cigarette smoke-induced COPD: Insights from animal models. *Am. J. Physiol. Lung Cell Mol. Physiol.* 294 (4):L612–31. doi:10.1152/ajplung.00390.2007.

Churg, A., R. D. Wang, H. Tai, X. Wang, C. Xie, and J. L. Wright. 2004. Tumor necrosis factor-α drives 70% of cigarette smoke–induced emphysema in the mouse. *Am. J. Respir. Crit. Care Med.* 170 (5):492–98. doi:10.1164/rccm.200404-511OC.

Churg, A., S. Zhou, X. Wang, R. Wang, and J. L. Wright. 2009. The role of interleukin-1β in murine cigarette smoke–Induced emphysema and small airway remodeling. *Am. J. Respir. Cell Mol. Biol.* 40 (4):482–90. doi:10.1165/rcmb.2008-0038OC.

Churg, A., S. Zhou, and J. L. Wright. 2012. Series "matrix metalloproteinases in lung health and disease": Matrix metalloproteinases in COPD. *Eur. Respir. J.* 39 (1):197–209. doi:10.1183/09031936.00121611.

Cochard, M., F. Ledoux, and Y. Landkocz. 2020. Atmospheric fine particulate matter and epithelial mesenchymal transition in pulmonary cells: State of the art and critical review of the in vitro studies. *J. Toxicol. Environ. Health B* 23 (7):293–318. doi:10.1080/10937404.2020.1816238.

Coultas, D. B., D. Mapel, R. Gagnon, and E. Lydick. 2001. The health impact of undiagnosed airflow obstruction in a national sample of United States adults. *Am. J. Respir. Crit. Care Med.* 164 (3):372–77. doi:10.1164/ajrccm.164.3.2004029.

Cowan, K. H., G. Batist, A. Tulpule, B. K. Sinha, and C. E. Myers. 1986. Similar biochemical changes associated with multidrug resistance in human breast cancer cells and carcinogen-induced resistance to xenobiotics in rats. *Proc. Natl. Acad. Sci. U.S.A.* 83 (24):9328–32. doi:10.1073/pnas.83.24.9328.

Davis, B. B., Y. H. Shen, D. J. Tancredi, V. Flores, R. P. Davis, K. E. Pinkerton, and M. Königshoff. 2012. Leukocytes are recruited through the bronchial circulation to the lung in a spontaneously hypertensive rat model of COPD. *PLoS. ONE* 7 (3):e33304. doi:10.1371/journal.pone.0033304.

Davis, B. B., A. A. Zeki, J. M. Bratt, L. Wang, S. Filosto, W. F. Walby, N. J. Kenyon, T. Goldkorn, E. S. Schelegle, and K. E. Pinkerton. 2013. Simvastatin inhibits smoke-induced airway epithelial injury: Implications for COPD therapy. *Eur. Respir. J.* 42 (2):350–61. doi:10.1183/09031936.00042512.

Desanctis, G. T., S. M. Kelly, M. P. Saetta, R. J. Shiner, J. L. Stril, T. S. Hakim, M. G. Cosio, M. King, and G. T. Desanctis. 1987. Hyporesponsiveness to aerosolized but not to infused methacholine in cigarette-smoking dogs. *Am. Rev. Respir. Dis.* 135 (2):338–44. doi:10.1164/arrd.1987.135.2.338.

De Torres, J. P., A. Campo, C. Casanova, A. Aguirre-Jaime, and J. Zulueta. 2006. Gender and chronic obstructive pulmonary disease in high-risk smokers. *Respiration* 73 (3):306–10. doi:10.1159/000090051.

D'Hulst, A. I., T. Maes, K. R. Bracke, I. K. Demedts, K. G. Tournoy, G. F. Joos, and G. G. Brusselle. 2005. Cigarette smoke-induced pulmonary emphysema in scid-mice. Is the acquired immune system required? *Respir. Res.* 6 (1):147. doi:10.1186/1465-9921-6-147.

Doggrell, S. A., and L. Brown. 1998. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovasc. Res.* 39 (1):89–105. doi:10.1016/S0008-6363(98)00076-5.

Dominguez-Fandos, D., C. Valdes, E. Ferrer, R. Puig-Pey, I. Blanco, O. Tura-Ceide, T. Paul, V. I. Peinado, and J. A. Barbera. 2015. Sildenafil in a cigarette smoke-induced model of COPD in the guinea-pig. *Eur. Respir. J.* 46 (2):346–54. doi:10.1183/09031936.00139914.

Duffy, S. P., and G. J. Criner. 2019. Chronic obstructive pulmonary disease: Evaluation and management. *Med. Clin. North Am.* 103 (3):453–61. doi:10.1016/j.mcna.2018.12.005.

Easter, M., S. Bollenbecker, J. W. Barnes, and S. Krick. 2020. Targeting aging pathways in chronic obstructive pulmonary disease. *Int. J. Mol. Sci.* 21 (18):6924. doi:10.3390/ijms21186924.

Effah, F., B. Taiwo, D. Baines, A. Bailey, and T. Marczylo. 2022. Pulmonary effects of e-liquid flavors: A systematic review. *J. Toxicol. Environ. Health B* 25 (7):343–71. doi:10.1080/10937404.2022.2124563.

Eisner, M. D. 2007. Indoor air, passive smoking, and COPD. *Am. J. Respir. Crit. Care Med.* 176 (5):426–27. doi:10.1164/rccm.200705-684ED.

Eisner, M. D., N. Anthonisen, D. Coultas, N. Kuenzli, R. Perez-Padilla, D. Postma, I. Romieu, E. K. Silverman,

and J. R. Balmes. 2010. An official American thoracic society public policy statement: Novel risk factors and the global burden of chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 182 (5):693–718. doi:10.1164/rccm.200811-1757ST.

Eppert, B. L., B. W. Wortham, J. L. Flury, and M. T. Borchers. 2013. Functional characterization of T cell populations in a mouse model of chronic obstructive pulmonary disease. *J. Immunol.* 190 (3):1331–40. doi:10.4049/jimmunol.1202442.

Fairclough, L., R. A. Urbanowicz, J. Corne, and J. R. Lamb. 2008. Killer cells in chronic obstructive pulmonary disease. *Clin. Sci.* 114 (8):533–41. doi:10.1042/CS20070356.

Fedosenko, S. V., L. M. Ogorodova, E. N. Il'ina, M. E. Senina, E. S. Lisitsina, M. A. Karnaushkina, E. S. Kostryukova, V. M. Govorun, I. A. Deev, E. S. Kulikov, et al. 2015. Genetic determinants of antibiotic resistance in oropharyngeal streptococci in patients with chronic obstructive pulmonary disease and in those with asthma. *Ter. Arkh.* 87 (8):51–57. doi:10.17116/terarkh201587851-57.

Ferrer, E., V. I. Peinado, M. Diez, J. L. Carrasco, M. M. Musri, A. Martinez, R. Rodriguez-Roisin, and J. A. Barbera. 2009. Effects of cigarette smoke on endothelial function of pulmonary arteries in the guinea pig. *Respir. Res.* 10 (1):76. doi:10.1186/1465-9921-10-76.

Finkelstein, J., E. Cha, and S. M. Scharf. 2009. Chronic obstructive pulmonary disease as an independent risk factor for cardiovascular morbidity. *Int. J. Chron Obstruct Pulmon Dis.* 4:337–49. doi:10.2147/COPD.S6400.

Fischer, B. M., J. A. Voynow, and A. J. Ghio. 2015. COPD: Balancing oxidants and antioxidants. *Int. J. Chron Obstruct Pulmon Dis.* 10:261–76. doi:10.2147/COPD.S42414.

Fisher, J. T., X. Liu, Z. Yan, M. Luo, Y. Zhang, W. Zhou, B. J. Lee, Y. Song, C. Guo, Y. Wang, et al. 2012. Comparative processing and function of human and ferret cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* 287 (26):21673–85. doi:10.1074/jbc.M111.336537.

