COPD: Preclinical models and emerging therapeutic targets

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COPD: PRECLINICAL MODELS AND EMERGING THERAPEUTIC TARGETS

Running title: Therapeutic potential of COPD experimental models

Key words: animal models; cigarette smoke exposure; COPD; lung regeneration potential; mesenchymal stromal cells; proteases and microorganisms; surfactant protein-D; systemic comorbidities of COPD

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ABSTRACT

Introduction: Chronic obstructive pulmonary disease (COPD) is a common disease and a leading cause of morbidity and mortality worldwide. Preclinical models of COPD have been developed lately. In those models, COPD/emphysema has been commonly induced using different toxic agents such as elastase, proteolytic enzymes, and chronic exposure to cigarette smoke (CS). **Areas Covered**: The most relevant features of preclinical models of COPD, namely chronic exposure to CS and other agents (proteases and microorganisms), cardiovascular effects, surfactant protein-D, airway remodeling and inflammation, lung regeneration potential, and mesenchymal stromal cell therapy are being described in the review below. The most relevant publications on the topic of interest were selected from P*ubMed* and used to write this review. **Expert opinion**: Preclinical models of COPD are key elements to understand the underlying biology and pathophysiology of the disease with the aim to design of therapeutic targets. Increased knowledge on the most relevant biological insights will help design therapeutic targets for the treatment of patients with COPD. This will enable health caregivers and doctors to better treat their patients, in which a more holistic approach will be used. This will benefit the patients and the society as the use of resources should be significantly diminished.

Word count: 200

HIGHLIGHTS

1. Cigarette smoke exposure is a relevant model of COPD to study the respiratory system and other organs

2. Proteases (elastase) and microorganisms or microbial components are useful models of COPD to study exacerbations

3. Cardiovascular manifestations are major comorbidities in COPD and may be studied using specific animal models

4. Animal models of surfactant protein-D help elucidate the role of this protein in COPD

5. Mouse models of airway remodeling are relevant to shed light into this biological process in COPD

6. Therapeutic strategies based on the use of mesenchymal stromal cells look promising in the near-future as evidenced from animal models

7. Preclinical models of COPD are key elements for the design of therapeutic targets: lungs and airways and the extra-pulmonary manifestations of the disease

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation together with an enhanced chronic inflammatory response to noxious particles or gases, usually inhaled cigarette smoke, in the airways and lungs of the patients. COPD is a common preventable and treatable disease, and the fourth leading cause of morbidity and mortality worldwide [1-4]. Most of these patients very often have concomitant diseases known as comorbidities which significantly impair their quality of life. The etiology of comorbidities has not been fully elucidated yet, since they share risk factors with the pulmonary disease (e.g. exposure to air pollution and smoking). It should also be mentioned that most of the disease burden and utilization of health-care resources are attributable to the extra-pulmonary manifestations and associated comorbidities of COPD patients. Furthermore, infections of the respiratory tract aggravate the course of the disease as they may precipitate acute exacerbations, which are very common in patients with COPD. Moreover, they have a substantial impact on the patients' quality of life, especially as a result of the documented muscle mass and bone mineral density loss after hospital discharge for COPD acute exacerbations [1-9].

Preclinical models of COPD have been developed in the last decades. In those models COPD/emphysema has been commonly induced by using different toxic agents such as elastase, proteolytic enzymes, and chronic exposure to cigarette smoke (CS). These models have proven to be useful to identify the cellular, molecular, biochemical, and biological structures that are altered in the lungs and airways of the animal models. Moreover, they are also useful to identify the structural and functional alterations taking place in the respiratory system. Knowledge emerging from the preclinical models contributes to a great extent to the design of therapeutic strategies that may be eventually used in clinical settings of patients with

COPD. In the current review, an overview on the preclinical models that have been most widely used so far to better characterize COPD and disease progression is given. The review has been based upon the most recent publications found in the literature in this specific field. The most relevant features of preclinical models of COPD, namely chronic exposure to CS and other agents (proteases and microorganisms), cardiovascular effects, surfactant protein-D, airway remodeling and inflammation, lung regeneration, and mesenchymal stromal cell therapy have been described in the review below (Figure 1).

