



Hypoxia-preconditioned mesenchymal stem cells attenuate bleomycin-induced pulmonary fibrosis

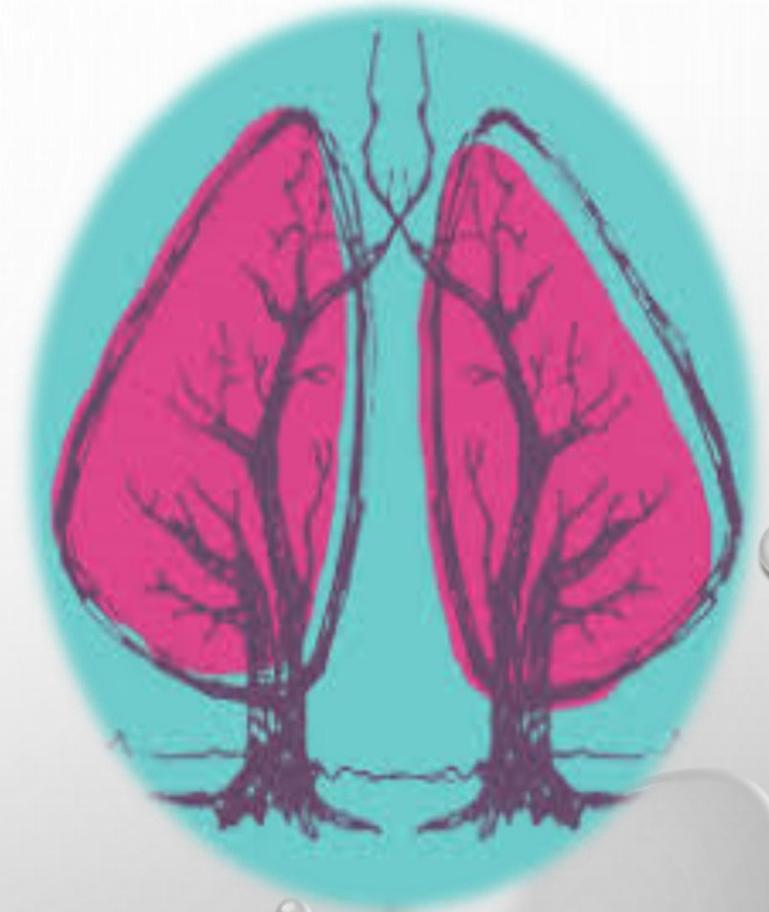
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RESEARCH

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Hypoxia-preconditioned mesenchymal stem cells attenuate bleomycin-induced pulmonary fibrosis



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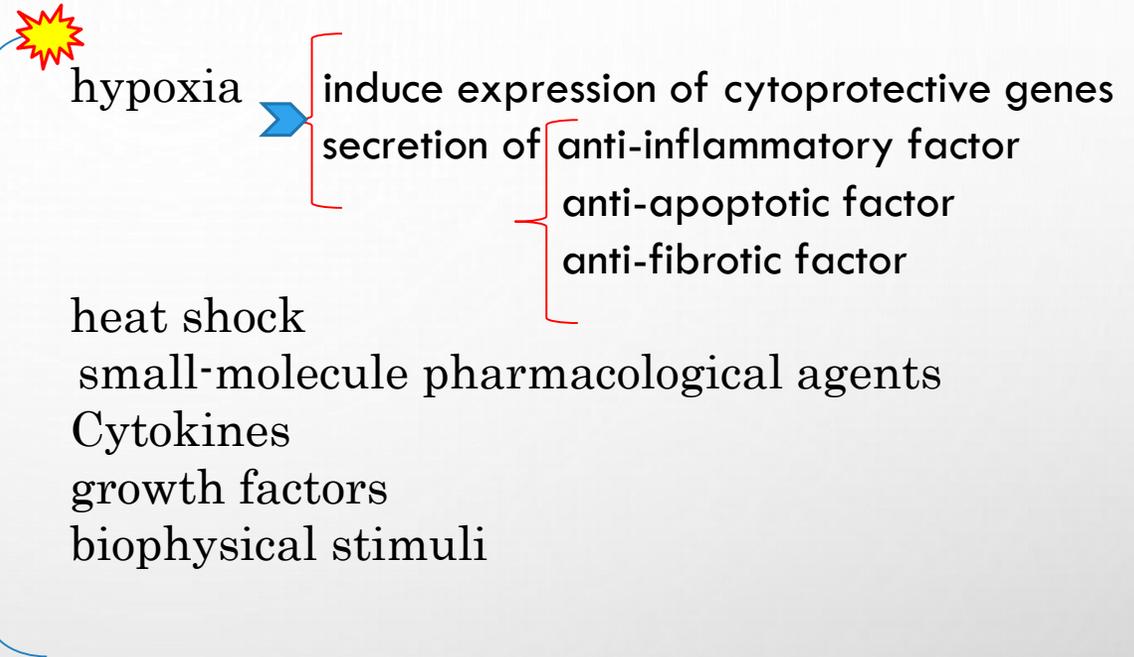
INTRODUCTION