Foronjy, R. F., B. A. Mercer, M. W. Maxfield, C. A. Powell, J. D'Armiento, and Y. Okada. 2005. Structural emphysema does not correlate with lung compliance: Lessons from the mouse smoking model. *Exp. Lung Res.* 31 (6):547–62. doi:10.1080/019021490951522.

Franssen, F. M., P. Alter, N. Bar, B. J. Benedikter, S. Iurato, D. Maier, M. Maxheim, F. K. Roessler, M. A. Spruit, C. F. Vogelmeier, et al. 2019. Personalized medicine for patients with COPD: Where are we? *Int. J. Chron. Obstruct Pulmon Dis.* 14:1465–84. doi:10.2147/COPD.S175706.

Frasca, J. M., O. Auerbach, H. W. Carter, and V. R. Parks. 1983. Morphologic alterations induced by short-term cigarette smoking. *Am. J. Pathol.* 111 (1):11–20.

Freeman, C. M., F. J. Martinez, M. K. Han, G. R. Washko, A. L. McCubbrey, S. W. Chensue, D. A. Arenberg, C. A. Meldrum, L. McCloskey, and J. L. Curtis. 2013. Lung CD8+ T cells in COPD have increased expression of bacterial TLRs. *Respir. Res.* 14 (1):13. doi:10.1186/1465-9921-14-13.

Fricker, M., A. Deane, and P. M. Hansbro. 2014. Animal models of chronic obstructive pulmonary disease. *Expert Opin. Drug Discov.* 9 (6):629–45. doi:10.1517/17460441.2014.909805.

Gamble, E., D. C. Grootendorst, K. Hattotuwa, T. O'Shaughnessy, F. S. Ram, Y. Qiu, J. Zhu, A. M. Vignola, C. Kroegel, F. Morell, et al. 2007. Airway mucosal inflammation in COPD is similar in smokers and ex-smokers: A pooled analysis. *Eur. Respir. J.* 30 (3):467–71. doi:10.1183/09031936.00013006.

Gharib, S. A., A. M. Manicone, and W. C. Parks. 2018. Matrix metalloproteinases in emphysema. *Matrix Biol.* 73:34–51. doi:10.1016/j.matbio.2018.01.018.

Ghorani, V., M. H. Boskabady, M. R. Khazdair, and M. Kianmeher. 2017. Experimental animal models for COPD: A methodological review. *Tob. Induc. Dis.* 15 (1):25. doi:10.1186/s12971-017-0130-2.

Ghosh, A., R. D. Coakley, A. J. Ghio, M. S. Muhlebach, C. R. Esther, N. E. Alexis, and R. Tarran. 2019. Chronic E-cigarette use increases neutrophil elastase and matrix metalloprotease levels in the lung. *Am. J. Respir. Crit. Care Med.* 200:1392–401.

Goco, R. V., M. B. Kress, and O. C. Brantigan. 1963. Comparison of mucus glands in the tracheobronchial tree of man and animals. *Ann. N. Y. Acad. Sci.* 106:555–71.

GOLD. 2020. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (2020 report). *Global Initiative for Chronic Obstructive Lung Disease.*

Goldklang, M. P., S. M. Marks, and J. M. D'Armiento. 2013. Second hand smoke and COPD: Lessons from animal studies. *Front Physiol.* 4:30.

Golovatch, P., B. A. Mercer, V. Lemaitre, A. Wallace, R. F. Foronjy, and J. D'Armiento. 2009. Role for cathepsin K in emphysema in smoke-exposed guinea pigs. *Exp. Lung Res.* 35:631–45.

Green, D. R., and T. A. Ferguson. 2001. The role of Fas ligand in immune privilege. *Nat. Rev. Mol. Cell Biol.* 2:917–24.

Green, C. E., and A. M. Turner. 2017. The role of the endothelium in asthma and chronic obstructive pulmonary disease (COPD). *Respir. Res.* 18:20.

Groneberg, D. A., and K. F. Chung. 2004. Models of chronic obstructive pulmonary disease. *Respir. Res.* 5:18. doi:10.1186/1465-9921-5-18.

Gross, T. J., L. S. Powers, R. L. Boudreau, B. Brink, A. Reisetter, K. Goel, A. K. Gerke, I. H. Hassan, and M. M. Monick. 2014. A microRNA processing defect in smokers' macrophages is linked to SUMOylation of the endonuclease DICER. *J. Biol. Chem.* 289 (18):12823–34. doi:10.1074/jbc.M114.565473.

Gu, Y., Z. W. Chen, A. Siegel, R. Koshy, C. Ramirez, T. D. Raabe, G. H. Devries, and A. A. Ilyas. 2012. Analysis of humoral immune responses to LM1 ganglioside in guinea pigs. *J. Neuroimmunol.* 246 (1–2):58–64. doi:10.1016/j.jneuroim.2012.03.001.

Guerassimov, A., Y. Hoshino, Y. Takubo, A. Turcotte, M. Yamamoto, H. Ghezzo, A. Triantafillopoulos,

K. Whittaker, J. R. Hoidal, and M. G. Cosio. 2004. The development of emphysema in cigarette smoke-exposed mice is strain dependent. *Am. J. Respir. Crit. Care Med.* 170 (9):974–80. doi:10.1164/rccm.200309-1270OC.

Gurumurthy, C. B., and K. C. K. Lloyd. 2019. Generating mouse models for biomedical research: Technological advances. *Dis. Model Mech.* 12 (1):dmm029462. doi:10.1242/dmm.029462.

Halappanavar, S., J. Nikota, D. Wu, A. Williams, C. L. Yauk, and M. Stampfli. 2013. IL-1 receptor regulates microRNA-135b expression in a negative feedback mechanism during cigarette smoke–induced inflammation. *J. Immunol.* 190 (7):3679–86. doi:10.4049/jimmunol.1202456.

Han, M. K., D. Postma, D. M. Mannino, N. D. Giardino, S. Buist, J. L. Curtis, and F. J. Martinez. 2007. Gender and chronic obstructive pulmonary disease: Why it matters. *Am. J. Respir. Crit. Care Med.* 176 (12):1179–84. doi:10.1164/rccm.200704-553CC.

Harada, T., and P. K. Basrur. 1998. Pulmonary macrophage mobilization in hamsters after cessation of smoking. *Exp. Animal* 47 (1):43–47. doi:10.1538/expanim.47.43.

Harting, J. R., A. Gleason, D. J. Romberger, S. G. Von Essen, F. Qiu, N. Alexis, and J. A. Poole. 2012. Chronic obstructive pulmonary disease patients have greater systemic responsiveness to ex vivo stimulation with swine dust extract and its components versus healthy volunteers. *J. Toxicol. Environ. Health Part A* 75 (24):1456–70. doi:10.1080/15287394.2012.722186.

Hawkins, N. M., S. Virani, and C. Ceconi. 2013. Heart failure and chronic obstructive pulmonary disease: The challenges facing physicians and health services. *Eur. Heart J.* 34 (36):2795–803. doi:10.1093/eurheartj/eht192.

Herfs, M., P. Hubert, A. L. Poirrier, P. Vandevenne, V. Renoux, Y. Habraken, D. Cataldo, J. Boniver, and P. Delvenne. 2012. Proinflammatory cytokines induce bronchial hyperplasia and squamous metaplasia in smokers: Implications for chronic obstructive pulmonary disease therapy. *Am. J. Respir. Cell Mol. Biol.* 47 (1):67–79. doi:10.1165/rcmb.2011-0353OC.

Hernandez, J. A., A. E. Anderson, W. L. Holmes, and A. G. Foraker. 1966. Pulmonary parenchymal defects in dogs following prolonged cigarette smoke exposure. *Am. Rev. Respir. Dis.* 93 (1):78–83. doi:10.1164/arrd.1966.93.1.78.