2. SEVERAL CONCEPTS ON THE RESPIRATORY SYSTEM

2.1. Anatomy: basic understanding

Transportation of air to the lungs and from regions of gas exchange takes place through complex structures: nose, larynx, trachea, and the bronchi. In the respiratory tract, several types of cells are found that confer structure and immune protection against microorganisms and toxics. In humans and small and large laboratory animals, the respiratory system is divided into left and right lungs at the bifurcation of the trachea. From a cellular standpoint, it should be mentioned that from the trachea to the midlevel intralobar airways, different cell types have been identified: ciliated, mucous, and basal cells together with submucosal glands. The different cellular types that conform the structure of the lungs play individual roles in the pathophysiology of acute and chronic conditions [10]. Moreover, differences exist in the type of cells and structures among several species. Despite the reported differences, laboratory animals are commonly used within the frame of basic and translational research. They enable scientists to study the pathophysiological mechanisms and biological effects of inhaled environmental particles and noxious gases in the respiratory tract, namely the lungs and bronchi [10].

2.2. Preclinical models of COPD

COPD is one of the most relevant causes of death worldwide. CS and air pollution represent the most relevant etiologic factors of COPD. In certain regions (Asia), the burden of COPD is already greater than that reported in Western countries [11]. Management of comorbidities in COPD patients imposes a huge burden in health-care utilization resources in our societies. Mostly available therapies have focused on the treatment of respiratory symptoms. However, therapies for COPD comorbidities are ineffective or inexistent. Therefore, there is a need for the identification of the mechanisms linking COPD to its comorbidities with the aim to design specific therapeutic strategies. On this basis, preclinical models of COPD and its comorbidities have been developed in the last few years. These concepts will be reviewed in the next sections.

3. ANIMAL MODELS OF COPD: CS EXPOSURE

3.1. Models of CS exposure with focus on the lungs

As CS is the most important etiologic agent in COPD, models of smoking animals are currently widely used to study alterations in lung physiology, pathology, and biology. Moreover, preclinical models of COPD are also broadly used to test the effects of drugs on animal models. Nonhuman primates have been used as model of environmental CS to study the effects of early-life exposure to second-hand smoke [10]. In fact, a wide variety of animals such as sheep, dogs, monkeys, guinea pigs, and rodents have been used in preclinical studies of COPD. Nonetheless, mice are counted among the most popular animals used by investigators worldwide.

The advantage in using mice relies on the ability to produce genetically modified animals that allow researchers to study the role of specific genes and proteins involved in the pathophysiology of COPD. Besides, certain strains of mice are more

sensitive to the effects of cigarette smoke, thus making them more useful to study lung pathobiology. In addition, the cost of mice is relatively low compared to that of other species and there are also many antibodies available to conduct laboratory experiments for the identification of antigens and cellular processes. Smoking mouse models have proven useful to analyze the effects of CS on the airways and lungs as well as to identify the potential beneficial effects of certain drugs [12-14].

3.2. Models of CS exposure with focus on the systemic comorbidities

In general, chronic exposure to CS induces loss of body weight and muscle mass in animal models [12-18]. As such mass of the hind limb muscles of mice chronically exposed to CS significantly decreased, especially of the fast-twitch gastrocnemius and tibialis anterior muscles and the slow-twitch soleus [18]. Importantly, grip strength and aerobic endurance were also reduced in the same animals [18]. Genes involved in the process of muscle anabolism and catabolism were also altered in the mice chronically exposed to CS [18]. In other investigations it was also shown that muscle capillarization and cross-sectional area were reduced in the muscles of mice chronically exposed to CS [16;18]. In summary, the reported studies have demonstrated that chronic exposure to CS in mice induces effects in organs other than the lungs in a similar fashion to what happens in patients with COPD. In this regard, the function, structure, and metabolism of the hind limb muscles were also affected in the mouse models of chronic exposure to CS. Other features of the systemic manifestations (metabolic alterations and cardiovascular manifestations) of COPD have also been studied using the same models of chronic exposure to CS [15;17]. These are very important findings, since they reveal that these models can be used to target therapeutically several aspects of COPD. These mouse models put

the line forward the validity and reliability of making them available for the study of the extrapulmonary manifestations of COPD.

3.3. Animal models of COPD other than CS exposure

Aqueous extracts of CS as well as intratracheal or intranasal administration of proteases (elastase or papain) (for review see [19] can also be used to study emphysema and the effects of COPD-associated systemic manifestations. Furthermore, other models in which mice are treated with antibodies against endothelial cells or the receptor of vascular endothelial growth factor (VEGF) have been also used to induce alveolar septal apoptosis, oxidative stress, and in the end emphysema in their lungs [19-21].

Other models are characterized by the exposure of the mice to microorganisms or to certain microbial components such as lypopolysaccharide (LPS), which also induce features of COPD [19;22]. Interestingly, COPD exacerbations may be studied in mouse models exposed to emphysema-inducing agents (e.g. elastase or CS exposure) along with infections induced as a result of inoculation of viruses or other microorganisms [19;23]. Exposure to air pollutants, ozone, and genetic models based on the overexpression or deletion of certain genes such as alfa-1 antitrypsin have also demonstrated to be of interest for the study of aspects of COPD both on the lungs and airways and the systemic manifestations [19;24].

