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Molecular changes underlying pulmonary emphysema and chronic bronchitis in Chronic Obstructive Pulmonary Disease: an updated review.

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Summary

The aim of this review is to update and synthesize the molecular mechanisms that lead to the heterogeneous effect on tissue remodeling observed in the two most important clinical phenotypes of chronic obstructive pulmonary disease (COPD), pulmonary emphysema (PE) and chronic bronchitis (CB). Clinical and experimental evidence suggests that this heterogeneous response to promote PE, CB, or both, is related to differentiated genetic, epigenetic, and molecular conditions. Specifically, a tendency toward PE could be related to a variant in the *DSP* gene, SIRT1 downregulation, macrophage polarization to M1, as well as the involvement of the noncanonical Wnt5A signaling pathway, among other alterations. Additionally, in advanced stages of COPD, PE development is potentiated by dysregulations in autophagy, which promotes senescence and subsequently cell apoptosis, through exacerbated inflammasome activation and release of caspases. On the other hand, CB or the pro-fibrotic phenotype could be potentiated by the downregulated activity of HDAC2, the activation of the TGF-β/Smad or Wnt/β-catenin signaling pathways, macrophage polarization to M2, upregulation of TIMP-1, and/or the presence of the epithelial-mesenchymal transition (EMT) mechanism. Interestingly, the upregulated activity of MMPs, especially MMP-9, is widely involved in the development of both phenotypes. Furthermore, MMP-9 and MMP-12 enhance the severity, perpetuation, and exacerbation of COPD, as well as the development of autoimmunity in this disease.

Abbreviations

Chronic Obstructive Pulmonary Disease (COPD), Pulmonary emphysema (PE), Chronic bronchitis (CB), Matrix metalloproteinase (MMP), Acute exacerbations of COPD (AE-COPD).

Introduction

The 2023 report of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) redefines COPD as a heterogeneous lung condition characterized by chronic respiratory symptoms due to abnormalities of the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema), which cause persistent, often progressive, airflow obstruction (GOLD, 2023).

Chronic obstructive pulmonary disease (COPD) etiology is multifactorial, tobacco smoke being the major risk factor in countries with a higher sociodemographic index (SDI), while environmental (including ambient particulate matter, biomass fuel, and ozone), as well as occupational exposures, are the main contributors in countries with lower SDIs (GBD, 2017; Li et al., 2017). Additionally, the use of agricultural pesticides, low birth weight, lower respiratory tract infections in childhood, as well as autoimmunity and genetic predisposition are factors that have been associated with COPD (Bagdonas et al., 2015; Antuni and Barnes, 2016; Hochberg and Sidhaye, 2017; Agustí et al., 2019).

Oxidative stress derived from these conditions induces structural, biochemical, and functional modifications in the respiratory epithelium of susceptible individuals. These modifications are recognized by alveolar macrophages and neutrophils, which react by increasing their synthesis of pro-inflammatory molecules and proteinases (Fischer et al., 2011; Martínez-Aguilar et al., 2017). These enzymes contribute substantially to the loss of normal lung architecture and have a pivotal dual effect on this pulmonary disease. On one side, these molecules promote the degradation of the extracellular matrix in the pulmonary interstitium and, on the other, they promote the development of peribronchial fibrosis (Zou et al., 2014; Barnes, 2021).

Unfortunately, COPD today remains an incurable disease, and pharmacological treatments are focused on symptom control. Therefore, this study aims to contribute to a better understanding of the molecular changes involved in the pathogenesis of COPD, which subsequently supports the identification of therapeutic targets and the design of more efficient treatments.

Search strategy for information

A bibliographical sources search was carried out in the PubMed and Scopus databases, using terms related to the aim of this review. Search filters were used to identify clinical and experimental studies, published in English from 2010 to 2022. Three reviewers worked independently and simultaneously to screen, review, and select the studies. The inclusion criteria used for the experimental research articles were studies including induction and validation of a COPD model (employing histological or functional measurements), as well as molecular evaluation by at least one laboratory technique. In the case of clinical research, the criteria considered sample calculation, the use of the GOLD parameters to diagnose and classify the severity of COPD, the correct selection of the control group, and molecular evaluation, among other considerations. Subsequently, 57 articles that fulfilled the aforementioned selection criteria were included in this review: 12 clinical studies (11 observational studies and one clinical trial) and 45 experimental studies (36 *in vivo* and 9 *in vitro* studies).

Epidemiology and diagnosis of COPD

According to data published by the Global Burden of Diseases Study, COPD is one of the leading causes of mortality worldwide. In 2017, it caused 3.2 million deaths (Li et al., 2017) and in 2015 affected 174.5 million people, with a higher prevalence in men than in women. Both parameters, mortality and prevalence rates, increase considerably as age augments (GBD, 2017).

COPD should be clinically suspected in any individual with dyspnea, chronic cough, sputum production, and/or a history of risk factors. In this clinical context, spirometry is the gold standard to diagnose COPD in patients who obtain a post-bronchodilator forced expiratory volume in one second and forced vital capacity (FEV1/FVC) ratio < 0.70. Spirometry detects this functional limitation by considering both small airway obstruction and pulmonary emphysema (GOLD, 2023).