Hersh, C. P. 2010. Pharmacogenetics of chronic obstructive pulmonary disease: Challenges and opportunities. *Pharmacogenomics* 11 (2):237–47. doi:10.2217/pgs.09.176.

Hikichi, M., K. Mizumura, S. Maruoka, and Y. Gon. 2019. Pathogenesis of chronic obstructive pulmonary disease (COPD) induced by cigarette smoke. *J. Thorac. Dis.* 11 (Suppl 17):S2129–40. doi:10.21037/jtd.2019.10.43.

Hizawa, N. 2016. Clinical approaches towards asthma and chronic obstructive pulmonary disease based on the heterogeneity of disease pathogenesis. *Clin. Exp. Allergy* 46 (5):678–87. doi:10.1111/cea.12731.

Hoang, L. L., Y. P. Nguyen, R. Aspee, S. J. Bolton, Y. H. Shen, L. Wang, N. J. Kenyon, S. Smiley-Jewell, and K. E. Pinkerton. 2016. Temporal and spatial expression of transforming growth factor-β after airway remodeling to tobacco smoke in rats. *Am. J. Respir. Cell Mol. Biol.* 54 (6):872–81. doi:10.1165/rcmb.2015-0119OC.

Hodge-Bell, K. C., K. M. Lee, R. A. Renne, K. M. Gideon, S. J. Harbo, and W. J. McKinney. 2007. Pulmonary inflammation in mice exposed to mainstream cigarette smoke. *Inhal. Toxicol.* 19 (4):361–76. doi:10.1080/08958370601144076.

Hodge, S., G. Matthews, V. Mukaro, J. Ahern, A. Shivam, G. Hodge, M. Holmes, H. Jersmann, and P. N. Reynolds. 2011. Cigarette smoke-induced changes to alveolar macrophage phenotype and function are improved by treatment with procysteine. *Am. J. Respir. Cell Mol. Biol.* 44 (5):673–81. doi:10.1165/rcmb.2009-0459OC.

Hotchkiss, R. S., A. Strasser, J. E. McDunn, and P. E. Swanson. 2009. Cell death. *N. Engl. J. Med.* 361 (16):1570–83. doi:10.1056/NEJMra0901217.

Hou, W., S. Hu, C. Li, H. Ma, Q. Wang, G. Meng, T. Guo, and J. Zhang. 2019. Cigarette smoke induced lung barrier dysfunction, EMT, and tissue remodeling: A possible link between COPD and lung cancer. *Biomed. Res. Int.* 2019:2025636. doi:10.1155/2019/2025636.

Hyde, D. M., N. E. Robinson, J. R. Gillespie, and W. S. Tyler. 1977. Morphometry of the distal air spaces in lungs of aging dogs. *J. Appl. Physiol.* 43 (1):86–91. doi:10.1152/jappl.1977.43.1.86.

Hylkema, M. N., P. J. Sterk, W. I. de Boer, and D. S. Postma. 2007. Tobacco use in relation to COPD and asthma. *Eur. Respir. J.* 29 (3):438–45. doi:10.1183/09031936.00124506.

Ito, K., and P. J. Barnes. 2009. COPD as a disease of accelerated lung aging. *Chest* 135:173–80. doi:10.1378/chest.08-1419.

Ito, H., Y. Yamashita, T. Tanaka, M. Takaki, M. N. Le, L. M. Yoshida, and K. Morimoto. 2020. Cigarette smoke induces endoplasmic reticulum stress and suppresses efferocytosis through the activation of RhoA. *Sci. Rep.* 10 (1):12620. doi:10.1038/s41598-020-69610-x.

Jang, J. H., H. S. Chand, S. Bruse, M. Doyle-Eisele, C. Royer, J. McDonald, C. Qualls, A. J. Klingelhutz, Y. Lin, R. Mallampalli, et al. 2017. Connective tissue growth factor promotes pulmonary epithelial cell senescence and is associated with COPD severity. *COPD* 14 (2):228–37. doi:10.1080/15412555.2016.1262340.

Jiang, J., Y. Xia, Y. Liang, M. Yang, W. Zeng, and X. Zeng. 2018. MiR-190a-5p participates in the regulation of hypoxia-induced pulmonary hypertension by targeting KLF15 and can serve as a biomarker of diagnosis and prognosis in chronic obstructive pulmonary disease complicated with pulmonary hypertension. *Int. J. Chron. Obstruct Pulmon Dis.* 13:3777–90. doi:10.2147/COPD.S182504.

Jimenez-Ruiz, C. A., S. Andreas, K. E. Lewis, P. Tonnesen, C. P. van Schayck, P. Hajek, S. Tonstad, B. Dautzenberg, M. Fletcher, S. Masefield, et al. 2015. Statement on smoking cessation in COPD and other pulmonary diseases and in smokers with comorbidities who find it difficult to quit. *Eur. Respir. J.* 46 (1):61–79. doi:10.1183/09031936.00092614.

Jin, S. W., J. S. Im, J. H. Park, H. G. Kim, G. H. Lee, S. J. Kim, S. J. Kwack, K. B. Kim, K. H. Chung, B. M. Lee, et al. 2021. Effects of tobacco compound 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) on the expression of epigenetically regulated genes in lung carcinogenesis. *J. Toxicol. Environ. Health Part A* 84 (24):1004–19. doi:10.1080/15287394.2021.1965059.

Jo, Y. S., C. K. Rhee, H. K. Yoon, C. K. Park, J. U. Lim, T. J. An, and J. Hur. 2022. Evaluation of asthma–chronic obstructive pulmonary disease overlap using a mouse model of pulmonary disease. *J. Inflamm (Lond)* 19 (1):25. doi:10.1186/s12950-022-00322-x.

Kabir, E. R., and N. Morshed. 2015. Different approaches in the treatment of obstructive pulmonary diseases. *Eur. J. Pharmacol.* 764:306–17. doi:10.1016/j.ejphar.2015.07.030.

Kaku, Y., H. Imaoka, Y. Morimatsu, Y. Komohara, K. Ohnishi, H. Oda, S. Takenaka, M. Matsuoka, T. Kawayama, M. Takeya, et al. 2014. Overexpression of CD163, CD204 and CD206 on alveolar macrophages in the lungs of patients with severe chronic obstructive pulmonary disease. *PLoS. ONE* 9:e87400. doi:10.1371/journal.pone.0087400.

Kamal, R., A. K. Srivastava, C. N. Kesavachandran, V. Bihari, and A. Singh. 2022. Chronic obstructive pulmonary disease (COPD) in women due to indoor biomass burning: A meta analysis. *Int. J. Environ. Health Res.* 32 (6):1403–17. doi:10.1080/09603123.2021.1887460.

Kameyama, N., S. Chubachi, A. E. Hegab, H. Yasuda, S. Kagawa, A. Tsutsumi, K. Fukunaga, M. Shimoda, Y. Kanai, K. Soejima, et al. 2018. Intermittent exposure to cigarette smoke increases lung tumors and the severity of emphysema more than continuous exposure. *Am. J. Respir. Cell Mol. Biol.* 59 (2):179–88. doi:10.1165/rcmb.2017-0375OC.

Kang, M. -J., R. J. Homer, A. Gallo, C. G. Lee, K. A. Crothers, S. J. Cho, C. Rochester, H. Cain, G. Chupp, H. J. Yoon, et al. 2007. IL-18 is induced and IL-18 receptor α plays a critical role in the pathogenesis of cigarette smoke-induced pulmonary emphysema and inflammation. *J. Immunol.* 178 (3):1948–59. doi:10.4049/jimmunol.178.3.1948.

Kasahara, Y., R. M. Tuder, L. Taraseviciene-Stewart, T. D. Le Cras, S. Abman, P. K. Hirth, J. Waltenberger, and N. F. Voelkel. 2000. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J. Clin. Invest.* 106 (11):1311–19. doi:10.1172/JCI10259.

