Journal of
Pathology and Allied Medicine

JScholar
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Research Article Open Access

Respiratory Bronchiolitis and Hypersensitivity Bronchiolitis– Early TGF-β Expression

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Received Date: March 15, 2022 **Accepted Date:** May 22, 2022 **Published Date:** May 24, 2022

Citation: Sá P, Figueiredo MI, Ladeirinha A, Alarcão A, Carvalho L (2022) Respiratory Bronchiolitis and Hypersensitivity Bronchiolitis– Early TGF-β Expression. J Pathol Allied Med 2: 1-10.

Abstract

Introduction: Clinical prevention of pulmonary fibrosis may benefit from morphological interpretation of early lesions in Bronchiolitis, focusing on epithelial and mesenchymal remodulation report. This study was designed to concise bronchiolar epithelial and parietal mesenchymal remodeling in Pathology reporting directed to preview therapy decisions when symptoms may benefit from clinical treatment and pulmonary rehabilitation.

Methods: Respiratory Bronchiolitis (RB) and Hypersensitivity Bronchiolitis (HB) cases were selected, and a series of 45 surgical biopsies was divided in two control groups: 1 - normal looking pulmonary parenchyma in surgical biopsy of patients with spontaneous pneumothorax (5 cases) and 2 - chronic bronchiolitis designated after bronchiolar scarce lymphocytic parietal infiltration (11 cases), then to be compared with RB (15 cases) and HB (14 cases). Morphological alterations were registered for bronchiolar luminal, basal and parietal cells. TTF-1, CK5/6, Vimentin, CD10 and TGF-β antibodies were applied to study epithelial-mesenchymal transition and cellular adaptation.

Results: RB and HB presented with Vimentin, TGF-β, CD10, and CK5/6 positive basal cells and cylindrical/ciliated luminal metaplasia, with lower TTF-1 expression in luminal cells, when compared with normal looking bronchioles and chronic bronchiolitis.

Conclusion: Although concerning a limited series, bronchial metaplasia of bronchiolar epithelium was relevant after CK5/6 positive basal cells and TTF-1 negative cylindrical ciliated cells. Expression of TGF-β and CD10 by parietal, basal and luminal cells indicated epithelial – mesenchymal modulation. New therapy implications to prevent interstitial fibrosis in early disease .stages may be on its way to become a routine procedure after patient counseling and respiratory-defined exercise

Keywords: TGF-β; CD10; Respiratory Bronchiolitis; Hypersensitivity Bronchiolitis

Introduction

Transforming growth factor-β (TGF-β) is a pleiotropic factor implicated in lung organogenesis, tissue regeneration and tumorigenesis [1]. TGF-β expression is also crucial for epithelial-mesenchymal interactions during lung morphogenesis and, after different types of bronchiolitis, several growth factors and ECM intermingle to originate the epithelial-mesenchymal transition (EMT) [1]. It possesses both immunomodulatory and fibrogenic properties, namely inducing Th17 cell differentiation and macrophage recruitment, thereby playing a fundamental role in airway remodeling [2]. TGF-β appears then to be a critical mediator of the balance between extracellular matrix (ECM) destruction and production, and alveolar epithelial cells were shown to have high expression of TGF-β in Idiopathic Interstitial Pneumonias (IIPs) [3].

CD10 (CALLA, common lymphoblastic leukemia antigen) is a cell surface endopeptidase expressed by fibroblasts that inactivates several growth stimulatory peptides and has been recognized in cellular stemness, immune surveillance and in tumoral stroma carcinogenesis, being related with an adverse prognosis in various epithelial malignancies [4,5]. As dynamic remodeling of the ECM in lung tissue is highly regulated by TGF-β, it has been postulated that TGF-β and CD10 expression in pulmonary mesenchymal and epithelial cells deserve interpretation for early therapeutic decisions, due to their function in EMT intermingling of epithelial, myoepithelial and myofibroblastic cells in persistent inflammatory stimulation [6,7].