Additionally, the GOLD strategy recommends computed tomography (CT) for surgical interventions (GOLD, 2023), however, from the COPD Genetic Epidemiology study

(COPDGene) emerged the proposal to integrate chest CT to diagnose COPD. This is relevant as CT would allow the early detection of individuals with emphysematous lesions but without spirometric alterations (Lowe et al., 2019). Pulmonary emphysema (PE) and chronic bronchitis (CB) do not necessarily occur simultaneously in the same COPD patient and are defined considering different pathological aspects, which are discussed below (Agustí et al., 2019).

Pulmonary emphysema

PE is a histopathological concept, defined since 1995 by the American Thoracic Society as abnormal and permanent enlargement of the airspaces distal to the terminal bronchioles, accompanied by destruction of their walls and without obvious fibrosis (ATS, 1995). Severe emphysema evaluated by high-resolution CT is related to worse quality of life, worse lung function, greater exacerbation frequency, and lower body mass index, among other clinical aspects (Wang et al., 2013). The presence of emphysema alone does not necessarily generate airflow obstruction (Hochberg and Sidhaye, 2017; Barnes, 2019), although, it is currently recognized that there may be a combined presence of emphysema and small airway obstruction (Kim et al., 2007).

Chronic bronchitis

CB is clinically defined as an active cough for at least three months in two or more consecutive years (ATS, 1995). These clinical signs are related to microscopic changes such as metaplasia (from ciliated cells to mucus-secreting cells) and hyperplasia of the subepithelial seromucous glands, smooth muscle hypertrophy, fibrosis of the bronchial wall, and inflammatory infiltrate. These alterations contribute significantly to reduced lung compliance and pulmonary function (Perotin et al., 2014; Zhang et al., 2020). CB is associated with worse quality of life, faster decline in lung function, greater exacerbation frequency, and a higher risk of death (Kim et al., 2016; Kim et al., 2021).

Pathophysiology and molecular changes involved in the development of COPD

Genetic alterations associated with COPD

The best-known genetic alteration in the development of COPD is alpha-1-antitrypsin (A1AT) deficiency. This disorder is caused by mutations in the *SERPINA1* gene, with autosomal recessive inheritance, which affects 0.5-1% of European smokers. It causes insufficient, null, or dysfunctional levels of this enzyme in serum. A1AT is the main inhibitor of neutrophil elastase (NE), whose enzymatic deregulation causes generalized hydrolysis of elastin in the lung parenchyma, causing early-onset panacinar-type PE. Currently, the therapeutic approach for this phenotype is the administration of purified human A1AT (Santangelo et al., 2017).

Genetic predisposition to COPD may also be mediated by mutations in telomerase components, such as telomerase reverse transcriptase (TERT) and telomerase RNA (TR). Stanley et al. (2015), in two independent cohorts, identified the presence of mutations in *TERT* in 1% of COPD patients. Three variants (Arg599Gln, Thr726Met, and His925Gln) affected telomerase enzymatic activity, generating short telomeres, inducing severe emphysema and higher incidence of pneumothorax, with predominance in women. Interestingly, families with the presence of PE along with pulmonary fibrosis exhibited an autosomal dominant inheritance pattern and, unlike the development of fibrosis, PE was only manifested if there was a history of tobacco smoking.

Furthermore, through a genome-wide association study (GWAS), Cho et al. (2015) identified the relation between greater susceptibility to moderate-severe COPD and six variants in the *CHRNA3/CHRNA5/IREB2, FAM13A, HHIP, MMP12, RIN3*, and *TGFB2* genes. These alterations are implicated in addictive behavior, and in the case of *RIN3* and *TGFB2*, their reduced gene expression favors damage severity. In another study, genetic variants were found related to manifestations of PE, such as the amplitude of the lung lesion (*SNRPF, PPT2, MAN1C1*) and the type of emphysema (centrilobular, *MYO1D*, or panlobular, *VMA8*) (Ragland et al., 2019). Interestingly, in non-Hispanic Whites and African Americans, a variant in the *DSP* gene (rs2076295 T > G) was found, which is associated with greater progression of PE, however, it exerts a protective effect against pulmonary fibrosis.

Desmoplakin, the protein encoded by this gene, has an important role in cell adhesion, therefore, its alteration favors the loss of normal lung architecture (Kim et al., 2019).

The knowledge of these genetic alterations contributes to a better understanding of COPD susceptibility and severity of lung damage. These findings are especially important considering that only 10-25% of people who smoke develop COPD (Álvarez-Sala et al., 2015), and that between 17-39% of COPD patients are non-smokers (Zeng et al., 2012).

Oxidative stress and epigenetic mechanisms in COPD

Chronic exposition to cigarette smoke (CS) is the most important risk factor associated with COPD development in higher SDI countries. However, COPD has also been associated with other noxious particles and gases, such as exposure to electronic cigarettes (Higham et al., 2016), ozone (Li F. et al., 2016; Li et al., 2018), fine particles of matter (PM) (Jiang et al., 2020), and wood smoke (Zou et al., 2014). These gases constitute an important exogenous source of free radicals, which interact with the respiratory tissue. Even after acute exposures to tobacco smoke (Shen et al., 2014; Shin et al., 2018) or other gases, free radicals produce protein, lipid, and nucleic acid oxidation, represented by increased levels of protein carbonyls (Singla et al., 2020), malondialdehyde (MDA) (Singla et al., 2020; Liu et al., 2021; Sokar et al., 2021), epoxycholesterol α and β, secosterol A and B (Speen et al., 2016), 8-iso-prostaglandin F2 (8-iso-PGF2)-α (Martins-Olivera et al., 2016), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Li F. et al., 2016; Li et al., 2018). These biochemical alterations lead to functional and metabolic cell damage, as well as activation of the immune system and inflammation. Specifically, 8-OHdG was positively associated with the development of severe emphysema in COPD patients, by promoting telomere shortening (Deslee et al., 2009), and mitochondrial dysfunction (Li et al., 2018).