Kaur-Knudsen, D., S. E. Bojesen, A. Tybjaerg-Hansen, and B. G. Nordestgaard. 2011. Nicotinic acetylcholine receptor polymorphism, smoking behavior, and tobacco-related cancer and lung and cardiovascular diseases: A cohort study. *J. Clin. Oncol.* 29 (21):2875–82. doi:10.1200/JCO.2010.32.9870.

Kim, Y. S., G. Hong, D. H. Kim, Y. M. Kim, Y. K. Kim, Y. M. Oh, and Y. K. Jee. 2018. The role of FGF-2 in smoke-induced emphysema and the therapeutic potential of recombinant FGF-2 in patients with COPD. *Exp. Mol. Med.* 50 (11):1–10. doi:10.1038/s12276-018-0178-y.

Kim, H. -Y., H. -S. Lee, I. -H. Kim, Y. K. Kim, M. Ji, S. Oh, D. -Y. Kim, W. Lee, S. -H. Kim, and M. -J. Paik. 2022. Comprehensive targeted metabolomic study in the lung, plasma, and urine of PPE/LPS-induced COPD mice model. *Int J Mol Sci* 23 (5):2748. doi:10.3390/ijms23052748.

King, M., A. Wight, G. T. DeSanctis, J. el-Azab, D. M. Phillips, G. E. Angus, M. G. Cosio, and G. T. De Sanctis. 1989. Mucus hypersecretion and viscoelasticity changes in cigarette-smoking dogs. *Exp. Lung Res.* 15 (3):375–89. doi:10.3109/01902148909087866.

Kodavanti, U. P., D. L. Costa, and P. A. Bromberg. 1998. Rodent models of cardiopulmonary disease: Their potential applicability in studies of air pollutant susceptibility. *Environ. Health Perspect.* 106 Suppl 1 (suppl 1):111–30. doi:10.1289/ehp.98106s1111.

Kodavanti, U. P., M. C. Schladweiler, A. D. Ledbetter, R. V. Ortuno, M. Suffia, P. Evansky, J. H. Richards, R. H. Jaskot, R. Thomas, E. Karoly, et al. 2006. The spontaneously hypertensive rat: An experimental model of sulfur dioxide-induced airways disease. *Toxicol. Sci.* 94 (1):193–205. doi:10.1093/toxsci/kfl087.

Kodavanti, U. P., M. C. Schladweiler, A. D. Ledbetter, W. P. Watkinson, M. J. Campen, D. W. Winsett, J. R. Richards, K. M. Crissman, G. E. Hatch, and D. L. Costa. 2000. The spontaneously hypertensive rat as a model of human cardiovascular disease: Evidence of exacerbated cardiopulmonary injury and oxidative stress from inhaled emission particulate matter. *Toxicol. Appl. Pharmacol.* 164 (3):250–63. doi:10.1006/taap.2000.8899.

Kotoulas, C., I. Panagiotou, P. Tsipas, M. Melachrinou, D. Alexopoulos, and D. Dougenis. 2014. Experimental studies in the bronchial circulation. Which is the ideal animal model? *J Thorac Dis* 6 (10):1506–12. doi:10.3978/j.issn.2072-1439.2014.09.32.

Kratzer, A., J. Salys, C. Nold-Petry, C. Cool, M. Zamora, R. Bowler, A. R. Koczulla, S. Janciauskiene, M. G. Edwards, C. A. Dinarello, et al. 2013. Role of IL-18 in second-hand smoke–Induced Emphysema. *Am. J. Respir. Cell Mol. Biol.* 48 (6):725–32. doi:10.1165/rcmb.2012-0173OC.

Langhammer, A., R. Johnsen, A. Gulsvik, T. L. Holmen, and L. Bjermer. 2003. Sex differences in lung vulnerability to tobacco smoking. *Eur. Respir. J.* 21 (6):1017–23. doi:10.1183/09031936.03.00053202.

Laniado-Laborin, R. 2009. Smoking and chronic obstructive pulmonary disease (COPD). Parallel epidemics of the 21 century. *Int. J. Environ. Res. Public Health* 6 (1):209–24. doi:10.3390/ijerph6010209.

Lee, J. H., D. S. Lee, E. K. Kim, K. H. Choe, Y. M. Oh, T. S. Shim, S. E. Kim, Y. S. Lee, and S. D. Lee. 2005. Simvastatin inhibits cigarette smoking–induced Emphysema and pulmonary hypertension in rat lungs. *Am. J. Respir. Crit. Care Med.* 172 (8):987–93. doi:10.1164/rccm.200501-041OC.

Leuenberger, C., C. Schuoler, H. Bye, C. Mignan, T. Rechsteiner, S. Hillinger, I. Opitz, B. Marsland, A. Faiz, P. S. Hiemstra, et al. 2016. MicroRNA-223 controls the

expression of histone deacetylase 2: A novel axis in COPD. *J. Mol. Med.* 94 (6):725–34. doi:10.1007/s00109-016-1388-1.

Liao, W., J. Dong, H. Y. Peh, L. H. Tan, K. S. Lim, L. Li, and W. -S.F. Wong. 2017. Oligonucleotide therapy for obstructive and restrictive respiratory diseases. *Molecules* 22:139. doi:10.3390/molecules22010139.

Lindberg, A., A. C. Jonsson, E. Ronmark, R. Lundgren, L. G. Larsson, and B. Lundback. 2005a. Prevalence of chronic obstructive pulmonary disease according to BTS, ERS, GOLD and ATS criteria in relation to doctor's diagnosis, symptoms, age, gender, and smoking habits. *Respiration* 72 (5):471–79. doi:10.1159/000087670.

Lindberg, A., A. C. Jonsson, E. Ronmark, R. Lundgren, L. G. Larsson, and B. Lundback. 2005b. Ten-year cumulative incidence of COPD and risk factors for incident disease in a symptomatic cohort. *Chest* 127 (5):1544–52. doi:10.1378/chest.127.5.1544.

Liu, Y., W. B. Liang, L. B. Gao, X. M. Pan, T. Y. Chen, Y. Y. Wang, H. Xue, L. S. Zhang, and L. Zhang. 2010. CTLA4 and CD86 gene polymorphisms and susceptibility to chronic obstructive pulmonary disease. *Human Immunol.* 71 (11):1141–46. doi:10.1016/j.humimm.2010.08.007.

Liu, Y., H. Liu, C. Li, C. Ma, and W. Ge. 2020. Proteome profiling of lung tissues in chronic obstructive pulmonary disease (COPD): Platelet and macrophage dysfunction contribute to the pathogenesis of COPD. *Int J Chron Obstruct Pulmon Dis.* 15:973–80. doi:10.2147/COPD.S246845.

Lofdahl, J. M., K. Cederlund, L. Nathell, A. Eklund, and C. M. Skold. 2005. Bronchoalveolar lavage in COPD: Fluid recovery correlates with the degree of emphysema. *Eur. Respir. J.* 25 (2):275–81. doi:10.1183/09031936.05.00033504.

Lugade, A. A., P. N. Bogner, T. H. Thatcher, P. J. Sime, R. P. Phipps, and Y. Thanavala. 2014. Cigarette smoke exposure exacerbates lung inflammation and compromises immunity to bacterial infection. *J. Immunol.* 192 (11):5226–35. doi:10.4049/jimmunol.1302584.

Mabley, J., S. Gordon, and P. Pacher. 2011. Nicotine exerts an anti-inflammatory effect in a murine model of acute lung injury. *Inflammation* 34 (4):231–37. doi:10.1007/s10753-010-9228-x.

Machida, H., S. Inoue, A. Igarashi, S. Saitoh, K. Yamauchi, M. Nishiwaki, T. Nemoto, Y. Otaki, M. Sato, K. Sato, et al. 2022. Role of CC chemokine ligand 17 in mouse models of chronic obstructive pulmonary disease. *Am. J. Respir. Cell Mol. Biol.* 66 (4):428–38. doi:10.1165/rcmb.2021-0069OC.

