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Immune response regulation by transduced mesenchymal stem cells with *decorin* gene on bleomycin-induced lung injury, fibrosis, and inflammation

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Abstract

Background: Pulmonary fibrosis is a pathological hallmark of lung injury. It is an aggressive disease that replaces normal lung parenchyma by fibrotic tissue. The transforming growth factor-beta-mothers against decapentaplegic homolog 3 (TGF-β1-Smad3) signaling pathway plays a key role in regulating lung fibrosis. *Decorin* (DCN), a small leucine-rich proteoglycan, has a modulatory effect on the immune system by reversibly binding with TGF-β and reducing its bioavailability. Mesenchymal stem cell (MSC) therapy is a new strategy that has an immune-modulatory capacity.

Objective: The aim of this study was to introduce a new therapeutic approach to harness remodeling in injured lung.

Material and Methods: Bone marrow MSCs were isolated and transduced by *decorin* gene. Lung injury was induced by bleomycin and mice were treated with MSCs, MSCs-*decorin*, and *decorin*. Then, oxidative stress biomarkers, remodeling biomarkers, bronchoalveolar lavage cells, and histopathology study were conducted.

Results: Reduced catalase and superoxide dismutase increased due to treatments. Elevated malondialdehyde, hydroxyproline, TGF-β levels, and polymorphonuclear cells count decreased

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in the treated groups. Additionally, the histopathology of lung tissues showed controlled inflammation and fibrosis.

Conclusion: Transfected *decorin* gene to MSCs and used cell therapy could control remodeling and bleomycin-induced lung injury.

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Introduction

Lung injury is caused by a wide range of harmful components and/or pathogens. In its chronic form, it can lead to the remodeling and fibrosis of lung tissue. Pulmonary fibrosis is a pathological hallmark of heterogeneous disorders in which excessive collagen accumulation results from a variety of insults to the lung. The pathogenetic mechanisms of lung fibrosis are hindered. In response to injury and oxidants, fibroblasts become active and produce collagen. In patients with pulmonary fibrosis, an imbalance between oxidant and antioxidant defense mechanisms is created in the microenvironment of target lung.¹ Lung fibrosis is one of the aggressive diseases and after the onset of symptoms, has a median survival of 3-5 years. The normal lung parenchyma is replaced by fibrotic tissue accompanied by fibroblast proliferation, inflammation, and excessive deposition of collagen. The scar tissue leads to irreversible decrease in the diffusion capacity of oxygen in the lungs. The cellular and molecular mechanisms involved in the pathogenesis of lung fibrosis are largely unclear, and presently no medications are available to prevent or reverse lung fibrogenesis. The transforming growth factor (TGF)- β 1-mothers against decapentaplegic homolog 3 (Smad3) signaling pathway plays a key role in regulating lung fibrosis. TGF- β 1 induces lung fibroblast differentiation into myofibroblasts, and deficiency of Smad3 attenuates bleomycin-induced lung fibrosis.² Tranilast, a macrophage inhibitor, reduces the severity of bleomycin-induced lung fibrosis.^{3,4} Fibroblasts, myofibroblasts, type II alveolar epithelial cells, macrophages, and eosinophils are the key cells that produce TGF- β 1 in fibrotic lungs.^{3,5-8} Novel therapeutic strategies through advances in gene-cell therapy are expected to become available soon. It is hoped that what was learned regarding the pathogenesis of fibrosis would soon be used in designing new ways to treat lung fibrosis.

Decorin as internal bio-molecule is small leucine-rich proteoglycan and has modulatory effect on the immune system. It is able to control tissue remodeling and in continuous, reduces fibrosis. *Decorin* is an extracellular matrix proteoglycan and is involved in the formation and stability of collagen fibers. It affects airway parenchymal interdependence, proliferation of airway smooth muscle cells, and bioavailability of TGF- β . *Decorin* reversibly binds with the TGF- β core protein and reduces its bioavailability.⁹

Mesenchymal stem cells regulate other cell functions, translocate to inflamed tissues, and help to repair injured tissues. MSCs promote repair and ameliorate injured tissues, such as lungs. Furthermore, MSCs have low immunogenicity and modulate proliferation of regulatory T cells (Tregs) and interleukin (IL)-10, TGF- β 1 secretions.^{10,11} MSCs therapy is a new strategy with immune-modulatory capacity. MSCs

through an immune system modulatory effect may control inflammation.¹² The aim of the current study was to introduce a new therapeutic approach with a long-term effect on lung fibrosis. In this study, immune-modulatory effect, harnessing of remodeling factors, and protective effect of transduced MSCs with the expression *decorin* gene in injured lung was evaluated.