Li et al. (2018) identified increased protein expression of the mitochondrial complexes II and V in mice chronically exposed to ozone. This change promoted the endogenous synthesis of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot}$-), hydrogen peroxide (H_2O_2), hydroxyl radical (*OH), and peroxynitrite (ONOO-). In the first stage of COPD, the endogenous antioxidant system is activated through a higher synthesis of the nuclear factor erythroid-2-related factor 2 (Nrf2) (Li et al., 2018). However, the continued

exposition of noxious particles and gases generates the subsequent insufficiency of the endogenous antioxidant barriers. Among the reported alterations are the reduced gene and protein expression of glutathione (GSH) (Shen et al., 2014; Singla et al., 2020), superoxide dismutase (SOD) (Li et al., 2016; Liu et al., 2021; Sokar et al., 2021; Wiegman et al., 2015), and heme-oxygenase (HO-1) (Liu et al., 2021). Moreover, a reduction in pro-resolving lipid mediators has also been identified, such as 17-hydroxy-docosahexaenoic acid (17-HDHA), 14-hydroxy-docosahexaenoic acid (14-HDHA), and protectin Dx (PDX), which promotes an anti-inflammatory phenotype and lung tissue regeneration (Kilburg-Basnyat et al., 2018).

Concomitantly, this condition increases the hypoxia-inducible factor (HIF)-1α activity (Li et al., 2018). This transcription factor enhances the expression of NADPH oxidases (NOX) and inducible nitric oxide synthase (iNOS). Particularly, NOX-2 was increased in emphysematous lung biopsies from humans and in the lungs of mice exposed to elastase. Subsequently, its activity was related to the enhanced activity of matrix metalloproteinase (MMP)-9 and the enlargement of the alveolar spaces (Trocme et al., 2015). Although this condition must be evaluated in patients with CB, this evidence suggests that NOX-2 activity could be more related to the emphysematous damage.

Regarding iNOS, its direct effect is the augmented production of nitric oxide (NO) and ONOO- in epithelial cells and alveolar macrophages (Theodoro-Júnior et al., 2017; Guan et al., 2018; Shin et al., 2018; Liu et al., 2021). These molecules with chemotactic activity (Martins-Olivera et al., 2016), also generate post-translational modifications, such as nitration, oxidation, carbonylation, and subsequent hydrolysis of two main deacetylases, NAD-dependent protein deacetylase sirtuin-1 (SIRT1) (Xu et al., 2012; Yao et al., 2013; Wang et al., 2014; Wang et al., 2020) and histone deacetylase (HDAC) (Perng et al., 2012; Sugiura et al., 2012; Chen et al., 2015; Wiegman et al., 2015; Kotnala et al., 2017; Jiang et al., 2020). Both enzymes maintain the condensed state of chromatin (silencing) of nuclear factor kappa B (NF-κB), the main pro-inflammatory transcription factor in several chronic diseases.

Besides the pro-inflammatory effect, SIRT1 downregulation also favors tissue remodeling, with a tendency towards the emphysematous phenotype. In rats exposed to CS and lipopolysaccharide (LPS), SIRT1 downregulation induced the activation of apoptotic mechanisms through the release of caspases 3 and 12 (Wang et al., 2020). In addition, it

promoted the imbalance between MMP-9 and its main tissue inhibitor, TIMP-1, resulting in greater loss of respiratory epithelium and worse lung function (Yao et al., 2013; Wang et al., 2020). Contrarily, in the presence of lung tissue fibrosis, SIRT1 expression can be activated through the transforming growth factor (TGF)-β/Smad signaling pathway (Xu et al., 2012).

In addition, the downregulated activity of HDAC2 could be more related to a pro-fibrotic COPD phenotype. The deficiency of HDAC2 increased the activation of MMP-9 (without TIMP-1 modification), through the TGF-β and NF-κB signaling pathways (Sugiura et al., 2012; Liu et al., 2021). Furthermore, Jiang et al. (2020) identified an important macrophage polarization to M2-like macrophages (M2) in HDAC2 deficient mice exposed to PM with a diameter of less than 2.5 microns (PM2.5). These animals overexpressed TGF-β, as well as MMP-9 and MMP-12, generating remodeled tissue and diminished lung function. The authors indicated that although the M2 response is related to a less inflammatory effect in comparison with M1-like macrophages (M1), it could be associated with a higher pro-fibrotic phenotype (Jiang et al., 2020).

Regarding HDAC3, Sun et al. (2020) reported a decreased activity of this enzyme in COPD models with mice and alveolar macrophages exposed to CS extract. This alteration led to a higher gene and protein expression of iNOS, interleukin (IL)-6, tumoral nuclear factor (TNF)-α, MMP-9, and CD40, representing the M1 phenotype. However, a slight increase in IL-10, TGF-β, and CD163 (markers of M2) was also found, indicating that both M1 and M2 phenotypes are activated in COPD but M1 at a higher proportion (Sun et al., 2020). This could partially explain the low degree of fibrosis present in CB rather than the extensive tissue fibrosis in other pathological entities, such as idiopathic pulmonary fibrosis.