4. MODELS OF CARDIOVASCULAR EFECTS ASSOCIATED WITH COPD

Cardiovascular complications are also major comorbidities associated with COPD in patients. In 2016, an excellent review [19] provided a comprehensive overview centered exclusively on the mouse models that are currently available for the study of

cardiovascular comorbidities associated with COPD. In the present review, a brief description of the most commonly used mouse models is provided below.

In patients, cardiovascular disease mostly results from atherosclerosis, characterized by the formation of atheromatous plaques (atherogenesis) that leads to injury of the endothelium, inflammation, oxidative stress, and migration of circulating monocytes into the intima [19]. Dyslipidemia and systemic inflammation are two major contributors to the process of atherosclerosis. Wild type mice do not develop dyslipidemia, thus genetic modifications have been induced in order to study the cardiovascular effects in the mouse models. For extensive review on the genes that are usually overexpressed or deleted in those models the authors recommend reading reference [19] for their interest. Briefly, the classical murine models to study atherosclerosis are the *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice. In these animals, the hepatic ApoE/LDLr-mediated lipoprotein clearance pathway is disrupted. This leads to increased levels of VLDL/LDL in the blood circulation of the mice [19;25].

Importantly, mouse models in which several aspects of COPD along with cardiovascular comorbidity are studied together have also been described in the literature. Again the authors recommend reference [19] to gain insight into the specific models and aspects analyzed in the different publications focused on the combinations of COPD and cardiovascular mouse models. Briefly, several models have been used with the aim to elucidate key aspects on the potential associations between COPD and cardiovascular comorbidity. In line with this, mouse models have been used in order to explore the potential role of CS along with respiratory infections in the development of systemic inflammation, as well as the role of chronic CS exposure in hyperlipidemia [19]. Other relevant aspects such as the study of oxidative stress and endothelial dysfunction as potential mechanisms linking COPD

and atherosclerosis have also been a matter of research in several important publications as previously reviewed [19].

Patients with COPD may also experience pulmonary hypertension, especially as disease progresses [26]. A well-known validated and reproducible experimental model of chronic heart failure in rats has been widely used in the literature [27-35]. As such, rats treated with monocrotaline, a pyrrolizidine alkaloid extracted from plants, experienced pulmonary hypertension, right ventricle hypertrophy, and severe muscle wasting in all the muscles, especially those from the limbs [27-35]. Monocrotaline induces pulmonary mononuclear vasculitis and arterial medial hypertrophy, dysregulation of nitric oxide signaling, and right ventricular hypertrophy in rats but not in other rodents. This model is well-suited to study therapeutic strategies targeted to attenuate the levels of pulmonary hypertension [27-35].

5. ANIMAL MODELS OF SURFACTANT PROTEIN-D

Surfactant protein (SP)-D is a pattern-recognition molecule that belongs to the collectin family of proteins. These proteins contain collagen-containing C-type lectins (carbohydrate-binding proteins) which play many roles in biological recognition [36]. The primary function of SP-D is represented by binding of bacteria, viruses, fungi, and helminthic parasites that are cleared via opsonization for phagocyte recognition by neutrophils for further bacterial and fungal cell-membrane lysis [36-39].

5.1. Animal models of SP-D modifications and effects

The SP-D knockout mice (transgenic *Sftpd-/-* mice) were generated to assess whether SP-D Met11 or Thr11 allelic variants are involved in disease. Mild

emphysema develops in the lungs of the transgenic mice along with the presence of foam cell-like macrophages [36;40-42]. Accumulation of surfactant lipids and proteins takes place in the lungs of these animals and are mildly obese [36]. Thus, the SP-D knockout mice represent a good model to study lung inflammation and innate immunity as well as to conduct investigations on lung surfactant homeostasis [36].

SP-D may experience several biochemical posttranslational modifications that are also involved in respiratory disease. Glycosylation variants, nitrosylation, oxidative damage or proteolytic degradation are counted among the most relevant biochemical modifications experienced by SP-D [36]. For instance, proteolytic degradation of SP-D has been documented during acute lung injury and in patients with cystic fibrosis [36]. All these alterations in the expression and activity of SP-D can be studied using specific animal models to reproduce human disease [36].