Materials and Methods

Isolation and transduction of MSCs

Bone marrow MSCs were isolated from the tibia and femur of mouse according to a previous study and transduced by *decorin* gene.^{9,10} The cells were analyzed after culture and passage. The expression vector containing a fully sequenced open reading frame was prepared and the expression vector of *decorin* gene was transduced to MSCs. The pAAV-CMV-DCN plasmid was used as an expression vector to contain the expressible mouse *decorin* gene. To express *decorin* gene, total RNA was isolated from transduced MSCs. After synthesizing complementary DNA (cDNA), real-time polymerase chain reaction was performed using specific primers for *decorin* gene.

Animals

Six 8-week-old male BALB/c mice (mean body weight: 21±2 g) were acclimatized for adaptation under standard conditions (24±2°C temperature, 65±15% humidity, and 12-h light/dark cycle) to pathogen-free environment in an animal room for 1 week prior to examination.

Animal modeling and treatment schedule

A total of 35 mice were allocated to five groups ($n = 7$). In four groups, lung injury was induced by bleomycin according to the method described in the past.² Animal groups included the sham group (group S), bleomycin-induced lung injury group (group B), MSCs treatment group (group M), MSCs-*decorin* treatment group (group M-D), and *decorin* treatment group (group D). Lung injury and fibrosis were induced by a single intratracheal instillation of bleomycin (5 mg/kg in 0.3-mL saline) in all four groups, and the sham group received an equal volume of saline. Beginning on day 14 after administration of bleomycin, mice in groups M, D, and M-D received MSCs, *decorin*, and transduced MSCs with *decorin* gene, respectively. On day 28, lung tissue, bronchoalveolar lavage (BAL) fluid, and blood samples were collected and animals were euthanized.

Oxidative stress biomarkers

For oxidative stress indicators, malondialdehyde (MDA) level in plasma, superoxide dismutase (SOD) pulmonary levels, and catalase (CAT) were measured using lung tissues.¹³ Level of SOD was calculated from the absorption features of lung tissue samples, and the results were expressed as (IU/mg protein). Level of CAT was evaluated by a colorimetric method, which was performed by incubating lung tissue with H₂O₂. The absorption of CAT was studied and the result was expressed as (μmol H₂O₂/min/mg protein).

Fibrotic (remodeling) biomarkers

In lung tissue, level of hydroxyproline (HP) was an important index for deposition of collagen fibers, which was measured by a colorimetric modified method.¹³ Lung tissue homogenates were hydrolyzed in HCL, and NaOH was added to hydrolyzed samples for neutralization. Chloramine T reagent was also added, and for inactivation, perchloric acid was added. Afterwards, Ehrlich's solution was also added until the appearance of a reddish color. The results were expressed as milligrams of HP per gram of lung tissue (mg/g).

In addition, TGF-β, one of the other remodeling factors, was measured in supernatants of lung tissue homogenate as described previously.¹³

BAL cells

Following the sampling of BAL, cytospin slides were prepared from BAL cells and stained subsequently. The presence of polymorphonuclear cells (PMNC) was evaluated and reported as a percentage of all white blood cells (WBC).^{9-13,29,31}

Histopathology

Parts of lung tissues were maintained in formalin solution until fixation; the sections were prepared and stained with hematoxylin and eosin (H&E) stain. Later, inflammation, remodeling, and fibrosis were evaluated in histopathological sections and determined in 10 randomly

selected microscopy fields of sections at magnification ×400. Histologic grading of lesions was conducted with the Szapiel method for extent of inflammation in lung parenchyma base, and the microscopic interstitial inflammation and degree of fibrosis were graded on a scale of 1-4 (absent, light, moderate, and strong). Collagen deposition was measured by Masson's trichrome staining. Percentage of fibrosis in the lung was determined by counting the number of pixels within the areas of stained collagen in digital images. Interstitial inflammation and fibrosis microscopic degrees were graded on a scale of 1-4 (absent, light, moderate, and strong). Photomicrographs were taken using microscope equipped with a digital camera.