Maeno, T., A. M. Houghton, P. A. Quintero, S. Grumelli, C. A. Owen, and S. D. Shapiro. 2007. CD8+ T cells are required for inflammation and destruction in cigarette smoke-induced emphysema in mice. *J. Immunol.* 178 (12):8090. doi:10.4049/jimmunol.178.12.8090.

Maiellaro, K., and W. R. Taylor. 2007. The role of the adventitia in vascular inflammation. *Cardiovasc. Res.* 75 (4):640–48. doi:10.1016/j.cardiores.2007.06.023.

Mall, M. A. 2016. Unplugging mucus in cystic fibrosis and chronic obstructive pulmonary disease. *Ann. Am. Thorac. Soc.* 13 (Suppl 2):S177–85. doi:10.1513/AnnalsATS.201509-641KV.

Mano, Y., M. Tsukamoto, K. Y. Wang, T. Nabeshima, K. Kosugi, T. Tajima, Y. Yamanaka, H. Suzuki, M. Kawasaki, E. Nakamura, et al. 2022. Oxidative stress causes muscle structural alterations via p38 MAPK signaling in COPD mouse model. *J. Bone Miner. Metab.* 40 (6):927–39. doi:10.1007/s00774-022-01371-1.

Maslarova, A., M. Alam, C. Reiffurth, E. Lapilover, A. Gorji, and J. P. Dreier. 2011. Chronically epileptic human and rat neocortex display a similar resistance against spreading depolarization *in vitro*. *Stroke* 42 (10):2917–22. doi:10.1161/STROKEAHA.111.621581.

Mauderly, J. L. 2000. Animal models for the effect of age on susceptibility to inhaled particulate matter. *Inhal Toxicol.* 12 (9):863–900. doi:10.1080/08958370050123216.

Meshi, B., T. Z. Vitalis, D. Ionescu, W. M. Elliott, C. Y. Liu, X. -D. Wang, S. Hayashi, and J. C. Hogg. 2002. Emphysematous lung destruction by cigarette smoke. *Am. J. Respir. Cell Mol. Biol.* 26 (1):52–57. doi:10.1165/ajrcmb.26.1.4253.

Miller, M., A. Pham, J. Y. Cho, P. Rosenthal, and D. H. Broide. 2010. Adiponectin-deficient mice are protected against tobacco-induced inflammation and increased emphysema. *Am. J. Physiol. Lung Cell Mol. Physiol.* 299 (6):L834–42. doi:10.1152/ajplung.00326.2009.

Miller, L. A., C. M. Royer, K. E. Pinkerton, and E. S. Schelegle. 2017. Nonhuman primate models of respiratory disease: Past, present, and future. *Ilsr J.* 58 (2):269–80. doi:10.1093/ilar/ilx030.

Mitzner, W., W. Lee, D. Georgakopoulos, and E. Wagner. 2000. Angiogenesis in the mouse lung. *Am. J. Pathol.* 157 (1):93–101. doi:10.1016/S0002-9440(10)64521-X.

Mossman, B. T., K. M. Lounsbury, and S. P. Reddy. 2006. Oxidants and signaling by mitogen-activated protein kinases in lung epithelium. *Am. J. Respir. Cell Mol. Biol.* 34 (6):666–69. doi:10.1165/rcmb.2006-0047SF.

Mullen, J. B., J. L. Wright, B. R. Wiggs, P. D. Pare, and J. C. Hogg. 1987. Structure of central airways in current smokers and ex-smokers with and without mucus hypersecretion: Relationship to lung function. *Thorax* 42 (11):843–48. doi:10.1136/thx.42.11.843.

Nadadur, S. S., K. E. Pinkerton, and U. P. Kodavanti. 2002. Pulmonary gene expression profiles of spontaneously hypertensive rats exposed to environmental tobacco smoke. *Chest* 121 (3 Suppl):83s–4s. doi:10.1378/chest.121.3_suppl.83S.

Nadziejko, C., K. Fang, A. Bravo, and T. Gordon. 2007. Susceptibility to pulmonary hypertension in inbred strains of mice exposed to cigarette smoke. *J. Appl. Physiol.* 102 (5):1780–85. doi:10.1152/japplphysiol.01076.2005.

Nakanishi, Y., D. Kobayashi, Y. Asano, T. Sakurai, M. Kashimura, S. Okuyama, Y. Yoneda, S. D. Shapiro, and K. Takayama. 2009. Clarithromycin prevents smoke-induced emphysema in mice. *Am. J. Respir. Crit.*

Care Med. 179 (4):271–78. doi:10.1164/rccm.200806-905OC.

Ng-Blichfeldt, J. P., R. Gosens, C. Dean, M. Griffiths, and M. Hind. 2019. Regenerative pharmacology for COPD: Breathing new life into old lungs. *Thorax* 74 (9):890–97. doi:10.1136/thoraxjnl-2018-212630.

O'Farrell, H. E., R. Brown, Z. Brown, B. Milijevic, Z. D. Ristovski, R. V. Bowman, K. M. Fong, A. Vaughan, and I. A. Yang. 2021. E-cigarettes induce toxicity comparable to tobacco cigarettes in airway epithelium from patients with COPD. *Toxicol in Vitro* 75:105204. doi:10.1016/j.tiv.2021.105204.

Oldham, M. J., C. R. Coggins, and W. J. McKinney Jr. 2013. A comprehensive evaluation of selected components and processes used in the manufacture of cigarettes: Approach and overview. *Inhal Toxicol.* 25 Suppl 2 (sup2):1–5. doi:10.3109/08958378.2013.854429.

Onor, I. O., D. L. Stirling, S. R. Williams, D. Bediako, A. Borghol, M. B. Harris, T. B. Darensburg, S. D. Clay, S. C. Okpechi, and D. F. Sarpong. 2017. Clinical effects of cigarette smoking: Epidemiologic impact and review of pharmacotherapy options. *Int. J. Environ. Res. Public Health* 14 (10):1147. doi:10.3390/ijerph14101147.

Overbeek, S. A., S. Braber, P. J. Koelink, P. A. Henricks, E. Mortaz, A. T. LoTam Loi, P. L. Jackson, J. Garssen, G. T. Wagenaar, W. Timens, et al. 2013. Cigarette smoke-induced collagen destruction; Key to chronic neutrophilic airway inflammation? *PLoS. ONE* 8 (1):e55612. doi:10.1371/journal.pone.0055612.

Padilla-Carlin, D. J., D. N. McMurray, and A. J. Hickey. 2008. The guinea pig as a model of infectious diseases. *Comp. Med.* 58 (4):324–40.

Park, S. S., Y. Kikkawa, I. P. Goldring, M. M. Daly, M. Zelefsky, C. Shim, M. Spierer, and T. Morita. 1977. An animal model of cigarette smoking in beagle dogs: Correlative evaluation of effects on pulmonary function, defense, and morphology. *Am. Rev. Respir. Dis.* 115 (6):971–79. doi:10.1164/arrd.1977.115.6.971.

Pelgrim, C. E., L. Wang, L. N. Peralta Marzal, S. Korver, I. van Ark, T. Leusink-Muis, S. Braber, G. Folkerts, J. Garssen, A. van Helvoort, et al. 2022. Increased exploration and hyperlocomotion in a cigarette smoke and LPS-induced murine model of COPD: Linking pulmonary and systemic inflammation with the brain. *Am. J. Physiol. Lung Cell Mol. Physiol.* 323 (3):L251–65. doi:10.1152/ajplung.00485.2021.

Perez-Rial, S., A. Giron-Martinez, and G. Peces-Barba. 2015. Modelos animales de enfermedad pulmonar obstructiva crónica. *Archivos de Bronconeumología* 51 (3):121–27. doi:10.1016/j.arbres.2014.06.016.

Pham, A. K., C. W. Wu, X. Qiu, J. Xu, S. Smiley-Jewell, D. Uyeminami, P. Upadhyay, D. Zhao, and K. E. Pinkerton. 2020. Differential lung inflammation and injury with tobacco smoke exposure in Wistar Kyoto and spontaneously hypertensive rats. *Inhal Toxicol.* 32 (8):328–41. doi:10.1080/08958378.2020.1805052.