Another epigenetic alteration present in COPD is the upregulation of bromodomain-containing protein 4 (Brd4), which has been found augmented in COPD patients. This protein binds to acetylated NF-κB and enhances its transcriptional activity and pro-inflammatory effect (Liu et al., 2021).

The inflammatory response in COPD

The structural alterations generated by harmful gases and particles in the respiratory tissue are recognized by resident macrophages and dendritic cells through their pattern recognition receptors (PRRs) (Owen et al., 2014; Cho et al., 2019). The two main families of PRRs are Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), primarily the NLR containing 3 pyrimidine domains (NLRP3) (Cho et al., 2019). NLRP3 is part of a multiprotein complex called the inflammasome since it activates pro-caspases 1 and 3, pro-IL-1β, and pro-IL-18. The activation of the inflammasome generates potent neutrophil and monocyte chemotaxis, as well as promoting the activation of cytotoxic lymphocytes and natural killer cells, followed by apoptosis and alveolar destruction (Chen et al., 2010; Suárez and Buelvas, 2015; Li F et al., 2016). Chen et al. (2010) reported that endothelin (ET)-1 is implicated in the activation of the inflammasome and MMPs, and demonstrated that administering ET-1 receptor antagonists reduced apoptosis in the lung tissue of mice.

Regarding TLRs, Tripathi et al. (2017) and Simpson et al. (2013) identified increased gene expression of *TLR2/4* in COPD patients compared with control individuals. In addition, they found higher protein levels of MMP-9, NE, and IL-8. In common, these studies supported that, from the early lung damage stage to advanced chronic disease, the immune system is activated along with other modulating molecules such as matrix proteinases.

The stimulation of NLRs and TLRs activates monocytes, macrophages, and epithelial cells through the mitogen-activated protein kinases (MAPK). The MAPK family includes extracellular signal-related kinases (ERKs), c-Jun N-terminal stress-activated kinases (JNKs), cellular stress-driven kinases, and the subfamily p38. Subsequently, MAPKs induce the activation of transcription factors such as NF-κB, activator protein (AP)‑1, and Janus kinase (JAK)/signal transducer and activator of transcription (STAT). These factors enhance inflammation as well as activation of the adaptive immune system (Betts et al., 2015; Liu et al., 2018).

Of note, clinical and experimental evidence shows a differentiated expression of MAPKs in COPD according to the scale and cause of the damage. For instance, p38 has been related not only to local lung damage but also to systemic inflammation. In a placebo-controlled clinical trial, one single dose of a p38 MAPK inhibitor administered to COPD patients re-

established gene expression and blood and sputum levels of IL-1β, MMP-9, STAT-1, caveolin 1, fibrinogen, and c-reactive protein (CRP) (Betts et al., 2015).

At the experimental level, Zhou et al. (2017) reported in rats exposed to CS, the proteinase/antiproteinase imbalance through overexpression of p38 and ERK1/2 but not through JNK. In addition, chronic ozone exposure in mice induced the activation of the inflammasome through p38 activity (Li F et al., 2016). Also, human neutrophils exposed to electronic cigarette extract augmented their inflammatory and gelatinase activity via p38 (Highman et al., 2016). In contrast, the exposure of bronchial epithelial cells and macrophages to LPS induced inflammation and proteinase activity through JNK and ERK1/2 but not by the p38 signaling pathway (Tian et al., 2018; Ko et al., 2019). Taken together, these results could suggest a different molecular response depending on whether the main COPD risk factor was exposure to harmful gases or respiratory infections.

Regarding NF-κB, this is a redox-sensitive transcription factor constituted by a heterodimer of 50-kDa (p50) and 65-kDa (p65). In the cytoplasm, the inactivated form of NF-κB is linked to its inhibitor (IκB); however, oxidative conditions promote the degradation of IκB, enhancing the translocation of NF-κB into the nucleus (Ariestanti et al., 2015; Games et al., 2016; Zhou et al., 2017; Ko et al., 2019).

Zhou et al. (2019) demonstrated in mice exposed to CS, significantly increased protein expression of the receptor activator of NF-κB (RANK) and its ligand (RANKL). Additionally, in alveolar macrophages, aberrant mechanisms were observed in the activity of lysosomes, followed by decreased degradation of RANK, and higher NF-κB activation. This was also related to the upregulation of MMP-9, but neither MMP-12 nor TIMP-1. Moreover, in alveolar macrophages from Ig-Hepta/GPR116-deficient mice, a protein essential for the homeostasis of pulmonary surfactant, a protein misbalance of p65 and IκB-α was found. Further alterations were lipid-peroxidation, as well as upregulation of MMPs, monocyte chemoattractant protein (MCP)-1, C-C motif chemokine ligand (CCL)-2 and -3, and the complement component C5a (Ariestanti et al., 2015).