Statistical analysis

All experiments were repeated thrice and the results were expressed as mean ± standard deviation (SD) of the indicated number of independent experiments. Correlation was analyzed using Pearson's method. The paired *t*-test was used to analyze differences between groups. In multiple comparisons, one-way ANOVA followed by Tukey's honestly significant difference (HSD) tests were used. *P* < 0.05 was considered as statistically significant. SPSS (ver. 22) was used for analysis and performed using GraphPad Prism (version 5.0).

Result

Oxidative stress biomarkers

Catalase decreased significantly in group B (*P* < 0.05), compared to group S (38±4 and 92±5 μmol H₂O₂/min/mg protein, respectively). Treatments increased significantly the levels of CAT (*P*<0.05), compared to group B (Figure 1). The increase was significant in group M-D (44±3 μmol H₂O₂/min/mg protein), compared to other treated groups (M and D groups).

Superoxide dismutase decreased significantly in group B (*P* < 0.05), compared to group S (31±5 and 96±4 IU/mg protein, respectively). Treatments could increase significantly the levels of SOD (*P* < 0.05), compare to group B (Figure 1). The increase was significant in group M-D

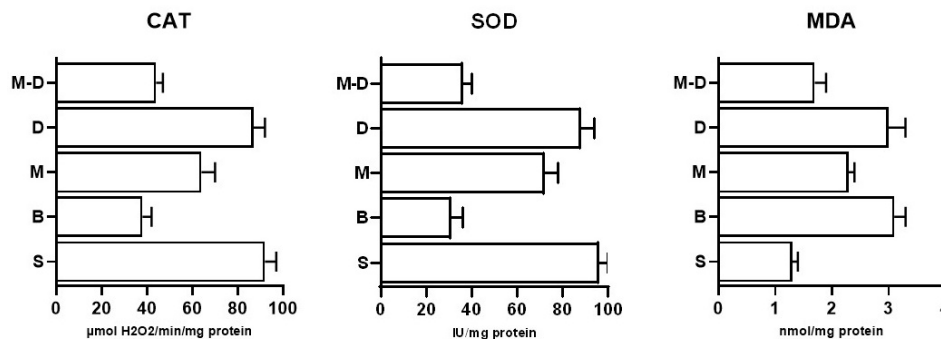


Figure 1 Oxidative stress biomarkers. Levels of CAT, SOD, and MDA were evaluated in all groups, and it was observed that treatments had significant effect on these biomarkers.

(36 ± 4 IU/mg protein), compared to other treated groups (M and D groups).

The levels of MDA were increased significantly in group B, compared to group S (3.1 ± 0.2 and 1.3 ± 0.1 nmol/mg protein, respectively; $P < 0.05$). Treatments decreased significantly the levels of MDA, compared to group B ($P < 0.05$) (Figure 1). The most significant decrease was observed in group M-D (1.7 ± 0.2 nmol/mg protein), compared to other treated groups (M and D groups).

Fibrotic (remodeling) biomarkers

Hydroxyproline elevated significantly in group B ($P < 0.05$), compare to group S (6.1 ± 0.4 and 1.6 ± 0.2 mg/g, respectively); treatments could significantly control the level of HP ($P < 0.05$), compare to group B (Figure 2). The controlling was significant in group M-D (2.7 ± 0.2 mg/g), compare to other treated groups (M and D groups).

TGF- β increased significantly in group B ($P < 0.05$), compare to group S (411 ± 16 and 76 ± 11 pg/mL, respectively); treatments could significantly decrease the levels of TGF- β ($P < 0.05$), compare to group B (Figure 2). The decrease was significant in group M-D (92 ± 8 pg/mL), compared to other treated groups (M and D groups).

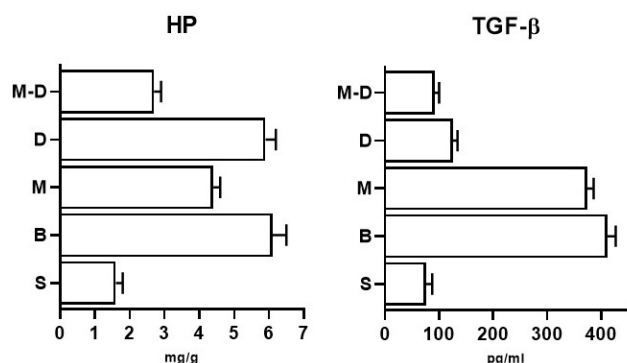


Figure 2 Remodeling biomarkers. HP and TGF- β levels were measured in all groups, and it was observed that treatments had significant effect on these biomarkers.