- **Idiopathic Pulmonary Fibrosis (IPF)** ➤ A progressive diffuse parenchymal lung disorder of unknown etiology.
- **Bleomycin (BLM)** ➤ A chemotherapeutic agent has undesirable side-effects in inducing lung injuries and triggering apoptosis of alveolar epithelial cells
- **Preconditioning** ➤ genetic manipulation , overexpression of anti-apoptotic proteins, chemokine receptors , growth factors or pro-survival genes prior to transplantation



Preconditioning

Exposure of cells to **physiological** stimuli

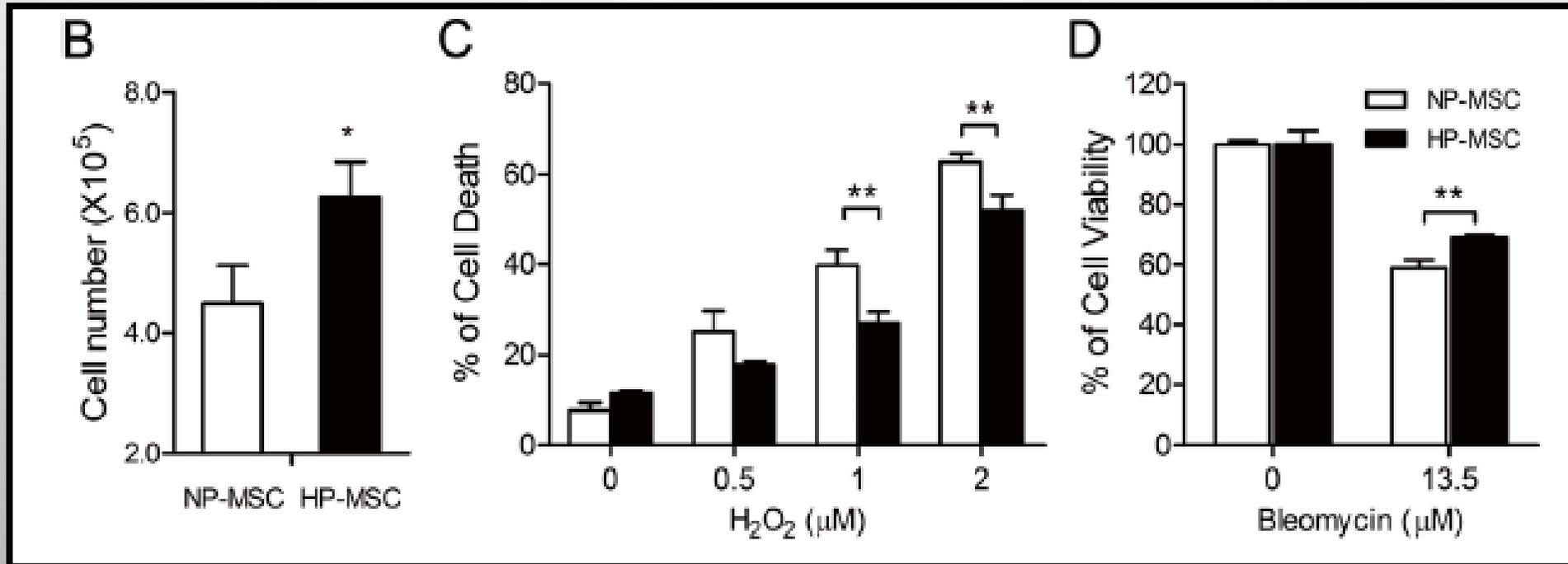


Adopting appropriate preconditioning strategies → provide a effective way of promoting **survival** and **regenerative** properties, and also the **tissue repair** capability of transplanted cells in stem cell-based therapy