The deficiency of vitamin D has also been associated with lung disease and steroid resistance. Sundar et al. (2011) identified the spontaneous activation of the immune system via the NF-κB signaling pathway in mice lacking the nuclear hormone vitamin D receptor (VDR), with a significant increase in neutrophils in bronchoalveolar fluid (BALF) and

macrophages in the lung interstitium, through MCP-1 and keratinocyte chemoattractant mediators. Although COPD pathogenesis is closely related to the innate immune system, VDR deficiency also induced lymphoid aggregates with lymphocytes T CD4+, CD8+, and B-cells. This has been linked to higher epithelial destruction in COPD's advanced phases. In VDR-deficient mice, these inflammatory conditions generated early declined lung function, PE, and peribronchial fibrosis, associated with an imbalance between MMPs/TIMPs (Sundar et al., 2011).

Furthermore, eosinophilic inflammation, which is a typical trait of asthma, also may be found in 32-40% of COPD patients. However, the pathogenic role seems to be different in each type of disease, and it remains unknown why only some patients with COPD develop this alteration. Eosinophilic COPD patients have been found to have increased levels of MMP-12 and an emphysematous phenotype compared with non-eosinophilic patients (Mycroft et al., 2020).

Autophagy and senescence in COPD

Autophagy is an essential process to degrade damaged organelles and aberrant cell structures. Physiologically, to restrict cell damage, the autophagic process directed by two important proteins, LC3B and Beclin-1, is increased in the early stages of COPD. However, oxidative stress produces alterations in the coupling between autophagosomes and lysosomes, causing the accumulation of damaged mitochondria and ubiquitinated proteins. In this context, autophagy alterations lead to cellular senescence and, in advanced stages of COPD, to cell death through apoptosis and necroptosis (Maciel-Herrerías and Cabrera-Benítez, 2016). Specifically, regarding the mitophagy mechanism, Araya et al. (2019) studied the deficiency of parkin RBR E3 ubiquitin protein ligase (PRKN) in mice exposed to CS. Compared with WT mice, the authors found increased levels of β-galactosidase (β-gal), DNA damage (phospho-histone H2AFX), cyclin-dependent kinase inhibitors (p16 and p21), and senescence-associated secretory phenotype (SASP), such as CXCL1, IL-6, and IL-1β. These alterations promoted higher emphysematous damage, airway wall thickening, and macrophage count (Araya et al., 2019).

Similar results were obtained by Woldhuis et al. (2020) in fibroblasts from COPD patients. Compared with older COPD patients and matched controls, patients with severe early-onset (SEO)-COPD exhibited increased levels of β-gal, DNA damage (γ-H2A.X), and oxidative stress (MGST1). These results show that β-gal, γ-H2A.X, and MGST1 could be specific markers of SEO-COPD patients, who experienced severe lung damage at a younger age and with a relatively low number of pack-years of smoking in comparison with most older COPD patients (Woldhuis et al., 2020).

Another alteration implicated with senescence in COPD is the downregulation of club cell protein 16 (Cc16). Laucho-Contreras et al. (2018) demonstrated the presence of emphysema, small airway fibrosis, and accelerated lung function decline in a Cc16-deficient murine model. Compared with wild-type mice, higher macrophage activation and increased senescence biomarkers (p16 and p21) were observed in Cc16 KO mice. Besides higher oxidative stress levels and higher lung levels of CCL-2, and CCL-5, Il-10, MMP-9, and CRP were reported. Nevertheless, there was no significant difference in TGF-β1, IL-6, or MMP-2 levels. In this case, it seems that tissue fibrosis occurred in a TGF-β independent manner; this alternative induction could be related to Smad and the wingless-type MMTV integration site (Wnt)/β-catenin signaling (Ortiz-Zapater et al., 2022).

Finally, the elevation of CRP and fibrinogen levels in COPD patients reflects the influence of respiratory damage on systemic inflammation and its relation to comorbidities, such as atherosclerosis, myocardial infarction, diabetic vascular damage, and renal fibrosis (Betts et al., 2015; Wang et al., 2016; Laucho-Contreras et al., 2018). Furthermore, in COPD patients, the plasminogen activator inhibitor (PAI)-1 has also been associated with senescence, systemic inflammation, lung function decline, and CB related to an MMP-9/TIMP-1 imbalance (Wang et al., 2016; Barnes 2021).

Metalloproteinases and tissue remodeling in COPD

COPD-associated proteases can be classified according to their catalytic site characteristics into three different types: serine-, metallo-, and cysteine- proteases. Included in the group of serine proteases are NE, cathepsin G, and proteinase 3, which are involved in the destruction of alveolar tissue. MMP-2, MMP-9, MMP-12, and MMP-13 are involved in

the degradation of the ECM and the severity and exacerbation of COPD. Finally, cysteine proteases, such as caspases-3, -8, and -9, regulate cell apoptosis in pulmonary tissue. Proteinases maintain a collaborative effect with each other, such as that exerted by NE, inhibiting TIMPs. Likewise, MMPs can degrade alpha 1-antitrypsin (Fischer et al., 2011).

MMP-9/TIMP-1 and MMP-12 have a relevant effect on PE and CB, as well as their contribution to autoimmunity, perpetuation, and exacerbation of COPD. MMP-9, also known as gelatinase B, substantially contributes in COPD to the generation of PE, as well as the deposition of connective tissue or fibrosis in the peribronchial compartment. The role of MMP-9 in COPD involves its upregulated catalytic activity on the components of the ECM, however, additionally, it exerts an essential modulation of the immune response due to its ability to hydrolyze and activate pro-TNF-α, pro-IL-1β, pro-IL-8, and pro-TGF-β (Vandooren et al., 2013; Li et al., 2020).