The classification of IIPs concerns airway-centered, lobular-centered and combined diseases affecting differently the bronchioles, and respiratory bronchiolitis – interstitial lung disease (RB-ILD), airway-centered interstitial fibrosis (ACIF), cryptogenic organizing pneumonia (COP) and hypersensitivity pneumonitis are recognized as mainly related to inhaled agents. Interstitial pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP), desquamative interstitial pneumonia (DIP), acute interstitial pneumonia (AIP), and probably pleuropulmonary fibroelastosis, either idiopathic and multifactorial, may correlate with clinicopathological relevance for the host´s immune characteristics [8–10]. The combined interstitial lung diseases group, where smoking related interstitial fibrosis (SRIF) and hypersensitivity pneumonitis (HP) form the largest group of interstitial lung diseases, microscopically show chronic bronchiolar-pulmonary inflammation along with epithelial remodeling, which might provide background for carcinogenesis [3,11,12].

There have been recognized two subtypes of HP: nonfibrotic/cellular HP, and fibrotic HP. The histopathological diagnosis of nonfibrotic HP requires: bronchiolocentric cellular interstitial pneumonia, cellular chronic bronchiolitis, poorly formed non-necrotizing granulomas with clusters of epithelioid cells and macrophages, and absence of features suggesting an alternative diagnosis [12]. Fibrotic HP differs from the nonfibrotic by the presence of fibrosis [12].

Bronchiolitis, as a generic term applied to many inflammatory diseases affecting the bronchioles [13], encompasses a wide spectrum of non-tumoral pulmonary diseases with heterogenic clinic and pathological features, as well as distinct and complex etiopathogenesis. Bronchiolitis are also commonly recognized as idiopathic, primary or secondary types, related or not to bronchopulmonary or extrapulmonary diseases [13,14]. Clinical behavior varies from insidious and asymptomatic to profound dyspnea with restrictive or obstructive patterns on pulmonary function studies, and chest imaging may range from minimal subtle lesions until diffuse parenchymal infiltrates [13]. Bronchiolitis management demand clinical interdisciplinary comprehension and raise currently an intricate challenge to clinicians, radiologists, surgeons and pathologists.

The terminal respiratory unit (TRU) concept of Noguchi, where alveolar cells and nonciliated epithelium from distal bronchioles are supplied by common stem cells, also runs with the concept of cellular repair and remodeling in adult lung, similar to the process of organogenesis [15,16]. TTF-1, being considered the most sensitive and specific biomarker of adenocarcinomas originating in the TRU, when co-expressed with p63, has been suggested by Cabibi et al. to represent the capability of differentiation into several histogenetic lines [15].

TGF-β1 expression has been demonstrated in small airway epithelial cells among smokers and chronic obstructive pulmonary disease (COPD) patients, which may suggest that TGF-β signaling pathologically activated may be involved in the pathogenesis of emphysema [1]. However, tobacco-related morphological patterns of bronchiolar diseases and respiratory bronchiolitis (RB) and HP-related initial bronchiolar lymphocytic infiltrate, inflammatory myofibroblastic polyps/Masson bodies/foci of bronchiolitis obliterans and noncaseating granulomas, keep being patterns still not clearly integrated in clinical and therapeutic strategies [17-19].

This retrospective study was developed as an approach and attempt to comprehend parietal and epithelial bronchiolar remodeling in smokers bronchiolitis and hypersensitivity bronchiolitis (HB) samples. Archival surgical biopsies (study cases of the Anatomical Pathology of the University Hospital of Coimbra, Portugal) were used to search specific histopathological differential alterations, to be supported by routine immunohistochemistry either for clinical, early therapeutic and pulmonary rehabilitation decisions.