Hypoxic preconditioning

- ↑ cell proliferation
- ↓ hydrogen peroxide-induced cytotoxicity in MSCs
- ↓ bleomycin-induced alveolar epithelial cell death





Hypoxic preconditioning

- The effects of hypoxic culture **on** → MSC proliferation and Expansion efficiency
 - a) sources of MSCs
 - b) oxygen concentration
 - c) seeding density MSCs
 - d) culturing Duration of the MSCs



Treatment

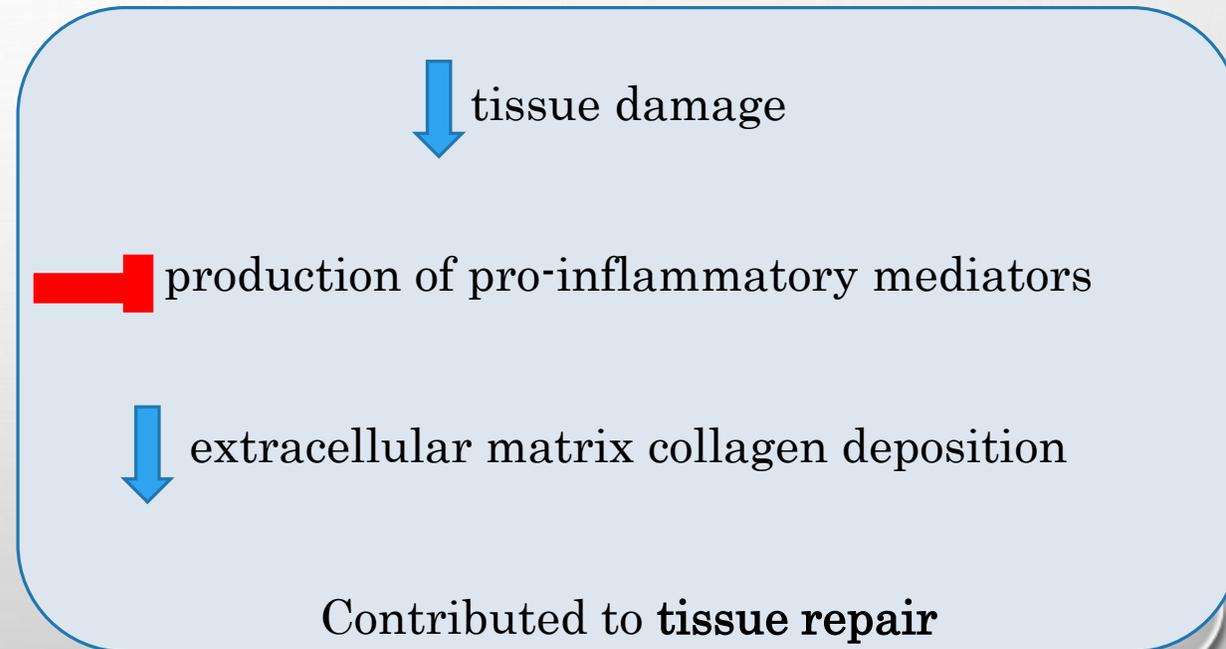
- ✓ Mesenchymal stem cell (MSC)-based therapy is a novel approach with great therapeutic potential for the treatment of Lung diseases

MSCs **secrete paracrine factors** with functions

anti-inflammatory

anti-apoptotic

anti-fibrotic





Treatment

- Hypoxic Preconditioning (MSC)-based therapy

Short-term exposure of MSCs to sub-lethal hypoxia before transplantation

↑ Cell survival ↓ apoptosis related pathways

HP ➔ induces the expression of markers

Pro-survival
Chemo attractants
Growth factors involved in cell proliferation
Anti-oxidation factor
Anti-apoptosis factor
Angiogenesis factor





Hypoxic Preconditioning MSCs

- ❖ **Ischemic diseases** such as stroke and myocardial infarction
- ❖ Traumatic brain injuries
- ❖ Diabetes mellitus
- ❖ Inflammatory Bowel Disease (IBD)
- ❖ Acute kidney
- ❖ Liver injuries



PURPOSE

- Prolong the duration of survival of engrafted MSCs
- enhance the effectiveness of idiopathic Pulmonary fibrosis transplantation therapy by the use of **hypoxia-preconditioned MSCs.**



the therapeutic potency and duration of survival of engrafted MSCs.