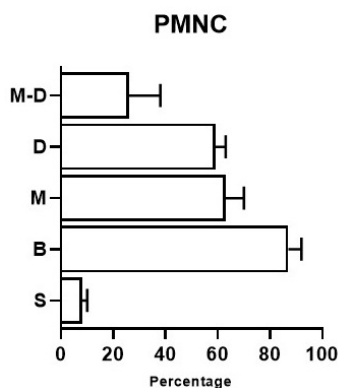


Figure 3 BAL fluid cells. The count of PMNC in BAL fluid was done in all groups. It was observed that treatments had significant effect on cell count.

BAL cells

The PMNCs were observed to increase significantly in group B ($P < 0.05$), compare to group S ($87 \pm 5\%$ and $8 \pm 2\%$, respectively); however, treatments could control PMNC of BAL cells (Figure 3). This control was significant in group M-D ($P < 0.05$), compare to groups M and D ($26 \pm 12\%$, $63 \pm 7\%$, and $59 \pm 4\%$, respectively).

Histopathology

In the histopathology evaluation of lung tissues, it was observed that inflammation and fibrosis were increased significantly ($P < 0.05$) in group B (3.7 ± 0.2 and 3.5 ± 0.4 , respectively), compared to group S (0.5 ± 0.1 and 0.5 ± 0.0 , respectively); however, treatments could significantly control inflammation and fibrosis ($P < 0.05$), compare to group B (Figure 4). The controlling was significant in group M-D (1.1 ± 0.2 and 0.9 ± 0.3 , respectively), compare to other treated groups (M and D groups).

Discussion

Bleomycin as antitumor factor and antibiotic was isolated from the fungus *Streptomyces verticillus*, which exerts antitumor activity by inducing tumor cell death and tumor angiogenesis inhibition. It is used as an antitumor therapy for different tumors, such as cervical cancer, Kaposi's sarcoma, and squamous cell carcinomas of the head and neck. Bleomycin has a major limitation, as it usually causes pulmonary toxicity. Several distinct pulmonary syndromes are linked to bleomycin usage, including eosinophilic hypersensitivity, bronchiolitis obliterans with organizing pneumonia, and, most commonly, interstitial pneumonitis that may progress to fibrosis. The bleomycin-induced lung injury mechanism is not clear and potentially involves oxidative damage, elaboration of inflammatory cytokines, genetic susceptibility, and relative deficiency of the deactivating enzyme bleomycin hydrolase.

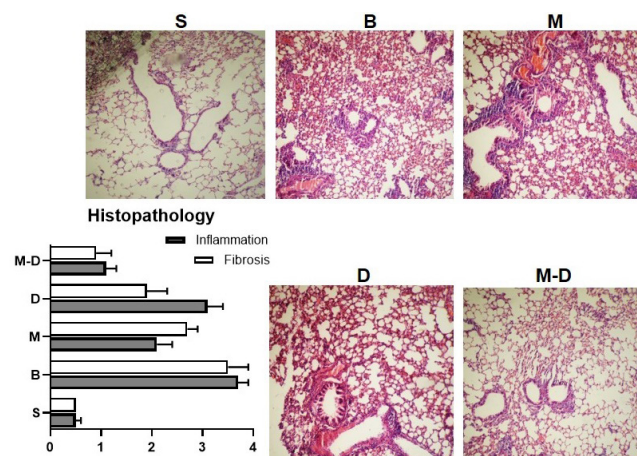


Figure 4 Histopathology, inflammation, and fibrosis were evaluated in all groups, and it was observed that treatment by MSCs and decorin gene had significant effect on these factors.

In animal models of bleomycin toxicity, dust cells (alveolar macrophages) release cytokines, such as IL-1, macrophage inflammatory proteins (MIP)-1, platelet-derived growth factor (PDGF), and TGF- β , which can lead to fibrosis. The activation of epithelial cells and damage causes the release of cytokines and growth factors, which stimulate the proliferation of myofibroblasts and the secretion of pathologic extracellular matrix, finally leading to fibrosis. Tumor necrosis factor (TNF)- α , TGF- β , and PDGF receptor- α (PDGFR- α) stimulate the transformation, proliferation, and accumulation of fibroblasts, leading to the deposition of extracellular matrix. This progressive accumulation of collagen matrix results in the distortion and destruction of alveolar structures, leading to a loss of lung function.