Pinkerton, K. E., M. Harbaugh, M. K. Han, C. Jourdan Le Saux, L. S. Van Winkle, W. J. Martin 2nd, R. J. Kosgei,

E. J. Carter, N. Sitkin, S. M. Smiley-Jewell, et al. 2015. Women and lung disease. Sex differences and global health disparities. *Am. J. Respir. Crit. Care Med.* 192 (1):11–16. doi:10.1164/rccm.201409-1740PP.

Pinkerton, K. E., and M. E. Poindexter. 2018. Harmful interruptions: Impact of smoking patterns on tumorigenesis and emphysema. *Am. J. Respir. Cell Mol. Biol.* 59 (2):133–34. doi:10.1165/rcmb.2018-0151ED.

Plopper, C. G., and D. M. Hyde. 2008. The non-human primate as a model for studying COPD and asthma. *Pulm Pharmacol Ther* 21 (5):755–66. doi:10.1016/j.pupt.2008.01.008.

Polverino, F., B. R. Celli, and C. A. Owen. 2018. COPD as an endothelial disorder: Endothelial injury linking lesions in the lungs and other organs? (2017 Grover Conference Series). Pulm Circ, Lost Valley Conference Center, Sedalia, CO, USA. Vol. 8, 2045894018758528.

Polverino, F., M. Doyle-Eisele, J. McDonald, J. A. Wilder, C. Royer, M. Laucho-Contreras, E. M. Kelly, M. Divo, V. Pinto-Plata, J. Mauderly, et al. 2015. A novel nonhuman primate model of cigarette smoke–induced airway disease. *Am. J. Pathol.* 185 (3):741–55. doi:10.1016/j.ajpath.2014.11.006.

Postma, D. S., K. F. Rabe, and J. M. Drazen. 2015. The Asthma–COPD overlap syndrome. *N. Engl. J. Med.* 373 (13):1241–49. doi:10.1056/NEJMra1411863.

Qiu, S. L., Q. X. Sun, J. P. Zhou, H. J. Tang, Y. Q. Chen, F. S. Chen, T. Feng, Z. Q. He, H. J. Qin, and M. C. Duan. 2022. IL-27 mediates anti-inflammatory effect in cigarette smoke induced emphysema by negatively regulating IFN-γ producing cytotoxic CD8 + T cells in mice. *Eur. J. Immunol.* 52 (2):222–36. doi:10.1002/eji.202049076.

Rab, A., S. M. Rowe, S. V. Raju, Z. Bebok, S. Matalon, and J. F. Collawn. 2013. Cigarette smoke and CFTR: Implications in the pathogenesis of COPD. *Am. J. Physiol. Lung Cell Mol. Physiol.* 305 (8):L530–41. doi:10.1152/ajplung.00039.2013.

Raju, S. V., H. Kim, S. A. Byzek, L. P. Tang, J. E. Trombley, P. Jackson, L. Rasmussen, J. M. Wells, E. F. Libby, E. Dohm, et al. 2016. A ferret model of COPD-related chronic bronchitis. *JCI Insight* 1 (15):e87536. doi:10.1172/jci.insight.87536.

Raju, S. V., V. Y. Lin, L. Liu, C. M. McNicholas, S. Karki, P. A. Sloane, L. Tang, P. L. Jackson, W. Wang, L. Wilson, et al. 2016. The cystic fibrosis transmembrane conductance regulator potentiator ivacaftor augments mucociliary clearance abrogating cystic fibrosis transmembrane conductance regulator inhibition by cigarette smoke. *Am. J. Respir. Cell Mol. Biol.* 56 (1):99–108. doi:10.1165/rcmb.2016-0226OC.

Raju, S. V., J. H. Tate, S. K. Peacock, P. Fang, R. A. Oster, M. T. Dransfield, and S. M. Rowe. 2014. Impact of heterozygote CFTR mutations in COPD patients with chronic bronchitis. *Respir. Res.* 15 (1):18. doi:10.1186/1465-9921-15-18.

Ramirez-Venegas, A., C. A. Torres-Duque, N. E. Guzman-Bouilloud, M. Gonzalez-Garcia, and R. H. Sansores. 2019.

Small airway disease in COPD associated to biomass exposure. *Rev. Invest. Clin.* 71 (1):70–78. doi:10.24875/RIC.18002652.

Rangasamy, T., C. Y. Cho, R. K. Thimmulappa, L. Zhen, S. S. Srisuma, T. W. Kensler, M. Yamamoto, I. Petrache, R. M. Tuder, and S. Biswal. 2004. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke–induced emphysema in mice. *J. Clin. Invest.* 114 (9):1248–59. doi:10.1172/JCI200421146.

Reece, W. O., and R. A. Ball. 1972. Inhaled cigarette smoke and treadmill-exercised dogs. *Arch. Environ. Health* 24 (4):262–70. doi:10.1080/00039896.1972.10666081.

Roehrs, J. D., W. R. Rogers, and W. G. Johanson. 1981. Bronchial reactivity to inhaled methacholine in cigarette-smoking baboons. *J, Appl Physiol. Respir. Environ. Exerc. Physiol.* 50 (4):754–60. doi:10.1152/jappl.1981.50.4.754.

Saha, S., and C. E. Brightling. 2006. Eosinophilic airway inflammation in COPD. *Int J Chron Obstruct Pulmon Dis* 1 (1):39–47. doi:10.2147/copd.2006.1.1.39.

Salvi, S., and P. J. Barnes. 2010. Is exposure to biomass smoke the biggest risk factor for COPD globally? *Chest* 138:3–6. doi:10.1378/chest.10-0645.

Sana, A., S. M. A. Somda, N. Meda, and C. Bouland. 2018. Chronic obstructive pulmonary disease associated with biomass fuel use in women: A systematic review and meta-analysis. *BMJ Open Respir. Res.* 5 (1):e000246. doi:10.1136/bmjresp-2017-000246.

Schikowski, T., I. C. Mills, H. R. Anderson, A. Cohen, A. Hansell, F. Kauffmann, U. Krämer, A. Marcon, L. Perez, J. Sunyer, et al. 2014. Ambient air pollution: A cause of COPD? *Eur. Respir. J.* 43 (1):250–63. doi:10.1183/09031936.00100112.

Schuliga, M. 2015. NF-kappaB signaling in chronic inflammatory airway disease. *Biomolecules* 5 (3):1266–83. doi:10.3390/biom5031266.

Selman, M., M. Montano, C. Ramos, B. Vanda, C. Becerril, J. Delgado, R. Sansores, R. Barrios, and A. Pardo. 1996. Tobacco smoke-induced lung emphysema in guinea pigs is associated with increased interstitial collagenase. *Am. J. Physiol.* 271 (5):L734–43. doi:10.1152/ajplung.1996.271.5.L734.

Shan, M., X. Yuan, L. -Z. Song, L. Roberts, N. Zarinkamar, A. Seryshev, Y. Zhang, S. Hilsenbeck, S. H. Chang, C. Dong, et al. 2012. Cigarette smoke induction of osteopontin (SPP1) mediates T H 17 Inflammation in human and experimental emphysema. *Sci Transl Med* 4 (117):117ra9. doi:10.1126/scitranslmed.3003041.

Shapiro, S. D., N. M. Goldstein, A. M. Houghton, D. K. Kobayashi, D. Kelley, and A. Belaaouaj. 2003. Neutrophil elastase contributes to cigarette smoke-induced emphysema in mice. *Am. J. Pathol.* 163 (6):2329–35. doi:10.1016/S0002-9440(10)63589-4.

Shaykhiev, R. 2019. Emerging biology of persistent mucous cell hyperplasia in COPD. *Thorax* 74 (1):4–6. doi:10.1136/thoraxjnl-2018-212271.