Material

- **Chemicals** → **Bleomycin sulfate** from streptomyces verticillus
- Bleomycin sulfate stock was prepared by dissolving in **sterile PBS** at **10 mg/ml** and storing in small aliquots at **4°C**
- **Cell lines**
 - **MSCs (B.M)** of female mice (C57BL/6)
 - female murine alveolar epithelial cell line (**MLE-12; CRL-2110**)
 - Human 14-week male embryonal lung cell line (**MRC-5; BCRC-60023**)



Material

- **Animal model of bleomycin-induced lung fibrosis** → Eight-week old male C57BL/6jnar1 mice
↓ anesthetized
Isoflurane inhalation

All animals received **BLM (1.5 mg/kg** body weight)on day 0. on day 3

Normoxia-pretreated MSCs
Hypoxia-pretreated MSCs
Just PBS

day 4 and day 18

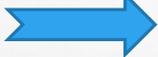
in the whole-body **plethysmograph** Tanks

Analysis of pulmonary functions

Noninvasive measurement

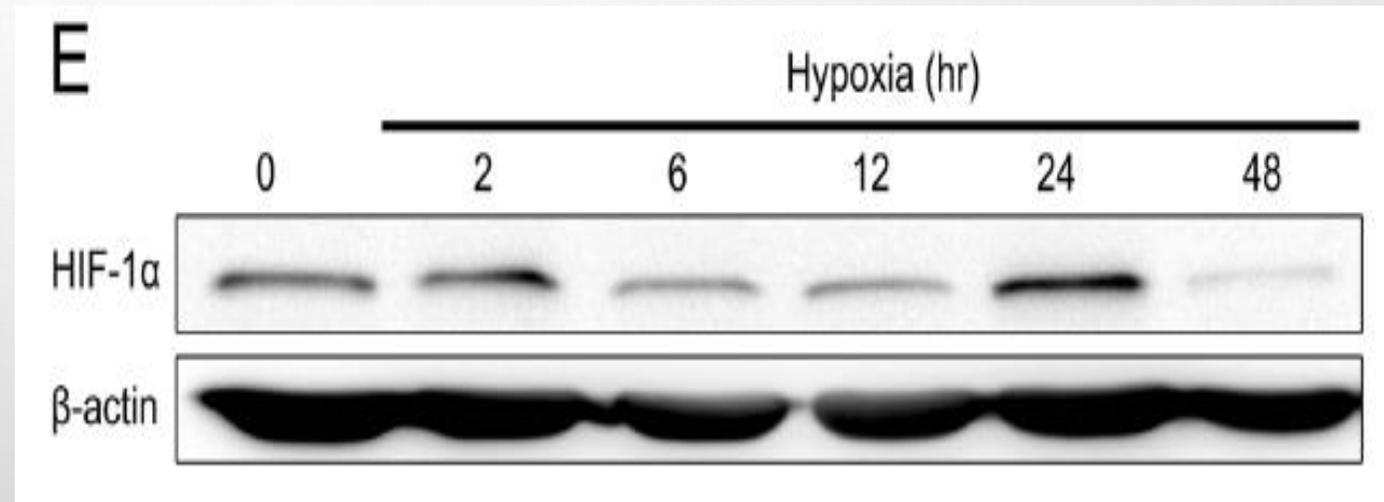
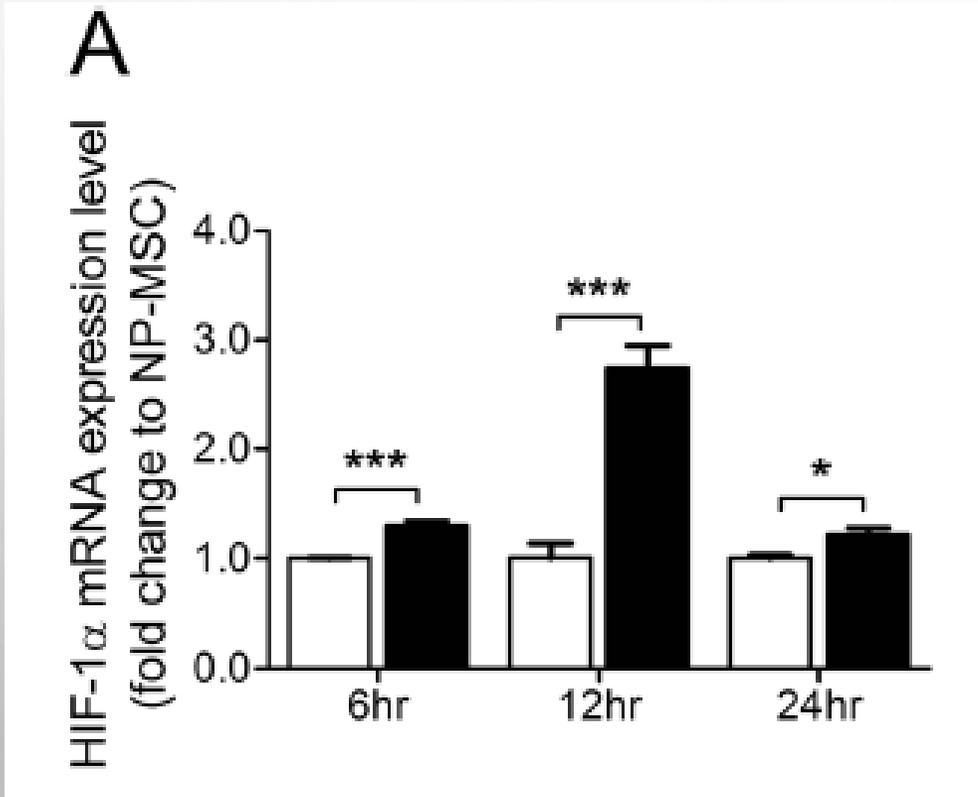