Regarding TIMP-1, it is a glycoprotein produced by macrophages, fibroblasts, and tumoral cells (Li Y. et al., 2016; Li et al., 2020). Its mechanism of inhibition on MMP-9 consists of coupling to its catalytic site through non-covalent interactions, obstructing the interaction with zinc ions, which is essential for MMP-9 catalytic activation. TIMP-1 interacts with MMP-9 at a 1:1 ratio through the hemopexin domain of MMP-9, being capable of inhibiting both the zymogen and active form of the gelatinase. In COPD, the ratio between MMP-9/TIMP-1 is dysregulated. If MMP-9 expression is increased, it mainly indicates an inflammatory-emphysematous reaction but, on the contrary, if TIMP-1 increases, it reflects a pro-fibrotic process in the airways (Li et al., 2020).

Most of the experimental articles analyzed herein reported increased TIMP-1 protein levels in lung tissue (Sundar et al., 2011; Wang et al., 2014; Ariestanti et al., 2015; Games et al., 2016; Martins-Olivera et al., 2016; Sugiura et al., 2016; Theodoro-Junior et al., 2017; Zou et al., 2014). In contrast, fewer studies identified reduced TIMP-1 protein expression (Yao et al., 2013; Shen et al., 2014; Kotnala et al., 2017; Wang et al., 2017; Zhou et al., 2017; Singla et al., 2020). In humans, the augment in TIMP-1 is directly associated with COPD exacerbations (Papakonstantinou et al., 2015), systemic inflammation (Zhang et al., 2020), and old age in both COPD patients and healthy controls, as well as smoking history in control individuals (Linder et al., 2015).

Regarding MMP-9, its upregulation contributes to PE and CB, however, a set of differentiated molecular pathways direct its impact toward one, the other, or both tissue remodeling effects. In the case of PE, Xu et al. (2012) and Kotnala et al. (2017) demonstrated MMP-9 upregulation via the deficiency or decreased levels of TGF-β/Smad and SIRT1-mediated signaling pathways, enhancing the development of alveolar emphysema. This effect was followed by reduced mRNA expression of ECM proteins (Kotnala et al., 2017). Additionally, Gao et al. (2012) reported decreased levels of mRNA and activity of α1,6-fucosyltransferase (FUT8) in a CS murine model. This alteration induced a deficient activation of TGF-βR and subsequent gene overexpression of *Mmp-9*, but not of *Mmp-12*. Interestingly, FUT8 downregulation also diminished the interaction with epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF), contributing to alveolar cell apoptosis (Gao et al., 2012).

By contrast, one primary mechanism involved in the development of peribronchial fibrosis is epithelial-mesenchymal transition (EMT), in which inflammation and cell senescence induce metaplasia of epithelial cells into profibrogenic fibroblasts (Lachapelle et al., 2018). This transformation involves the loss of phenotypic epithelial characteristics and the acquisition of other mesenchymal features. In comparison with normal fibroblasts, cells resulting from EMT express a higher amount of MMP-9 and MMP-2, TGF-β, collagen, fibronectin, CXCL-8, vimentin, and α-smooth muscle actin (α-SMA), with decreased expression of E-cadherin (Zou et al., 2014; Guan et al., 2017; Jiang et al., 2018). MMP-9 constitutes a key molecule in EMT due to hydrolysis of the type IV collagen present in the basement membrane of the respiratory parenchyma. This breakdown allows the migration of epithelial cells to the airways, enhancing their subsequent transformation to fibroblasts. Moreover, MMP-9 catalyzes the active form of TGF-β, enhancing tissue fibrosis (Zou et al., 2014).

In a case-control study, Zhang et al. (2020) reported small airway morphological changes in COPD patients, including cilia cluttered, subepithelial fibrosis, and basement membrane fragmentation. In this study, MMP-9 and sestrin2 were significantly augmented in the COPD group. Sestrin2 is a protein involved in antioxidant defense but it is also related to the development of tissue fibrosis through upregulation of the TGF-β signaling pathway.

Additionally, a positive correlation between MMP-9 and sestrin2 was found, as well as between sestrin2 and quantitative CT parameters (Zhang et al., 2020).

Lung fibrosis and EMT development in COPD involve the upregulation of the TGF-β/Smad signaling pathway (Sugiura et al., 2012; Shen et al., 2014; Guan et al., 2017; Jiang et al., 2018; Laucho-Contreras et al., 2018; Jiang et al., 2020; Liu et al., 2021). Furthermore, in airway fibroblasts from COPD rats, the activation of Wnt/β-catenin and Wnt/Ras homolog family member A (RhoA), followed by MMP-9 production and lung tissue fibrosis were found (Ge et al., 2019). Interestingly, Wnt5A, a noncanonical Wnt signaling, not only promotes tissue fibrosis through ECM synthesis in small airways, it also enhances alveolar enlargement and obstructs tissue repair in the lung parenchyma (Baarsma et al., 2017).

Importantly, the cellular and molecular characteristics of CB, such as overexpression of MMP-9, the presence of EMT, chronic inflammation, senescence, and cell migration constitute common pathological traits between COPD and lung cancer. Indeed, both pathologies also share other molecular mechanisms, for example, the TGF-β/Smad, PI3K/Akt/mTOR, and Akt/p38 MAPK signaling pathways (Jiang et al., 2018), as well as the intercellular adhesion molecule 1 (Sokar et al., 2021), and the downregulation of the hyperplasia suppressor gene (*HSG*), whose reduction induces cell survival and pulmonary fibrosis through the Wnt signaling pathway (Ge et al., 2019).