Materials and Methods

A group of 45 pulmonary representative surgical biopsies was selected, regularly collected from upper and middle lobes of patients whose clinical evolution demanded that approach: 15 cases belonged to smokers with histopathological pattern of RB and 14 cases belonged to patients with inhaling history after contact with chickens/birds, where the different patterns of HP had been identified. Two control groups were also collected: 11 cases of Chronic Bronchiolitis (CB) and 5 cases of normal lung tissue from patients with spontaneous pneumothorax (due to sub-pleural infancy infarct scars fragility). CB, classically referred as cellular bronchiolitis, was considered when few lymphocytes and plasma cells infiltration was present in bronchioles walls without morphological alterations of either epithelium or surrounding alveolar septae, concerning cases without other morphologic alterations.

The 4 groups of study were then represented by: spontaneous pneumothorax 3 males and 2 women between 16 and 87 years old; CB of 6 females and 5 males between 35 and 74 years old; RB of 13 males and 2 women between 21 and 54 years old; and HB of 8 females and 6 males between 44 and 72 years old. Figure 1 represents the four morphological patterns of each group of selected cases to integrate this study.

Rules defined by the Faculty of Medicine of the University of Coimbra Ethical Committee for retrospective studies were followed and patients definitive anonymity was guaranteed.

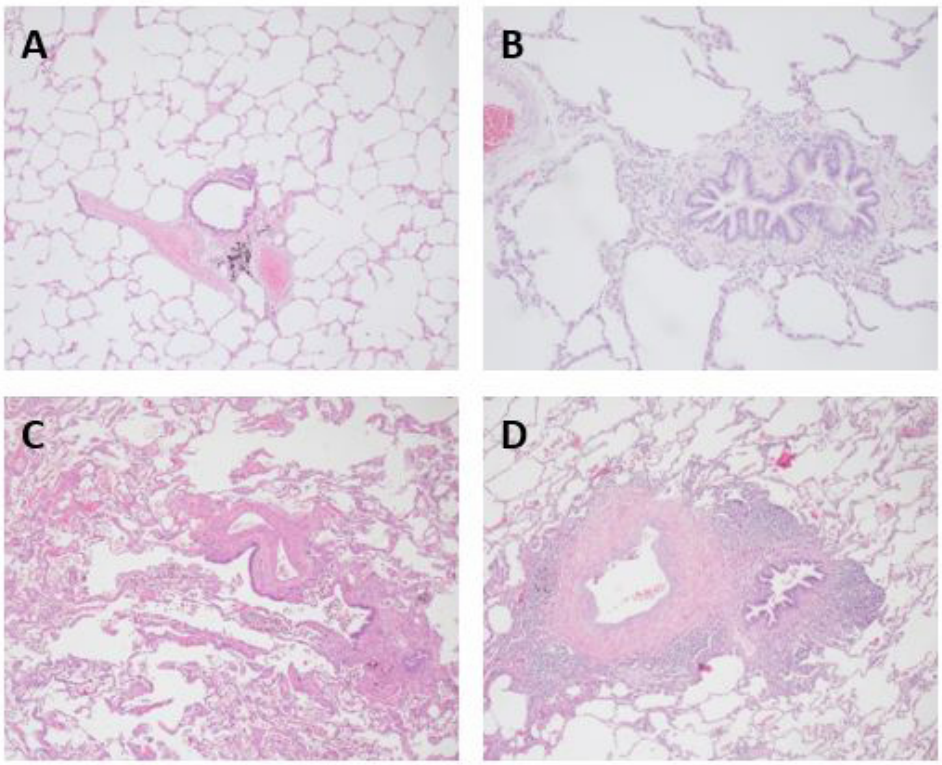

Figure 1: Normal looking bronchiole with thin wall and mainly linear unicellular epithelium with ambiental antracotic pigment retention, HE x 40 (A); Chronic bronchiolitis: parietal lymphocytic infiltration and fusiform cells hyperplasia, with cylindrical cells hyperplasia, HE x 100 (B); Respiratory bronchiolitis: scarce lymphocytic parietal infiltration and luminal pigmented macrophages, HE x 40 (C); Hypersensitivity bronchiolitis: lymphocytic parietal infiltration also with commitment of arteriolar wall, HE x 200 (D).