5.2. Increased SP-D levels in serum

Loss of integrity of the air-blood barrier leads to the outward intravascular leakage of lung secreted proteins, while an inward flooding to the interstitium and air spaces takes place [36;43]. Serum SP-D levels may increase in response to acute and chronic exposure to CS [36]. In fact, smoking status is a strong predictor of the translocation of SP-D from the lungs to the bloodstream [36;43;44]. Increased levels of circulating SP-D are associated with mortality in respiratory diseases such as COPD, idiopathic pulmonary fibrosis, and acute respiratory distress syndrome [36;45-47].

Importantly, genetic or phenotypic SP-D variation was also shown to be associated with acute lung injury/acute respiratory distress syndrome ([36;47], lung injury in critical illness [48], community-acquired pneumonia [49;50], viral infections, asthma, lung cancer, pulmonary aspergillosis, interstitial lung disease, and COPD [36].

Different aspects of SP-D expression and activity in the lung and blood that are associated with human respiratory disease (e.g. smoking and COPD) can also be studied using the SP-D knockout mice [36].

5.3. Biological and clinical implications of SP-D on COPD

Importantly, SP-D regulates the function of a wide array of cells such as immune, epithelial, and smooth muscle cells, and fibrocytes. SP-D exerts its effects through the action of several receptors in different cell types. We recommend reference [36] for a comprehensive overview on the different receptors and cell types.

Associations of polymorphisms with COPD and emphysema and even survival have been identified and extensively reviewed [36]. The most relevant conclusions from the different results were that variants associated with greater SP-D levels were associated with a lower risk of COPD and delayed the decline of lung function in the patients [36;51;52]. CS exposure induces a significant reduction in the alveolar levels of SP-D in patients [36;52;53]. In vitro observations have also demonstrated that nicotine causes a reduction in SP-D levels in human airway epithelial cells [36;52;54]. Whether SP-D might be used as a biomarker to differentiate smokers from COPD patients or even from those with asthma remained debatable as published results were not entirely conclusive [36;52]. In COPD patients, disease severity was not associated with the levels of SP-D as reported in the ECLIPSE study [36;52].

Emphysema, progressive septal wall thickness, and subpleural fibrosis developed in *Sftpd*$^{-/-}$ mice [36;42;52], thus suggesting that these animals presented a combined phenotype of emphysema and fibrosis in the lungs. Other biological effects attributable to the reduced levels of SP-D in the lungs were its potential role in alveolar macrophage activation, oxidant production, and the activity of matrix metalloproteinases, which may lead to emphysema-like and fibrotic changes in the

lungs of the animals [36;42;52]. More specific details on the underlying biology of these genetically deficient mice can be found in reference [36].

6. AIRWAY REMODELING: ROLE OF INFLAMMATION

COPD is characterized by nonreversible airway obstruction and neutrophilic inflammation in response to CS and/or environmental exposure. Structural remodeling of the airways takes place in patients with COPD. Acute exacerbations play a significant role in disease progression and loss of lung function in patients with COPD. Furthermore, airway remodeling may also be influenced by the severity and number of acute exacerbations. Remodeling of the airways is defined by the structural changes taking place as a result of collagen deposition in the subepithelial basement membrane, disruption of the epithelial barrier, mucous metaplasia and/or mesenchymal transition, and smooth muscle hypertrophy [55;56]. These structural events lead to a narrowing of the airways, especially of the smallest ones, along with obstruction and reduced lung compliance [56]. Morbidity and mortality of the patients is increased as these events progress. Inflammation is a strong mediator of these events.

6.1. Preclinical models of airway remodeling: role of inflammation

Models of RNA viruses and other allergens have demonstrated that these molecules trigger the innate inflammatory response, which is characterized by an initial neutrophilic response and activation of CD8 memory T cells [56]. Different types of cells, events, and structures are involved in the inflammatory response: airway epithelial cells, bronchiolar-derived epithelial cells, oxidative damage, and mesenchymal transition, and finally airway remodeling [56]. All these particular

aspects of airway remodeling that are so relevant to disease progression and prognosis can be studied using specific animal models.

7. ROLE OF THE MESENCHYME IN COPD

Mesenchymal transition is the result of repetitive innate stimuli mediated by nuclear factor (NF)-KB and bromodomain-containing protein (BRD)4, which in turn alters histone acetyltransferase activity [56]. Fibrosis of airways is the result of the action of myofibroblasts, which are mesenchymal-derived cells responsible for the excessive deposition of extracellular matrix in the subepithelial basement membrane. Myofibroblasts are highly dynamic cells that are distributed throughout the subepithelium and stroma [56]. Its rapid progression takes place in response to viral infections and in the airways of asthma patients [56;57]. Different types of cells such as resident mesenchymal cells, epithelial and endothelial cells undergoing epithelial-mesenchymal transition (EMT), and circulating bone marrow stem cells (fibrocytes) may contribute to the formation of myofibroblasts in the lungs and airways of patients with chronic obstructive pulmonary diseases [56;58;59] or during acute exacerbations of their disease [56;57].