The role of metalloproteinases in autoimmunity and exacerbations of COPD

Acrolein, a reactive αβ-unsaturated aldehyde from environmental pollution, has been involved in emphysema and COPD perpetuation. This molecule enhances the expression and activity of MMP-9 in neutrophils, leading to collagen hydrolysis. The collagen fragments are degraded by another enzyme increased by acrolein exposition, prolyl endopeptidase, which produces tripeptides, also known as matricines, such as proline-glycine-proline (PGP). This amino acid sequence shares structural homology with an IL-8 region and is able to interact with the CXC receptor 1 (CXCR1) from neutrophils and macrophages, inducing in these cells MMP and chemokine upregulation. This positive feedback among peptidases and matricines can be disrupted by PGP degradation and catalyzed by leukotriene A4 hydrolase (LTA4H);

however, LTA4H is downregulated under oxidative conditions (Noerager et al., 2015; Wells et al, 2015).

Consistently, Zhou et al. (2020) found that, after CS exposure in COPD patients and mice, MMP-12 produced elastin peptides that induced chemotactic activity and stimulated T cells to synthesize antibodies against elastin fragments. This autoimmune process involved differentiation to Th1 and Th17 and contributes to the perpetuation of lung damage even after tobacco smoking cessation.

Regarding acute exacerbations of COPD (AE-COPD), MMP-9 has been widely associated with this condition, which is characterized by increased dyspnea, cough, sputum, worsening of lung function, and increased susceptibility to respiratory infections. Wells et al. (2018) performed a complementary analysis between Outcome Measures in COPD Study (SPIROMICS) and COPDGene. The authors reported that higher MMP-9 levels were associated with the development of AE-COPD (odds ratio [OR], 1.71; 95%CI, 1.00–2.90; and OR, 3.03; 95%CI, 1.02–9.01), as well as augmented AE-COPD frequency (incidence rate ratio [IRR], 1.45; 95%CI, 1.23–1.7; and IRR, 1.24; 95% CI, 1.03–1.49), in SPIROMICS and COPDGene, respectively. In addition, a significant negative correlation has been reported between MMP-9 levels (measured in BALF and serum), FEV1%, and FEV1/FVC ratio in patients with AE-COPD, as well a higher activation of pro-MMP-9 in these patients compared with stable COPD patients (Perotin et al., 2014; Linder et al., 2015; Papakonstantinou et al., 2015).

Additionally, higher gene expression of *MMP-9* and gelatinase activity in BALF has been found in patients with advanced disease (GOLD IV stage) in comparison with patients with mild-moderate illness (GOLD II). These findings were associated with increased neutrophils in BALF (Bchir et al., 2017; Vlahos et al., 2012). All this information suggests that MMPs are deeply involved in the process of the perpetuation of COPD through maintaining the pro-inflammatory and damage conditions, besides the continued exposure to oxidative stimuli. Therefore, MMP-9 seems to be a specific molecule in COPD worsening and exacerbation.

Finally, **Figure 1** summarizes the most important changes and molecular alterations in COPD reviewed in this article. In this scheme, the relevant pathways in the development of PE and fibrosis in CB are highlighted.

Conclusions

Oxidative stress, inflammation, and protease imbalance contribute to the development of the two main phenotypes of COPD, PE and CB. In PE, powerful inflammatory and senescent processes develop that induce the loss of the alveolar epithelium, while in CB, pro-fibrotic mechanisms are activated, related to the EMT and TGF-β or Wnt/β-catenin signaling pathways. Both pathological processes share intricate molecular changes, which ultimately lead to the decline in lung function of COPD patients. However, the heterogeneous manifestation of this disease makes it necessary to sharpen our understanding of the pathways that are deregulated in a particular way in each COPD phenotype. Elucidating these mechanisms increases the possibility of success in generating treatments that are increasingly efficient and personalized for patients.

Limitations of the study

Concerning the process of this review, only two databases were searched for information, nevertheless, it is considered that both databases belong to the most relevant academic sources for obtaining high-quality scientific information on the topic of interest.

Other information Disclosure statement

No potential conflict of interest was reported by the authors.