Bleomycin may also up-regulate collagen synthesis by modulating fibroblast proliferation via TGF- β response, which results in irreversible pulmonary fibrosis.¹⁴ It was reported that the treatment of bleomycin-induced lung injury mouse model with montelukast reduced fibrotic area and HP content in fibrotic lungs. Montelukast exhibits its effects by inhibition of the IL-6, IL-10, IL-13, and TGF- β 1 overexpression.^{14,15}

In a study, the effect of all-trans retinoic acid on bleomycin-induced lung fibrogenesis and the role of TGF- β 1-Smad3 signaling pathway were investigated. It was observed that bleomycin increased thickness of the alveolar wall, infiltration of inflammatory cells, and formation of collagen fiber. The all-transretinoic acid ameliorated bleomycin-induced lung fibrosis, and these anti-fibrotic effects could be mediated by inhibition of epithelial-mesenchymal transition (EMT) through the down-regulation of TGF- β 1-Smad3 signaling pathway. In addition, it ameliorates bleomycin-induced EMT by inhibiting α -SMA-expressing myofibroblasts and expression of collagen and enhancing expression of E-cadherin in fibroblasts.² In lung fibrosis, fibroblasts differentiate into myofibroblasts that exhibit enhanced fibrotic and contractile activities. Lung fibrosis is regulated by TGF- β 1-Smad3 signaling pathway activated by contraction of myofibroblast, and this pathway promotes fibrotic transformation of the lung through EMT activation.² Bleomycin increases PMNC in BAL of injured lungs. In our study, the cells were increased in BAL of nontreated bleomycin-induced lungs injury controlled by cell therapy, *decorin* therapy, and especially in treated group, with transduced MSCs with *decorin* gene.

The oxidative lung injury and pulmonary fibrosis can be induced via bleomycin administration, and the related pathophysiology typically consists of two overlapping phases. The early inflammatory phase (leucocyte infiltration) leads to alveolar epithelial cells injury and the subsequent fibroproliferative phase with matrix remodeling and fibrosis.^{1,16} Pulmonary fibrosis is commonly mediated by TGF- β , which enhances collagen gene expression and protein deposition.^{1,17,18} In addition, TGF- β mediates the depletion of alveolar glutathione stores and promotes acute lung injury.^{1,19} CAT and SOD are the main biomarkers in oxidative stress pathway, which were decreased in the bleomycin-induced lung and in this study, treatments could increase CAT and SOD levels. The increasing CAT and SOD levels were significant in group M-D, compare to other treated groups, and it was demonstrated that transduced MSCs with *decorin* gene had a strong effect. In this study,

we found that MDA levels were increased in the bleomycin-induced group. However, treatments were effective in controlling MDA levels. Notably, group M-D showed a significant decrease in MDA levels, compared to other two treated groups (MSCs and *decorin* groups).

Collagen fibers as connective tissue elements are clearly responsible for fibrosis in bleomycin-induced lungs, and collagen type I gene expression is preceded by collagen type VI gene expression.^{3,20} Fibroblast and myofibroblast cells synthesize extracellular matrix proteins, which are the core of fibrotic transformation in the lung.³ *Decorin* deficiency enhances collagen deposition and airway remodeling in the lung.^{9,21,22} *Decorin* modulates essential biological processes and biofactors, including collagen fibrillogenesis, and affects smooth muscle contractility, immune system responsiveness, and cell migration. In fibrotic diseases, TGF- β initiates remodeling and fibrosis, which is inhibited by *decorin*.^{9,23,24} *Decorin* can down-regulate TGF- β and inhibit elevated expression of TGF- β -driven profibrogenic genes, formation of myofibroblasts, fibrosis, tenascin, fibronectin, and collagen types I and IV.^{9,25} Furthermore, *decorin* regulates both inflammation and fibrillogenesis processes.^{9,26-28} *Decorin* as a natural product has no immune response and can be used as a therapeutic agent to control lung remodeling. In addition, *decorin* gene therapy inhibits TGF- β -driven fibrosis.⁹ In bleomycin-induced lung, both HP and TGF- β were elevated significantly; however, treatments controlled HP and TGF- β levels. The controlling was more notable in group M-D, compare to other treated groups (M and D groups).