7.1. Preclinical models of mesenchymal transition inhibition

Nonselective inhibitors of BRD are currently being used for the treatment of cancer and metabolic and cardiovascular diseases [56;60]. Recently, more selective inhibitors of BRD4 that specifically target airway remodeling have been developed in which a pharmacophore model was used [56;60;61]. It should be mentioned that by definition a pharmacophore is a group of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target to trigger (or block) its biological response [62]. The purpose of those drugs is

to decrease the levels of neutrophilic inflammation in the airways as well as to prevent the change of the epithelial cell state to avoid/reduce myofibroblast growth in response to viral and/or allergen exposures in patients.

7.2. Mesenchymal stromal cells

Recently, the line has also been put forward that mesenchymal stromal cells (MSCs) may also be used as a therapeutic strategy for the treatment of COPD (for extensive review see reference [63]). MSCs are non-hematopoietic cells that have the ability to differentiate into multiple lineages of the mesenchyme, namely chondrocytes, osteoblasts, and adipocytes. Data emerging from preclinical investigations have demonstrated that they can be used for the treatment of several respiratory diseases including asthma and COPD [63].

In lung injury models, therapy with MSCs have shown to exert several beneficial effects such as reduced inflammation, antimicrobial actions, and promote lung epithelial and endothelial repair [63].

In preclinical models of COPD, in which emphysema was induced using different agents (elastase, proteolytic enzymes, and chronic exposure to CS) promising effects (greater numbers of proliferating cells, reduced apoptosis, along with improvements in gas exchange and exercise tolerance of the animals) were also seen in response to treatment with MSCs [63]. Other positive effects have also been described in response to treatment with MSCs in preclinical models of COPD (for extensive review, see reference [63]): anti-inflammatory effects, promotion of lung tissue repair, paracrine effects, and restoration of endothelium integrity. Nevertheless, the field is moving cautiously as more placebo-controlled will still be needed in order to better define the efficacy of MSCs in COPD patients. In keeping with, animal models of COPD used so far were not entirely suited to be applied in

clinical research. Several aspects of the models such as the great numbers of cells and the lack of more acute models that enhance the efficacy of the MSCs accounted for the lack of translation of MSC therapy from preclinical results to actual COPD patients [63]. Additionally, more invasive readouts were used in the mouse models of MSC therapy than in clinical studies (e.g. quality of life and lung function testing) [63]. Nonetheless, despite these concerns treatment of COPD patients with MSCs looks promising given the ability of these cells to favor airway and endothelial repair as well as to restore lung tissue architecture in emphysematous lungs as demonstrated in the animal models [63].

7.3. Lung regeneration strategies

Given the regenerative potential of the lungs and airways, alternative therapeutic strategies targeted to boost that potential would be of interest for the treatment of COPD patients in the near-future. Preclinical investigations have yield interesting results showing the regeneration potential of the lungs in animal models of emphysema [64;65]. However, these approaches need more confirmatory results emerging from clinical investigations. More research should be devoted to identify the molecular players involved in the regenerative potential of the lungs and its control.

8. CONCLUDING REMARKS

COPD is a leading cause of morbidity and mortality worldwide. Although we understand better the pathophysiology of the disease especially that concerning the airways and lungs, more research is still needed to identify the factors that explain the extra-pulmonary manifestations of the disease. Despite that currently available therapies have demonstrated to be useful for the relief of the respiratory symptoms and exercise tolerance, effective therapies are yet to be identified for the treatment of

the systemic manifestations of the disease. Preclinical models based on the use of laboratory animals, with a special emphasis on mice have proven useful to conduct mechanistic research that has shed light into pathophysiological insights and biological pathways that have helped design effective therapeutic drugs in the last decades. Nonetheless, more research is still warranted to identify novel mechanisms, especially those attempting to foster the regenerative potential of the lungs and airways. Elucidation of the key molecules and pathways that control lung regeneration will definitely contribute to the design of specific therapeutic targets for the better treatment and care of patients with COPD (Figure 2).