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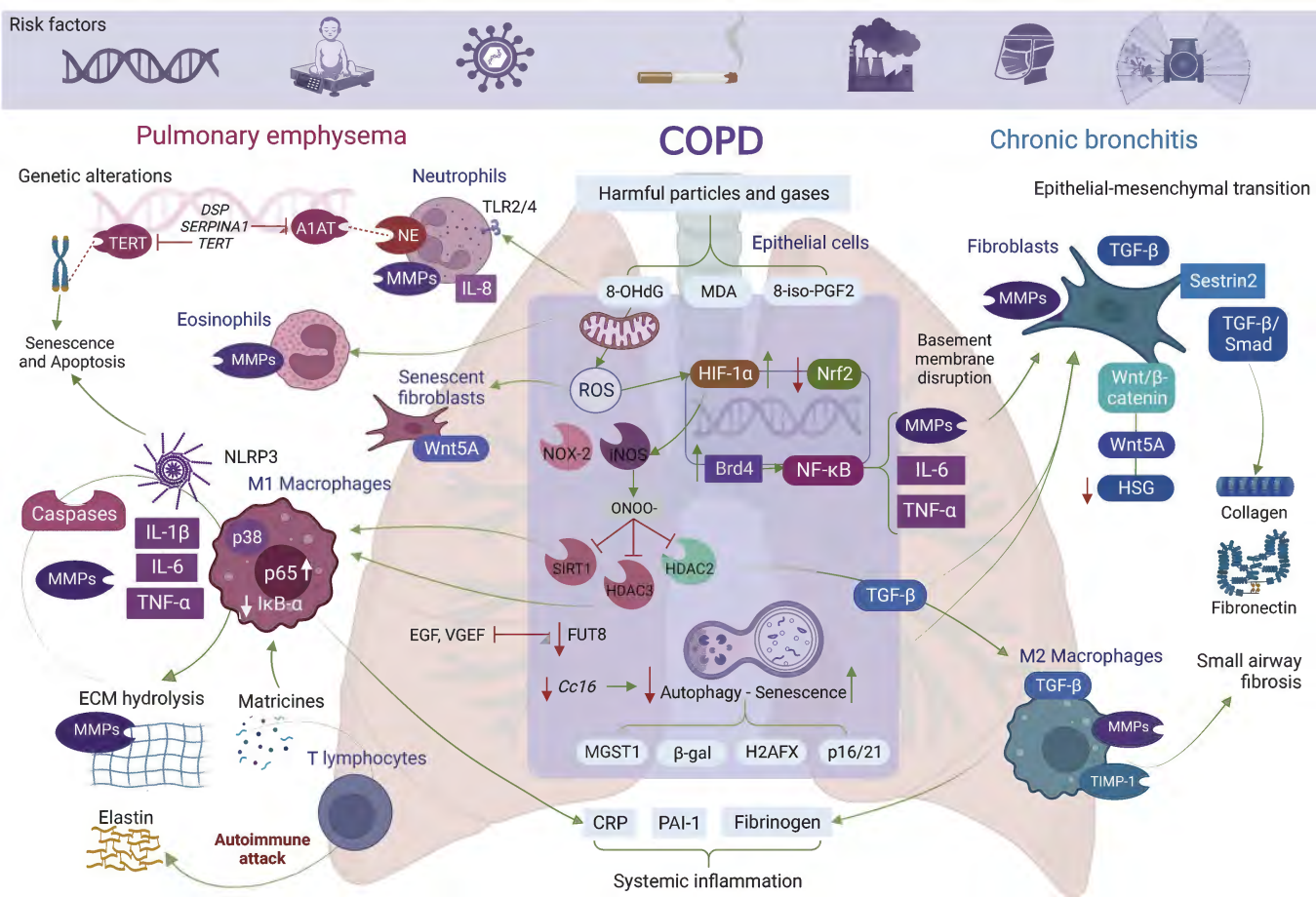
Figure 1. Schematic representation of the dysregulated molecular mechanisms in COPD, and its clinical phenotypes, pulmonary emphysema (PE) and chronic bronchitis (CB). In PE, more severe inflammation is generated, with an important role for M1 macrophages and inflammasome activation, which enhance apoptosis and ECM degradation. In the case of CB, the polarization to M2 macrophages and the epithelial-mesenchymal transition (EMT) process promote tissue fibrosis mainly via the TGF-β signaling pathway. Interestingly, matrix metalloproteinases 9 and 12 play a relevant role in the severity, perpetuation, and exacerbation of COPD. Green arrows indicate a molecule or molecular mechanism's stimulation, or upregulation. Red arrows represent the downregulation of a molecule or molecular mechanism. Red lines with a cross line represent inhibition of a molecule or a molecular mechanism. Each red dotted line indicates a loss of function. This figure was created with BioRender (BioRender.com (accessed on July 13, 2023), and created during the free trial period for this software).


Risk factors
Pulmonary emphysema
COPD
Chronic bronchitis
Genetic alterations
DSP
SERPINA1
TERT
TERT
A1AT
NE
Neutrophils
TLR2/4
MMPs
IL-8
Eosinophils
MMPs
Senescence and Apoptosis
Senescent fibroblasts
Wnt5A
NLRP3
M1 Macrophages
Caspases
IL-1β
IL-6
TNF-α
MMPs
p38
p65
IκB-α
ECM hydrolysis
MMPs
Matricines
T lymphocytes
Elastin
Autoimmune attack
Harmful particles and gases
Epithelial cells
8-OHdG
MDA
8-iso-PGF2
ROS
HIF-1α
Nrf2
NOX-2
iNOS
Brd4
NF-κB
MMPs
IL-6
TNF-α
ONOO-
SIRT1
HDAC3
HDAC2
TGF-β
EGF, VGEF
FUT8
Cc16
Autophagy - Senescence
MGST1
β-gal
H2AFX
p16/21
CRP
PAI-1
Fibrinogen
Systemic inflammation
Basement membrane disruption
Epithelial-mesenchymal transition
Fibroblasts
TGF-β
MMPs
Sestrin2
TGF-β/ Smad
Wnt/β-catenin
Wnt5A
HSG
Collagen
Fibronectin
M2 Macrophages
TGF-β
MMPs
TIMP-1
Small airway fibrosis