Method

- (HP-MSCS)  inject bleomycin-induced pulmonary fibrosis mice  3 day
- lung functions ,Cellular , molecular and pathological changes were assessed at 7 and 21 days after bleomycin administration.



The reason why 1.5% O₂ concentration was chosen in this study





Method

- Normoxia-treated MSCs (control) ➔ 95% atmospheric air and 5% CO₂ for 24 h

MSCs 100000 cells/well + **MRC-5** cells 200000 cells/well were cultured overnight.

MSCs [O₂] in hypoxic treatment for 24 h

MRC-5 Cells with or without 2.5 ng/ml (TGF)-β1 24 h

- **hypoxia pretreated MSCs co-cultured** with the **MRC-5** cells for 24 h
- The MRC-5 cells were harvested ➔ fibronectin mRNA expression level
Quantitative RT-PCR.



Method

- Cell proliferation and viability test
- MSCs 50000 cells/well in a 12-well culture plate

↓ incubation

[O₂] For 24 h

↓ detached

trypsinization

Countess™ automated cell counter

0.4% (w/v) Trypan-blue and Countess™ software

Cell viability MTT assay

MLE-12 cells 100000 cells/well
in a 24-well culture plate

↓ treated

BLM (13.5 μM)

(NP-MSC) or (HP-MSC) for 48 h

At a wavelength of 540 nm by
versamax spectrophotometry



Method

- **Annexin V / PI double staining assay**
- 1 hour post-H₂O₂ treatment (0.5, 1, 2 μ m)
- cell viability of NP-MSCs and HP-MSCs
- 100000 cells +1 μ l Annexin V /PI in a binding buffer For 15 minutes.
- The percentage of cell numbers in each quadrant was calculated using
- **CELLQUEST** software

RNA isolation and qRT-PCR

Total RNA



treated

DNase I

RNAs  cDNAs

at 42°C for 60 minutes

Immuno histochemical staining of β -gal

Paraffin-fixed sections were microwave-heated (750 W, three 5-minute cycles) in 0.01 M citrate (pH 6.0) or 1-mM EDTA (pH 8.0) solution.