9. EXPERT OPINION

As we have learned from different epidemiological studies COPD is a very prevalent disease which is already a leading cause of morbidity and mortality worldwide [2;4], with special emphasis in certain geographical areas like in Asia. Therefore, the burden imposed by the disease into the health-care systems is very high and worrisome. COPD is also a preventable disease as its main etiologic agent is tobacco exposure. Additionally, occupational exposure and/or air pollution may also contribute to the development or aggravation of COPD. In the last decades, clinical and basic research has identified relevant pathways that have enabled scientists to design very effective therapeutic targets for the treatment of the respiratory symptoms in patients with COPD. The candidate drugs may also induce beneficial effects on the exercise capacity component in the patients as a result of decreased pulmonary hyperinflation [2;4]. Nevertheless, currently available drugs mainly target airway bronchoconstriction and inflammation but do not exert any effects on the destruction of the airways or lung parenchyma, which are key biological events in the emphysematous lungs. In fact, knowledge on the underlying biology of the

regenerative potential of the lungs and airways is scarce and further research is still needed in order to identify key molecules and regulatory pathways that could be targeted therapeutically.

On the other hand, most of COPD patients also experience non-respiratory symptoms derived from alterations of organs other than the lungs [2;4;19]. A wide range of extra-pulmonary manifestations have been described in the patients with a different degree of involvement in their exercise capacity, quality of life, and mortality. Skeletal muscle dysfunction and mass loss or sarcopenia, malnutrition, and cardiovascular events are counted among the most prominent comorbidities in patients with COPD [2;4;19]. They have been clearly demonstrated to negatively influence disease progression and prognosis irrespective of the degree of the airway obstruction (event used for the classification of COPD severity). Furthermore, in the clinical assessment of COPD, the patients usually refer complaints related to their systemic manifestations, namely difficulty in the performance of their daily life activities as a result of exercise intolerance, in which factors such as sarcopenia, cachexia, congestive heart failure, and dyspnea play a determinant role [2;4;19]. Taken together, this scenario creates a vicious circle in which muscles become progressively weaker due to sedentarism and immobilization. Surprisingly, very little attention has been and is still being devoted to the systemic manifestations of COPD in the literature or scientific symposia. The lack of effective drugs targeted to palliate these comorbidities may account for such a shortcoming in COPD research, both clinical and basic [2;4;19].

Other common comorbidities in COPD are lung cancer, pulmonary hypertension, diabetes, and osteoporosis [2;4;19]. All these comorbidities have a great impact on the patients' survival and quality of life. Animal models in which these comorbidities can be studied simultaneously simply do not exist. Indeed, this is a major limitation of

the animal models of COPD, since it is not possible to reproduce what actually happens in clinical settings with a great amount of the patients. In this regard, specific animal models must be used to study particular aspects of COPD comorbidities. An approach that enables us the evaluation of the most common comorbidities in a single animal model of COPD is not as yet possible. This represents a major weakness in the use of preclinical models of COPD, especially if the impact of comorbidities on disease prognosis and quality of life are to be thoroughly studied. In fact, this is a major limitation in translational research in general, especially in the study of chronic respiratory diseases. Furthermore, other aspects inherent to the life style of the patients, medication, active smoking (despite this being the first step in the treatment of COPD), daily physical activity, alcohol intake, and the type of diet/nutritional status are also factors that play a substantial role in the development/progression of the systemic comorbidities in COPD [2;3; 4;6]. All these factors are not usually taken into consideration in the design of preclinical models of COPD. Indeed, they should be analyzed independently in different animal models with the corresponding control groups, which represent a real challenge/burden in translational respiratory research.

On the other hand, preclinical models based on the use of laboratory animals have also proven very useful to acquire knowledge on the pathophysiological and biological mechanisms involved in the development of COPD and its progression [18]. In basic respiratory research, mice are the most commonly used animal model as they are simple to handle, they can be easily manipulated genetically, many strains are accessible, and probes and other laboratory reagents are also commercially available to conduct biological experiments [18]. Indeed, in the last few decades COPD research conducted on the basis of mouse models has shed light into a great deal of biological mechanisms and regulatory pathways that could be

eventually therapeutically targeted. The most relevant and promising pathways are those involving the study of the regenerative potential of the airways and lungs in these mouse models of COPD, namely emphysema [63]. However, these investigations have yield very preliminary results that will need further research in actual patients with COPD, in which ethical constraints of the existing policies may jeopardize the progress of its clinical applicability in the very near-future. Furthermore, more research is also needed in order to fully characterize the precise pathways and mechanisms whereby MSCs act in the lungs of COPD patients. Preclinical models other than animals such as microfluidic lung-on-a-chip, organoids, and ex vivo lung perfusion models may help find answers to unresolved questions with regards to the cure of COPD [63].