Methods

Morphological Registry

Pulmonary surgical biopsies had been formalin-fixed paraffin-embedded and redundant tissue was selected to evaluate morphological parameters and apply immunohistochemistry where bronchiolar remodeling concerning epithelial and parietal cells was present.

In each group of selected cases and due to the different volume of tissue available, a minimal number of twenty bronchiolar-vascular axes was considered to validate each case in order to register morphological alterations.

Bronchiolar epithelium changes in RB and HB were scored after the following parameters: ciliated cells presence, to be compared with Control Groups either said normal bronchioles and CB alterations, corresponding to bronchial metaplasia. Bronchiolar parietal fusiform cells were graded after less or more than 4 layers presence. Subepithelial and bronchiolar parietal lymphocytes and plasma cells were also registered.

Luminal inflammatory myofibroblastic polyps/Masson bodies were seen in some cases of HB.

Immunohistochemical Study

To sustain epithelial and mesenchymal bronchiolar remodeling in morphological registration, the following panel of immunohistochemistry antibodies was selected to register cellular adaptations, following the methodology advised by the manufacturer.

Vimentin was selected to register bronchiolar parietal cells expression, CD10 was applied to characterize early progenitor/myoepithelial cells, and TGF-β, referred as mainly produced by macrophages, was searched to determine its expression in either fusiform and epithelial cells, to enlighten mesenchymal and epithelial remodeling.

CK5/6 and TTF1, two epithelial markers to follow bronchial basal cell metaplasia and hyperplasia in bronchioles, respectively, were also applied.

The applied antibodies expression was then registered in the following cell types: bronchiolar epithelial cells, ciliated or not; bronchiolar basal cells and bronchiolar parietal fusiform cells.

Statistical Analysis

The immunohistochemical antibodies frequency was analyzed according to the different cell types positivity by applying STATISTICA 9.1 (StatSoft, Inc., 2009) based on the Qi-Square Test (2x3 and 2x4) with p-value <0,05 significance.

Results

Histopathological Distinction

In normal looking bronchioles, scarce parietal lymphocytes and plasma cells were present in the bronchioles wall where fusiform cells appear in less than four layers. In general, the epithelium was flat concerning simple cuboidal epithelium.
In the cases of CB, inflammatory infiltration became relevant by forming a delicate rim of lymphocytes in the walls of the bronchioles. In general, the epithelium became hyperplastic (8/11 cases) with ciliated cells (7/11 cases), representing bronchial metaplasia.

In the group of RB, the characteristics of inflammation registered for CB were present. Fusiform cells showed their persistence in bronchiolar walls (7/15 cases) with more than four layers.

In the cases of HB, inflammatory myofibroblastic polyps/bronchiolitis obliterans were present focally. Epithelial ciliated cells were seen in 5/14 cases and fusiform cells were present in four or less layers, similar to normal looking bronchioles. Lymphocytic infiltration was similar to CB.

Integrated Immunohistochemical Results

Bronchiolar epithelial and basal cells expressed nuclear TTF1 in all group cases. Bronchial-type basal cells identified with CK5/6 were relevant in CB (5/11 cases), RB (7/15 cases) and HP (8/14 cases).

The expression of TGF-β was seen in RB (10/15 cases) and HB (4/14 cases) epithelial cells, where basal and cylindrical cells expression was registered separately.

Parietal fusiform cells identified with vimentin expression became relevant in all cases, mainly in RB cases. Fusiform cells TGF-β expression was irrelevant in normal looking bronchioles and in CB cases. For RB and HB, the expression of TGF-β was observed in 5/15 cases of RB and 4/14 cases of HP.

Considering CD10 expression, it was observed in epithelial cells in 1/5, 2/11, 3/15 and 2/14 cases in each study group, respectively, control and disease cases.

The described results for epithelial and mesenchymal cells´ characterization are emphasized in Figure 2.