Cell administration for immunomodulation is a recent therapeutic method for immune system-related diseases. Therefore, immune response modulation by cell therapy could be used as a treatment of inflammatory diseases.^{29,30} MSCs immunomodulation is a new therapeutic approach for a variety of immune-response diseases and is able to alleviate airway inflammation. In the presence of inflammation, MSCs suppress immune response and T cell proliferation by secreting prostaglandin E2 (PGE2) and indoleamine 2-3 dioxygenase (IDO). The immunomodulation is achieved through the secretion of TGF- β and PGE2, which decreases T cell proliferation and increases the proportion of T regulatory cells.^{10,31,32} Vector direct injection can stimulate immune response against vector that removes them prior to entering target cells, while MSCs are almost non-immunogenic. Therefore, using transfected MSCs with target expression of *decorin* gene is used to treat uncontrolled lung injury.¹⁰ Systemic administration of MSCs could suppress remodeling of airway.¹¹ MSCs have a high degree of plasticity and are capable of differentiating into bronchial, alveolar, and interstitial cell types in the lung. They play a significant role in modulating local inflammation and repairing injured lung tissue.¹¹ In the histopathology evolution of lung tissues in the current study, it was observed that inflammation and fibrosis as main problems in lung injury were increased by bleomycin; however, treatments controlled inflammation as well as fibrosis. The group that received MSCs containing *decorin* gene exhibited dominant control, compared to the group that received MSCs without *decorin* gene and the group that received only *decorin* gene.

Mesenchymal stem cells are immunomodulatory cells, and in asthmatic lungs could up-regulate IL-10. In

addition, MSCs promote immunosuppressive effect, and the intranasal administration of MSCs enhances immunosuppressive cytokine IL-10 production by lung interstitial macrophages.^{12,33} Inflammation has a notable role in lung injury, but MSCs have a protective effect in lung injury. It may be enhanced if combined with the protective effect of IL-35.¹⁰ It was reported in a study that transduced MSCs with IL-35 expression gene controlled immunopathology.¹⁰ Mechanism of inflammation in asthma and immunomodulatory potential of MSCs had synergism with IL-35 effect.¹⁰

In the current study, we transfected *decorin* gene into MSCs to control bleomycin-induced lung injury. Our results showed that transduced MSCs with *decorin* gene effectively controlled remodeling, inflammation, and fibrosis in the lung, thus preventing lung injury.

Our study had few limitations. First, we could not check the effect of transduced MSCs with *decorin* gene in chronic lung injury. In addition, we could not evaluate other related biofactors and immune system-related responses and molecules.

Ethics Approval and Consent to Participate

The current study was approved by the Ethics Committee of Animal House of ix.med.vet.dep, 2023 (No. IX.MED.VET.DEP.REC.2023.0100099.5). All methods were performed in accordance with the relevant guidelines and regulations.

Conflict of Interest

There was no conflict of interest to declare.

Author contributions

Wei Xu, Chang Kun Li, Li Sha Yang, Entezar Mehrabi Nasab, Seyyed Shamsadin Athari, and Wen Dong Gu participated in planning of the project, animal study, laboratory testing, analysis of data, and writing of the manuscript.