Despite the clear benefits of the preclinical models of COPD, several concerns should also be raised. The reported models do have important limitations that are worth mentioning. Changes in susceptibility to CS exposure of different mouse strains, differences in the anatomy of the respiratory system between patients and mice, and the lack of airway involvement in the CS exposure mouse models as compared to humans are counted among the most relevant weaknesses of the mouse models of COPD [63;66]. Other constraints such as tissue collection (more invasive procedures in animal models) and preparation may also influence the final results obtained from the preclinical models as opposed to tissue sampling (less invasive approaches) in COPD patients. In spite of these considerations, the mouse models of COPD are deemed suitable for the study of its underlying mechanisms and the design of potential therapeutic strategies for the better care of COPD patients [19;67;68].

Another important challenge in the translation of research conducted on preclinical models of COPD is that related to exacerbations. Despite that infections, especially

viral infections, constitute the most frequent etiology of exacerbations, several patient subtypes are more prone to suffer from COPD exacerbations than others or even experience more severe episodes. This scenario imposes a major challenge in the research devoted to the analyses of the particularities (severity, length, hospitalization stay, and therapies required) of COPD exacerbations using animal models [22;23]. Identification of COPD patients with a greater susceptibility to develop exacerbations is a paramount objective in clinical research. Likewise, elucidation of the underlying mechanisms that prompt COPD patients to develop exacerbations is also of utmost importance in translational research. Nonetheless, extrapolation of knowledge emerging from animal models is limited, since the inflammatory response of the host against viral/bacterial infections may vary widely among patients, which may also influence their prognosis following acute exacerbations [22;23]. Moreover, medications administered to the patients to resolve the acute episode also influence the severity and duration of the exacerbation and may, in turn, have a negative impact on the patients' skeletal muscle mass and function (e.g. corticosteroids). In fact, it has been well-established that the number and duration of the exacerbations are major contributors to sarcopenia and cachexia in patients with severe COPD. These specific aspects can be barely approached on the basis of preclinical models of COPD, thus representing another major challenge in COPD translational research.

As abovementioned COPD is a very heterogeneous disease with important manifestations in the lungs and airways as well as in other organs. Furthermore, as a chronic disorder the status of the patients may vary widely throughout their lives on the basis of factors such as therapies, physical activity, nutrition, and the number of exacerbations. In this respect, animal models usually enable us to study aspects of the disease mainly on the basis of cross-sectional investigations or with a very short

follow-up [17;18]. Longitudinal studies of COPD animal models, however, are costly, time-consuming and hard to be translated, since not all the aspects can be integrated in a single model as also discussed above [17;18].

In COPD research, identification of the most relevant clinical phenotypes is paramount as diverse therapeutic strategies will be applied to the different types of patients [1-4]. Moreover, research should also be devoted to enable the identification those active smokers who are placed at a greater risk to acquire COPD for the same degree of cigarette smoking burden, as not all smokers develop airflow limitation throughout their existence [12-14]. Elucidation of the underlying mechanisms that prompt some of the smokers to develop COPD is a major challenge in translational respiratory research. These mechanisms will help predict those smokers with a greater risk to develop airway obstruction related to cigarette smoking. Animal models of COPD do not enable us to fully establish the mechanisms that account for the phenotypes of patients with a greater risk to develop COPD due to the complexity of this disorder and the different compartments that are affected in the patients. In view of the currently available preclinical models of COPD it is not possible to study all the systemic manifestations of COPD along with the airways and lung disease on the basis of a single experimental model.

It should also be mentioned that the discoveries made in the animal models must be tested in the patients in clinical settings. This requires the design of clinical investigations in which the mechanisms identified in the animals must be confirmed in the patients. Importantly, when therapeutic strategies shown to be effective in the animals are to be tested in the patients, clinical trials must be carried out [17;18]. The design of clinical trials may be specifically tailored to target groups of patients according to their phenotype and degree of airway obstruction. However, this usually

implies a long process through which the translation emerging from the experimental models can be put into practice in clinical settings.

Despite all these concerns preclinical models of COPD, particularly those conducted on animals continue to be very valuable tools from which novel mechanisms will be discovered/identified. New therapeutic strategies will also be designed for the care and treatment of patients with COPD on the basis of mechanisms and pathways identified in the animal models [17;18]. The use of animal models is advantageous as certain types of samples are more easily available than in patients [17;18]. In addition, different types of drugs can also be tested in the animal models in an easier manner prior to the demonstration of their safeness and effectiveness in clinical settings [17;18].

In the field of COPD, the best scenario will be to continue working with preclinical models along with the translation of the target mechanisms and therapies in the patients. For the time being, these tools need to be made more available to all the community interested in the translation of research conducted in the field of COPD [17;18].