Shen, Y. H., A. K. Pham, B. Davis, S. Smiley-Jewell, L. Wang, U. P. Kodavanti, M. Takeuchi, D. J. Tancredi, and K. E. Pinkerton. 2016. Sex and strain-based inflammatory response to repeated tobacco smoke exposure in spontaneously hypertensive and Wistar Kyoto rats. *Inhal Toxicol* 28 (14):677–85. doi:10.1080/08958378.2016.1249812.

Shore, S., L. Kobzik, N. C. Long, W. Skornik, C. J. Van Staden, L. Boulet, I. W. Rodger, and D. J. Pon. 1995. Increased airway responsiveness to inhaled methacholine in a rat model of chronic bronchitis. *Am. J. Respir. Crit. Care Med.* 151 (6):1931–38. doi:10.1164/ajrccm.151.6.7767542.

Shu, J., D. Li, H. Ouyang, J. Huang, Z. Long, Z. Liang, Y. Chen, Y. Chen, Q. Zheng, M. Kuang, et al. 2017. Comparison and evaluation of two different methods to establish the cigarette smoke exposure mouse model of COPD. *Sci. Rep.* 7 (1):15454. doi:10.1038/s41598-017-15685-y.

Silverman, E. K. 2020. Genetics of COPD. *Annu. Rev. Physiol.* 82 (1):413–31. doi:10.1146/annurev-physiol-021317-121224.

Sin, D. D., and S. F. Man. 2005. Chronic obstructive pulmonary disease as a risk factor for cardiovascular morbidity and mortality. *Proc. Am. Thorac Soc.* 2 (1):8–11. doi:10.1513/pats.200404-032MS.

Smith, K. R., K. E. Pinkerton, T. Watanabe, T. L. Pedersen, S. J. Ma, and B. D. Hammock. 2005. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* 102 (6):2186–91. doi:10.1073/pnas.0409591102.

Snoderly, H. T., H. Alkhadrawi, D. M. Panchal, K. L. Weaver, J. N. Vito, K. A. Freshwater, S. P. Santiago, I. M. Olfert, T. R. Nurkiewicz, and M. F. Bennewitz. 2023. Short-term exposure of female BALB/cJ mice to e-cigarette aerosol promotes neutrophil recruitment and enhances neutrophil-platelet aggregation in pulmonary microvasculature. *J. Toxicol. Environ. Health Part A* 86 (8):246–62. doi:10.1080/15287394.2023.2184738.

Solomon, G. M., S. V. Raju, M. T. Dransfield, and S. M. Rowe. 2016. Therapeutic approaches to acquired cystic fibrosis transmembrane conductance regulator dysfunction in chronic bronchitis. *Ann. Am. Thorac. Soc.* 13 (Suppl 2):S169–76. doi:10.1513/AnnalsATS.201509-601KV.

Song, Q., P. Chen, and X. M. Liu. 2021. The role of cigarette smoke-induced pulmonary vascular endothelial cell apoptosis in COPD. *Respir. Res.* 22 (1):39. doi:10.1186/s12931-021-01630-1.

Sorroche, P. B., M. Fernandez Acquier, O. Lopez Jove, E. Giugno, S. Pace, B. Livellara, S. Legal, J. Oyhamburu, and M. S. Saez. 2015. Déficit de alfa 1 antitripsina en pacientes con EPOC: estudio de corte transversal. *Archivos de Bronconeumología* 51 (11):539–43. doi:10.1016/j.arbres.2015.01.008.

Stabile, L. P., and J. M. Siegfried. 2003. Sex and gender differences in lung cancer. *J. Gend. Specif Med.* 6:37–48.

Suki, B., S. Sato, H. Parameswaran, M. V. Szabari, A. Takahashi, and E. Bartolak-Suki. 2013. Emphysema and mechanical stress-induced lung remodeling. *Physiol. (Bethesda)* 28:404–13. doi:10.1152/physiol.00041.2013.

Suzuki, M., T. Betsuyaku, Y. Ito, K. Nagai, N. Odajima, C. Moriyama, Y. Nasuhara, and M. Nishimura. 2009. Curcumin attenuates elastase- and cigarette smoke-induced pulmonary emphysema in mice. *Am. J. Physiol. Lung Cell Mol. Physiol.* 296:L614–23. doi:10.1152/ajplung.90443.2008.

Takahashi, K., S. Yokota, N. Tatsumi, T. Fukami, T. Yokoi, and M. Nakajima. 2013. Cigarette smoking substantially alters plasma microRNA profiles in healthy subjects. *Toxicol. Appl. Pharmacol.* 272 (1):154–60. doi:10.1016/j.taap.2013.05.018.

Tamimi, A., D. Serdarevic, and N. A. Hanania. 2012. The effects of cigarette smoke on airway inflammation in asthma and COPD: Therapeutic implications. *Respir. Med.* 106 (3):319–28. doi:10.1016/j.rmed.2011.11.003.

Tanner, L., and A. B. Single. 2020. Animal models reflecting chronic obstructive pulmonary disease and related respiratory disorders: Translating pre-clinical data into clinical relevance. *J. Innate. Immun.* 12 (3):203–25. doi:10.1159/000502489.

Tashkin, D. P. 2015. Smoking cessation in chronic obstructive pulmonary disease. *Semin Respir. Crit Care Med.* 36 (04):491–507. doi:10.1055/s-0035-1555610.

Thomas, R. D., and T. J. Vigerstad. 1989. Use of laboratory animal models in investigating emphysema and cigarette smoking in humans. *Regul. Toxicol. Pharmacol.* 10 (3):264–71. doi:10.1016/0273-2300(89)90053-6.

Turino, G. M., S. Ma, Y. Y. Lin, and J. O. Cantor. 2018. The therapeutic potential of hyaluronan in COPD. *Chest* 153 (4):792–98. doi:10.1016/j.chest.2017.12.016.

Vandamme, T. F. 2014. Use of rodents as models of human diseases. *J. Pharm. Bioallied Sci.* 6 (1):2–9. doi:10.4103/0975-7406.124301.

Vesely, D. L., and G. S. Levey. 1978. Nicotine decreases guanylate cyclase activity. *Bull Environ. Contam Toxicol.* 20 (1):31–34. doi:10.1007/BF01683482.

Viegi, G., A. Scognamiglio, S. Baldacci, F. Pistelli, and L. Carrozzi. 2001. Epidemiology of chronic obstructive pulmonary disease (COPD). *Respiration* 68 (1):4–19. doi:10.1159/000050456.

Vlaykova, T., and D. Dimov. 2014. Polymorphisms of matrix metalloproteinases (MMP) in COPD. *Biotechnol. Biotechnol. Equip.* 26 (Supp1):111–19. doi:10.5504/50YRTIMB.2011.0021.

Vucic, E. A., R. Chari, K. L. Thu, I. M. Wilson, A. M. Cotton, J. Y. Kennett, M. Zhang, K. M. Lonergan, K. Steiling, C. J. Brown, et al. 2014. DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. *Am. J. Respir. Cell Mol. Biol.* 50 (5):912–22. doi:10.1165/rcmb.2013-0304OC.

Wallis, T. W., W. R. Rogers, and W. G. Johanson. 1982. Effects of acute and chronic exposure to nicotine aerosol on bronchial reactivity to inhaled methacholine. *J. Appl Physiol. Respir. Environ. Exerc. Physiol.* 52 (4):1071–76. doi:10.1152/jappl.1982.52.4.1071.

Wang, Z., S. J. Liu, J. Ma, G. B. Qu, X. Y. Wang, S. J. Yu, J. Y. He, J. F. Liu, T. Xia, and G. B. Jiang. 2013. Silver nanoparticles induced RNA polymerase-silver binding and RNA transcription inhibition in erythroid progenitor cells. *ACS Nano* 7 (5):4171–86. doi:10.1021/nn400594s.