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References

- Atzori L, Chua F, Dunsmore SE, Willis D, Barbarisi M, McAnulty RJ, et al. Attenuation of bleomycin-induced pulmonary fibrosis in mice using the heme oxygenase inhibitor Zn-deuteroporphyrin IX-2,4-bisethylene glycol. *Thorax*. 2004;59(3):217-23. <https://doi.org/10.1136/thx.2003.008979>
- Song X, Liu W, Xie S, Wang M, Cao G, Mao C, et al. All-trans-retinoic acid ameliorates bleomycin-induced lung fibrosis by downregulating the TGF- β 1/Smad3 signaling pathway in rats. *Lab Investigat*. 2013;93:1219-31. <https://doi.org/10.1038/labinvest.2013.108>
- Grande NR, Peão MND, de Sá CM, Águas AP. Lung fibrosis induced by bleomycin: structural changes and overview of recent advances. *Scan Microscopy*. 1998;12(3):487-94.
- Mori H, Tanaka H, Kawada K, Nagai H, Koda A. Suppressive effects of tranilast on pulmonary fibrosis and activation of alveolar macrophages in mice treated with bleomycin: role of alveolar macrophages in the fibrosis. *Jpn J Pharmacol*. 1995;67:279-89. <https://doi.org/10.1254/jjp.67.279>
- Khalil N, O'Connor RN, Flanders KC, Shing W, Whitman CI. Regulation of type II alveolar epithelial cell proliferation by TGF- β during bleomycin induced lung injury in rats. *Am J Physiol*. 1994;267:498-507. <https://doi.org/10.1152/ajplung.1994.267.5.L498>
- Madtes DK, Busby HK, Strandjord TP, Clark JG. Expression of transforming growth factor- α and epidermal growth factor receptor is increased following bleomycin-induced lung injury in rats. *Am J Resp Cell Mol Biol*. 1994;11:540-51. <https://doi.org/10.1165/ajrcmb.11.5.7524566>
- Santana A, Saxena B, Noble NA, Gold LI, Marshall BC. Increased expression of transforming growth factor beta isoforms in bleomycin induced pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 1995;13:34-44. <https://doi.org/10.1165/ajrcmb.13.1.7541221>
- Zhang K, Flanders KC, Phan SH. Cellular localization of transforming growth factor- β expression in bleomycin-induced pulmonary fibrosis. *Am J Pathol* 1995;147:352-61.
- Zhang R, Yin Z, Pan J, Zhai C, Athari SS, Dong L. Effect of transfected induced pluripotent stem cells with *decorin* gene on control of lung remodeling in allergic asthma. *J Invest Med*. 2023;71(3):235-43. <https://doi.org/10.1177/10815589221140590>
- Bao X-h, Gao F, Athari SS, Wang H. Immunomodulatory effect of IL-35 gene-transfected mesenchymal stem cells on allergic asthma. *Fundam Clin Pharmacol*. 2023;37(1):116-24. <https://doi.org/10.1111/fcp.12823>
- Huang M, Nasab EM, Athari SS. Immunoregulatory effect of mesenchymal stem cell via mitochondria signaling pathways in allergic asthma. *Saudi J Biol Sci*. 2021;28:6957-62. <https://doi.org/10.1016/j.sjbs.2021.07.071>
- Hou C, Sun F, Liang Y, Nasab EM, Athari SS. Effect of transduced mesenchymal stem cells with IL-10 gene on control of allergic asthma. *Allergol Immunopathol (Madr)*. 2023;51(2):45-51. <https://doi.org/10.15586/aei.v51i2.789>
- Hajimohammadi B, Athari SM, Abdollahi M, Vahedi G, Athari SS. Oral administration of acrylamide worsens the inflammatory responses in the airways of asthmatic mice through agitation of oxidative stress in the lungs. *Front Immunol*. 2020;11:1940. <https://doi.org/10.3389/fimmu.2020.01940>
- Reinert T, da Rocha Baldotto CS, Nunes FAP, de Souza Scheliga AA. Bleomycin-induced lung injury. *J Cancer Res*. 2013;Art ID:480608. <https://doi.org/10.1155/2013/480608>
- Shimbori C, Shiota N, Okunishi H. Effects of montelukast, a cysteinyl-leukotriene type 1 receptor antagonist, on the pathogenesis of bleomycin-induced pulmonary fibrosis in mice. *Eur J Pharmacol*. 2011;650(1):424-30. <https://doi.org/10.1016/j.ejphar.2010.09.084>
- Marshall RP, McAnulty RJ, Laurent GJ. The pathogenesis of pulmonary fibrosis: is there a fibrosis gene? *Int J Biochem Cell Biol*. 1997;29:107-20. [https://doi.org/10.1016/S1357-2725\(96\)00141-0](https://doi.org/10.1016/S1357-2725(96)00141-0)
- Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor- β on bleomycin-induced accumulation of lung collagen in mice. *Thorax*. 1993;48:959-66. <https://doi.org/10.1136/thx.48.10.959>
- Wang Q, Wang Y, Hyde DM, Gotwals PJ, Koteliansky VE, Ryan ST, et al. Reduction of bleomycin induced lung fibrosis