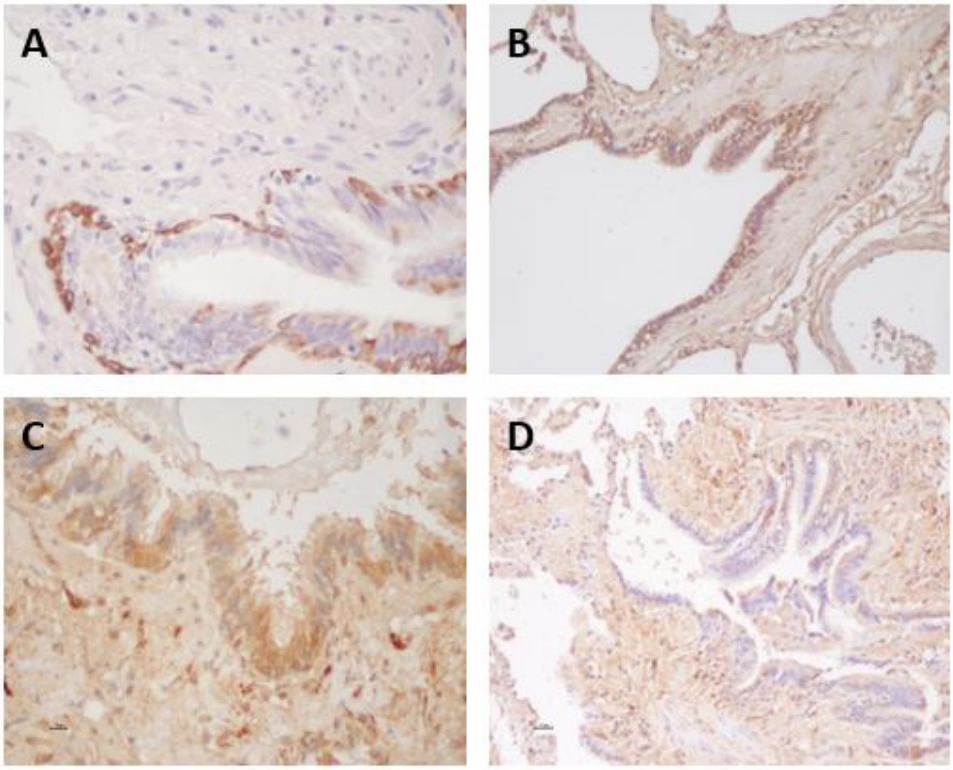

Figure 2: Chronic bronchiolitis: epithelial basal cells with CK5.6 expression corroborating bronchial metaplasia, CK5.6 x 400 (A); Respiratory bronchiolitis: TGF-β expression in epithelial cells, TGF-β x 200 (B); Hypersensitivity bronchiolitis: TGF-β expression in epitelial cells and wall macrophages, TGF-β x 400 (C); Hypersensitivity bronchiolitis: TGF-β expression in parietal fusiform cells and macrophages, TGF-β x 100 (D).

Statistical Cluster Analysis

When considering TGF-β and CD10, they were expressed randomly in cytoplasm and apical surface of epithelial cells.

In RB and HB, TGF-β has proved to be a bronchiolar basal cell marker with statistical value of p=0,003.

Discussion and Conclusion

Classically, the last segment of small airways concerns: membranous bronchiole still with smooth muscle cell sheath, terminal bronchiole with fibroblast-epithelial support, and respiratory bronchiole, already with the same function as the alveolar duct, without well-formed wall and without identifiable fusiform cells. Smoking-related diseases and HB chronic inflammation induced in small airways still deserve clearer morphological understanding as precursors of fibrotic diseases and carcinogenesis after mesenchymal and epithelial remodeling and cellular adaptation [20-24]. In clinical routine, both smoking-related diseases and hypersensitivity pneumonitis cause the largest number of complex cases discussed by Multidisciplinary Teams, with relevance to both pulmonary disfunction and development of pulmonary carcinoma in peripheral regions of the lung [11,25].