The stained sections were scanned by the HistoFAXS



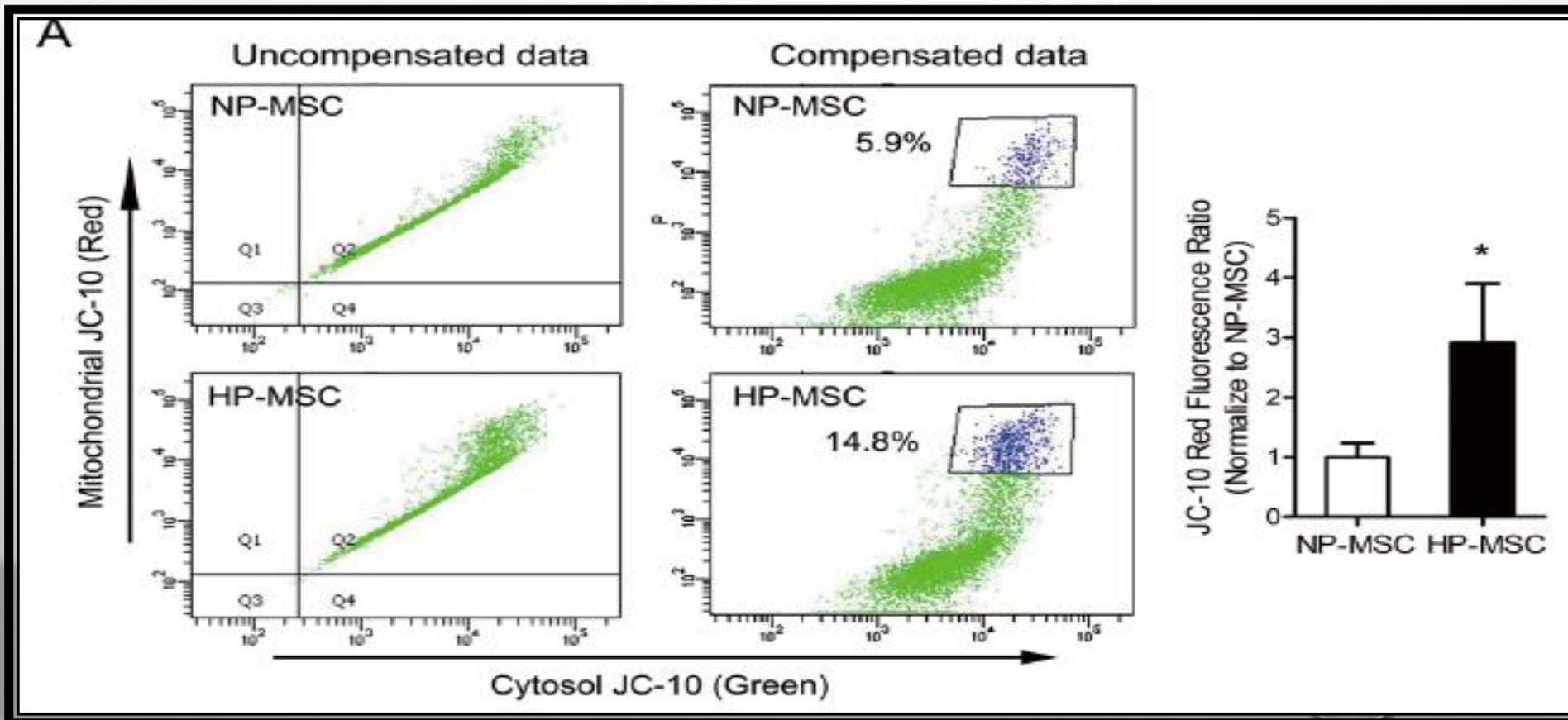
Hypoxic preconditioning

- stabilizes the **mitochondrial** membrane potential in MSCs

Antioxidant
defense

Cell survival

Anti-apoptosis