- transforming growth factor-B soluble receptor in hamsters. *Thorax*. 1999;54:805-12. <https://doi.org/10.1136/thx.54.9.805>
19. Pittet J-F, Griffiths MJD, Geiser T, Kaminski N, Dalton SL, Huang X, et al. TGF- β is a critical mediator of acute lung injury. *J Clin Invest*. 2001;107:1537-44. <https://doi.org/10.1172/JCI11963>
 20. Specks U, Nerlich A, Colby TV, Wiest I, Timpl R. Increased expression of type VI collagen in lung fibrosis. *Am J Respir Crit Care Med*. 1995;151:1956-64. <https://doi.org/10.1164/ajrccm.151.6.7767545>
 21. Marchica CL, Pinelli V, Borges M, Zimmer J, Narayanan V, Iozzo RV, et al. A role for decorin in a murine model of allergen-induced asthma. *Am J Physiol Lung Cell Mol Physiol*. 2011;300:863-73. <https://doi.org/10.1152/ajplung.00300.2009>
 22. Salerno FG, Pinelli V, Pini L, Tuma B, Iozzo RV, Ludwig MS. Effect of PEEP on induced constriction is enhanced in decorin-deficient mice. *Am J Physiol Lung Cell Mol Physiol*. 2007;293:1111-7. <https://doi.org/10.1152/ajplung.00095.2007>
 23. Markmann A, Hausser H, Schönherr E, Kresse H. Influence of decorin expression on transforming growth factor-B mediated collagen gel retraction and biglycan induction. *Matrix Biol*. 2000;19:631-6. [https://doi.org/10.1016/S0945-053X\(00\)00097-4](https://doi.org/10.1016/S0945-053X(00)00097-4)
 24. Schönherr E, Broszat M, Brandan E, Bruckner P, Kresse H. Decorin core protein fragment Leu 155-Val 260 interacts with TGF- β but does not compete for decorin binding to type I collagen. *Arch Biochem Biophys*. 1998;355:241-8. <https://doi.org/10.1006/abbi.1998.0720>
 25. Mohan RR, Gupta R, Mehan MK, Cowden JW, Sinha S. Decorin transfection suppresses profibrogenic genes and myofibroblast formation in human corneal fibroblasts. *Exper Eye Res*. 2010;91:238-45. <https://doi.org/10.1016/j.exer.2010.05.013>
 26. Allawadhi P, Singh V, Khurana I, Rawat PS, Renushe AP, Khurana A, et al. Decorin as a possible strategy for the amelioration of COVID-19. *Med Hypotheses*. 2021;152:110612. <https://doi.org/10.1016/j.mehy.2021.110612>
 27. Chen S, Young MF, Chakravarti S, Birk DE. Interclass small leucine-rich repeat proteoglycan interactions regulate collagen fibrillogenesis and corneal stromal assembly. *Matrix Biol*. 2014;35:103-11. <https://doi.org/10.1016/j.matbio.2014.01.004>
 28. Robinson PS, Huang TF, Kazam E, Iozzo RV, Birk DE, Soslowsky LJ. Influence of decorin and biglycan on mechanical properties of multiple tendons in knockout mice. *J Biomech Eng*. 2005;127:181-5. <https://doi.org/10.1115/1.1835363>
 29. Yin J, Yan F, Zheng R, Wu R, Athari SS. Immunomodulatory effect of IL-2 induced bone marrow mononuclear cell therapy on control of allergic asthma. *Allergol Immunopathol (Madr)*. 2023;51(1):110-5. <https://doi.org/10.15586/aei.v51i1.746>
 30. Lin WY, Oh SKW, Choo ABH, George AJT. Activated T cells modulate immunosuppression by embryonic- and bone marrow-derived mesenchymal stromal cells through a feedback mechanism. *Cytotherapy*. 2012;14:274-84. <https://doi.org/10.3109/14653249.2011.635853>
 31. Wang D, Nasab EM, Athari SS. Study effect of Baicalein encapsulated/loaded chitosan-nanoparticle on allergic asthma pathology in mouse model. *Saudi J Biol Sci*. 2021;28:4311-7. <https://doi.org/10.1016/j.sjbs.2021.04.009>
 32. Lin Y-D, Fan X-L, Zhang H, Fang S-B, Li C-L, Deng M-X, et al. The genes involved in asthma with the treatment of human embryonic stem cell-derived mesenchymal stem cells. *Mol Immunol*. 2018;95:47-55. <https://doi.org/10.1016/j.molimm.2018.01.013>
 33. Jiling R, Yongzhe L, Ye Y, Lifeng F, Xiaotong Z, Zongjin L, et al. Intranasal delivery of MSC-derived exosomes attenuates allergic asthma via expanding IL-10 producing lung interstitial macrophages in mice. *Int Immunopharmacol*. 2021;91:107288. <https://doi.org/10.1016/j.intimp.2020.107288>