In our study, commonly used antibodies in pathology routine were used to stratify early lesions of tobacco-related diseases and early bronchiolar HP: CK5/6 and TTF-1 identified bronchial basal cells and Clara cells/pneumocytes type II, respectively, as expected, being useful to quote bronchiolar epithelium where CK5/6 expressing cells are usually absent. Vimentin, as the commonest intermediary filament in the cytoskeleton of mesenchymal cells, was applied to quote fusiform cells in bronchioles walls, as well as epithelial-mesenchymal remodeling when expressed by epithelial cells. In the studied samples, bronchiolar epithelium acquired bronchial characteristics in RB cases by adopting ciliated cells and TTF-1 remained the classical marker of TRU, while CK5/6 allowed to highlight basal epidermoid metaplasia in bronchiolar basal cells, where TTF-1 expression was replaced.

Since 2010 Anna-Luise Kazenstein recommendations, relevance has been given to "severe interstitial fibrosis in cigarette smokers with no evidence of interstitial lung disease", referring to the absence of commonly recognized histopathological patterns that characterize IIP. Instead, a list of morphological distortions of bronchial-pulmonary normal morphology for smokers, namely interstitial inflammation and fibrosis, fibroblast foci, peribronchiolar metaplasia, honey-comb change, emphysema and RB, have been recognized [8,20,26-28].

In mice exposed to tobacco smoke, several clusters of activated macrophages have been demonstrated in subpleural emphysema, RB and adjacent alveolar structures expressing TGF-β protein, with the frequency of TGF-β-positive alveolar macrophages increasing significantly with the duration of smoke exposure [29].

Airway remodeling is a feature of chronic bronchial diseases characterized by accumulation of fibroblasts and deposition of ECM, causing fixed bronchial restriction. Recently, EMT has been proposed to contribute to the remodeling of the airways, namely through secretion of TGF-β by alveolar epithelial cells during chronic aggression of the epithelium (provoked by allergens, infections, cigarette smoking and atmospheric pollutants), such as in diseases like asthma, COPD and bronchiolitis obliterans (BO) [30].

Bronchiolitis as a broad designation for inflammatory and potentially fibrosing diseases affecting mainly the lobular respiratory and transitional small airways, occur commonly in clinical practice and corresponds to inflammatory processes of the small airways and surrounding ducts and alveolar septae, present as early lesions of usually known etiology [6,19]. The role of EMT in these keeps being an open field where a pool of mesenchymal cells may contribute to the activation and proliferation of fibroblasts and myofibroblasts responsible for the accumulation of ECM and consequent cellular adaptation and epithelial remodeling [30].

BO in transplanted lungs became a model to understand EMT activated by macrophages, where inflammation and fibrosis of bronchioles and alveoli contribute to obstruction followed by restriction and increased expression of mesenchymal proteins by epithelial cells as a clear indication of EMT activation [31]. Also TGF-β has been identified as one of the most important factors in the inflammatory environment of BO, involved in the fibrotic process together with other pro-inflammatory cytokines expressed in BO patients, such as TNFα, IL-1β and IL-8, that might become therapeutic targets to limit bronchial inflammation, as they act by raising Smad-depentent TGF-β1 EMT induction, verified in bronchial epithelial cells isolated from lung transplant patients [30,31]. Blocking TNFα, but not IL-1β, seems to inhibit EMT as anti-TNFα treatment improved forced expiratory volume in 1 second and 6-min walk distances in four patients included in a pilot study [32].