Wanner, A., J. A. Hirsch, D. E. Greeneltch, E. W. Swenson, and T. Fore. 1973. Tracheal mucous velocity in beagles after chronic exposure to cigarette smoke. *Arch. Environ. Health* 27 (6):370–71. doi:10.1080/00039896.1973.10666405.

Whittemore, A. S., S. A. Perlin, and Y. DiCiccio. 1995. Chronic obstructive pulmonary disease in lifelong nonsmokers: Results from NHANES. *Am. J. Public Health* 85 (5):702–06. doi:10.2105/AJPH.85.5.702.

WHO. 2014. Global status report on noncommunicable diseases. *World Health Organization.*

Wright, J. L., and A. Churg. 1990. Cigarette smoke causes physiologic and morphologic changes of emphysema in the guinea pig. *Am. Rev. Respir. Dis.* 142 (6_pt_1):1422–28. doi:10.1164/ajrccm/142.6_Pt_1.1422.

Wright, J. L., and A. Churg. 2002. Animal models of cigarette smoke-induced COPD. *Chest* 122 (6 Suppl):301s–6s. doi:10.1378/chest.122.6_suppl.301S.

Wright, J. L., and A. Churg. 2008a. Animal models of COPD: Barriers, successes, and challenges. *Pulm Pharmacol Ther* 21 (5):696–98. doi:10.1016/j.pupt.2008.01.007.

Wright, J. L., and A. Churg. 2008b. Short-term exposure to cigarette smoke induces endothelial dysfunction in small intrapulmonary arteries: Analysis using guinea pig precision cut lung slices. *J. Appl. Physiol.* 104 (5):1462–69. doi:10.1152/japplphysiol.00520.2007.

Wright, J. L., M. Cosio, and A. Churg. 2008. Animal models of chronic obstructive pulmonary disease. *Am. J. Physiol. Lung Cell Mol. Physiol.* 295 (1):L1–15. doi:10.1152/ajplung.90200.2008.

Wright, J. L., S. G. Farmer, and A. Churg. 2002. Synthetic serine elastase inhibitor reduces cigarette smoke–induced emphysema in guinea pigs. *Am. J. Respir. Crit. Care Med.* 166 (7):954–60. doi:10.1164/rccm.200202-098OC.

Wright, J. L., T. Ngai, and A. Churg. 1992. Effect of long-term exposure to cigarette smoke on the small airways of the guinea pig. *Exp. Lung Res.* 18 (1):105–14. doi:10.3109/01902149209020654.

Wright, J. L., and J. P. Sun. 1994. Effect of smoking cessation on pulmonary and cardiovascular function and structure: Analysis of guinea pig model. *J. Appl. Physiol.* 76 (5):2163–68. doi:10.1152/jappl.1994.76.5.2163.

Wright, J. L., S. Zhou, O. Preobrazhenska, C. Marshall, D. D. Sin, I. Laher, S. Golbidi, and A. M. Churg. 2011. Statin reverses smoke-induced pulmonary hypertension and prevents emphysema but not airway remodeling. *Am. J. Respir. Crit. Care Med.* 183 (1):50–58. doi:10.1164/rccm.201003-0399OC.

Wu, J., and D. D. Sin. 2011. Improved patient outcome with smoking cessation: When is it too late? *Int J Chron Obstruct Pulmon Dis* 6:259–67. doi:10.2147/COPD.S10771.

Wu, D. D., J. Song, S. Bartel, S. Krauss-Etschmann, M. G. Rots, and M. N. Hylkema. 2018. The potential for targeted rewriting of epigenetic marks in COPD as a new therapeutic approach. *Pharmacol. Ther.* 182:1–14. doi:10.1016/j.pharmthera.2017.08.007.

Wu, C. -W., T. Yau, C. C. Fulgar, S. M. Mack, A. M. Revilla, N. J. Kenyon, and K. E. Pinkerton. 2020. Long-term sequelae of smoking and cessation in spontaneously hypertensive rats. *Toxicol Pathol* 48 (3):422–36. doi:10.1177/0192623319893312.

Xi, S., H. Xu, J. Shan, Y. Tao, J. A. Hong, S. Inchauste, M. Zhang, T. F. Kunst, L. Mercedes, and D. S. Schrump. 2013. Cigarette smoke mediates epigenetic repression of miR-487b during pulmonary carcinogenesis. *J. Clin. Invest.* 123:1241–61. doi:10.1172/JCI61271.

Yang, H. J., W. H. Tsou, M. C. Shen, C. Y. Liu, H. M. Saunders, K. Y. Wang, and F. L. Douglas. 2022. The effects of hydrogen treatment in a cigarette smoke solution-induced chronic obstructive pulmonary disease-like changes in an animal model. *J Thorac Dis* 14 (11):4246–55. doi:10.21037/jtd-22-324.

Yu, B., U. P. Kodavanti, M. Takeuchi, H. Witschi, and K. E. Pinkerton. 2008. Acute tobacco smoke-induced airways inflammation in spontaneously hypertensive rats. *Inhal Toxicol* 20 (7):623–33. doi:10.1080/08958370701861538.

Yu, X. F., J. Wang, O. UYang, S. G. N, H. Sun, J. Tong, T. Chen, and J. Li. 2019. The role of miR-130a-3p and SPOCK1 in tobacco exposed bronchial epithelial BEAS-2B transformed cells: Comparison to A549 and H1299 lung cancer cell lines. *J. Toxicol. Environ. Health Part A* 82 (15):862–69. doi:10.1080/15287394.2019.1664479.

Zeki, A. A., M. Schivo, A. Chan, T. E. Albertson, and S. Louie. 2011. The asthma-COPD overlap syndrome: A common clinical problem in the elderly. *J Allergy (Cairo)* 2011:861926. doi:10.1155/2011/861926.

Zeng, H., T. Li, X. He, S. Cai, H. Luo, P. Chen, and Y. Chen. 2020. Oxidative stress mediates the apoptosis and epigenetic modification of the Bcl-2 promoter via DNMT1 in a cigarette smoke-induced emphysema model. *Respir. Res.* 21 (1):229. doi:10.1186/s12931-020-01495-w.

Zhang, L., H. Valizadeh, I. Alipourfard, R. Bidares, L. Aghebati-Maleki, and M. Ahmadi. 2020. Epigenetic modifications and therapy in chronic obstructive pulmonary disease (COPD): An update review. *COPD* 17 (3):333–42. doi:10.1080/15412555.2020.1780576.

Zhong, C. Y., Y. M. Zhou, G. C. Douglas, H. Witschi, and K. E. Pinkerton. 2005. MAPK/AP-1 signal pathway in tobacco smoke-induced cell proliferation and squamous metaplasia in the lungs of rats. *Carcinogenesis* 26 (12):2187–95. doi:10.1093/carcin/bgi189.

Zhong, C. Y., Y. M. Zhou, and K. E. Pinkerton. 2008. NF-κB inhibition is involved in tobacco smoke-induced apoptosis in the lungs of rats. *Toxicol. Appl. Pharmacol.* 230 (2):150–58. doi:10.1016/j.taap.2008.02.005.

Zhou, X., and E. D. Frohlich. 2007. Analogy of cardiac and renal complications in essential hypertension and aged SHR or L-NAME/SHR. *Med Chem* 3 (1):61–65. doi:10.2174/157340607779317634.

Zhou, J. S., Z. Y. Li, X. C. Xu, Y. Zhao, Y. Wang, H. P. Chen, M. Zhang, Y. F. Wu, T. W. Lai, C. H. Di, et al. 2020. Cigarette smoke-initiated autoimmunity facilitates sensitisation to elastin-induced COPD-like pathologies in mice. *Eur. Respir. J.* 56 (3):2000404. doi:10.1183/13993003.00404-2020.

Zwicker, G. M., R. E. Filipy, J. F. Park, S. M. Loscutoff, H. A. Ragan, and D. L. Stevens. 1978. Clinical and pathological effects of cigarette smoke exposure in beagle dogs. *Arch. Pathol. Lab. Med.* 102 (12):623–28.