In summary, despite the limitations abovementioned, preclinical models of COPD are key elements for the design of therapeutic targets with a special focus on the identification of regulatory pathways of the regenerative potential of the lungs and airways as well as on the mechanisms involved in the extra-pulmonary manifestations of the disease. Increased knowledge on the most relevant biological insights will help design therapeutic targets for the treatment of patients with COPD. This will enable health caregivers and doctors to better treat their patients, in which a more holistic approach will be used. This will benefit the patients and the society as the use of resources due to COPD should be significantly diminished (Figure 2).

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FIGURE LEGENDS

Figure 1: Schematic representation of the different models of COPD that have been reviewed in the article.

Figure 2: Schematic representation of the usefulness of the animal models of COPD to identify mechanisms that may lead to the design of therapeutic targets that will benefit the exercise capacity and quality of life of patients with COPD. Animal models should be designed to target aspects of the respiratory system (lungs and airways) as well as the systemic manifestations, which greatly contribute to the quality of life and prognosis in COPD patients.

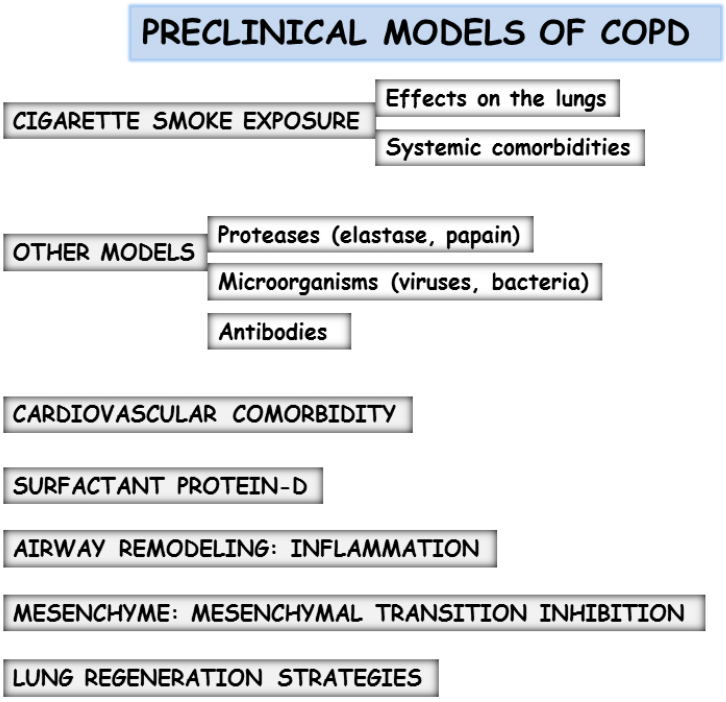

Figure 1

Figure 1

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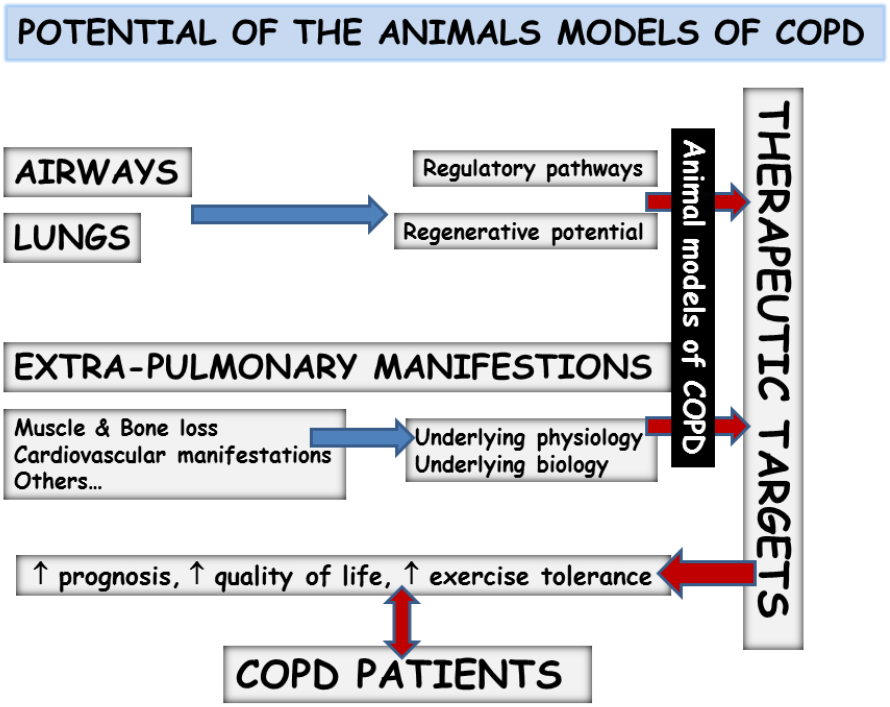

Figure 2

Figure 2

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