Smoking-related COPD is characterized by irreversible airway flow limitation secondary to subepithelial airway fibrosis, clinically encompassing emphysema, small airway remodeling, chronic bronchitis and pulmonary hypertension [33,34]. In smokers and patients with COPD, a fragmented clefted reticular basement membrane became a hallmark of EMT presence in the airways, also expressing EMT markers such as vimentin [31]. Recent investigations reported that nicotine and tobacco smoke are correlated with increased levels of TGF-β1 in chronically exposed mice and can also induce EMT in bronchial epithelial cells in a TGF-β-dependent manner through the phosphorylation of Smad2 and Smad3, thus, targeted interventions of TGF-β may be a suitable therapeutic option in COPD [34,35]. HB can progress to pulmonary disease and fibrosis when patients remain persistently exposed to the offending agents, with the formation of honeycomb cysts, which are common in other interstitial lung diseases, mainly in IPF. This process has been recognized also as dependent of TGF-β1 regulation of inflammation and connective tissue synthesis [6].

In this study, RB showed underestimated inflammatory infiltration scored as sub-epithelial lymphocytes and plasma cells, while it appeared to be a constant marker for HB and CB. EMT seemed to be activated either in epithelial cells of both RB and HB through TGF-β and CD10 expression, also demonstrated in parietal fusiform cells, corroborating the results from previous studies [30,31].

Prevention of bronchiolitis evolution to chronic fibrosing pulmonary interstitial diseases with adequate early treatment may be a clinical point of care, where pirfenidone anti-fibrotic, anti-inflammatory and antioxidative actions through cytokines and growth factors modulation, including TGF-β1, TNF-α, bFGF, IFN-γ, IL-1β, and IL-18 might be considered to exert inhibitory effects in the pathogenesis of pulmonary fibrosing diseases evolution [26,28]. A study by Minagawa et al. demonstrated that an antibody to human integrin αvβ8, a receptor for latent TFG-β fundamental for its activation, may be a promising therapeutic approach for the treatment of airway remodeling in COPD, through the blockade of inflammatory and fibrotic responses induces by tobacco smoke [36].

Alternative therapeutic approaches are also emerging, based on the advances of TGF-β -target therapies in several diseases, namely in rehabilitation medicine. For example, the inhibition of TGF-β in striate muscle improves muscle regeneration and recovery from injury, by reducing the levels of creatine kinase and fibrosis related to muscle damage: postdamage administration of suramin, which inhibits TGF-β signaling, revealed to be associated with reduction of scar tissue formation and promotion of myoblasts and muscle-derived stem cells differentiation, resulting in an improvement of muscle strength [37]. TGF-β is also a promising target for the treatment of osteoarthritis, as it stimulates proteoglycan synthesis and inhibits hypertrophic chondrocyte differentiation, and clinical trials have shown significant improvement in clinical scores compared with placebo [38]. Finally, pulmonary rehabilitation mixture combining extracts from traditional Chinese medicines resulted in EMT phenotypic reversion and prevention of pulmonary fibrosis progression in Idiopathic Pulmonary Fibrosis through the inhibition of TGF-β1 induced EMT via decreased vimentin, increased E-cadherin and modulation of the HMGB1/RAGE pathway [39].

This study purpose, committed with bronchiolar epithelial remodeling in RB and HB, needs further studies to be incorporated and adapted for routine practice and disease prevention in patients follow-up and treatment, where cryobiopsy samples will play an important role in fibrotic evolution interpretation and interstitial disease prevention.

Acknowledgments

The authors thank Dr. Paula António, Dr. Marisa Vilela and Dr. Ana Rita Rocha for the laboratory work provided.

Author Contributions

Dr. Pedro Sá, as the first author, and Dr. Maria Inês Figueiredo were responsible for the redaction of the manuscript. Dr. Ana Ladeirinha and Dr. Ana Alarcão contributed equally to the manuscript and performed the statistical analysis and technical laboratory work described in the Methods section. Dr. Lina Carvalho participated in the study's research, design and coordination. All authors read and approved the manuscript.

Ethics Approval

The study fulfilled the rules for archival retrospective study defined by the Faculty of Medicine of the University of Coimbra Ethical Committee.

Funding

The authors did not receive funding for conducting this study.

Conflicts of Interest

The authors report no conflicts of interest.

Data Availability

Data sets from this study are available upon request from the corresponding author.

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