Measurement of mitochondrial membrane potential

Sensitive fluorescent probe **JC-10**

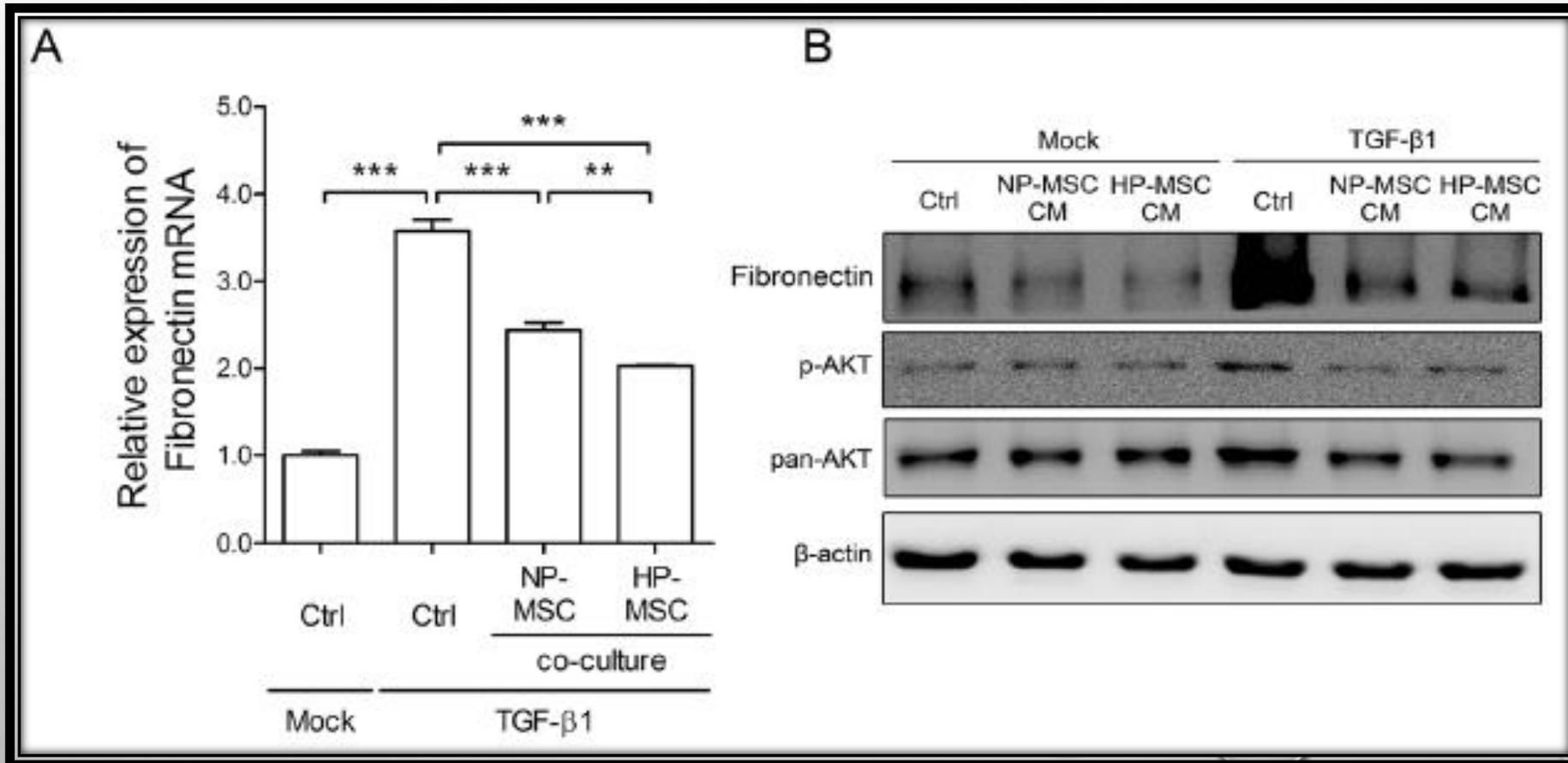
green (JC-10 monomeric form)
Orange (JC-10 aggregate form)

MSCs were incubated with JC-10 (1 μ M) at 37°C for 30 minutes

(Hp-MSCs)-conditioned medium

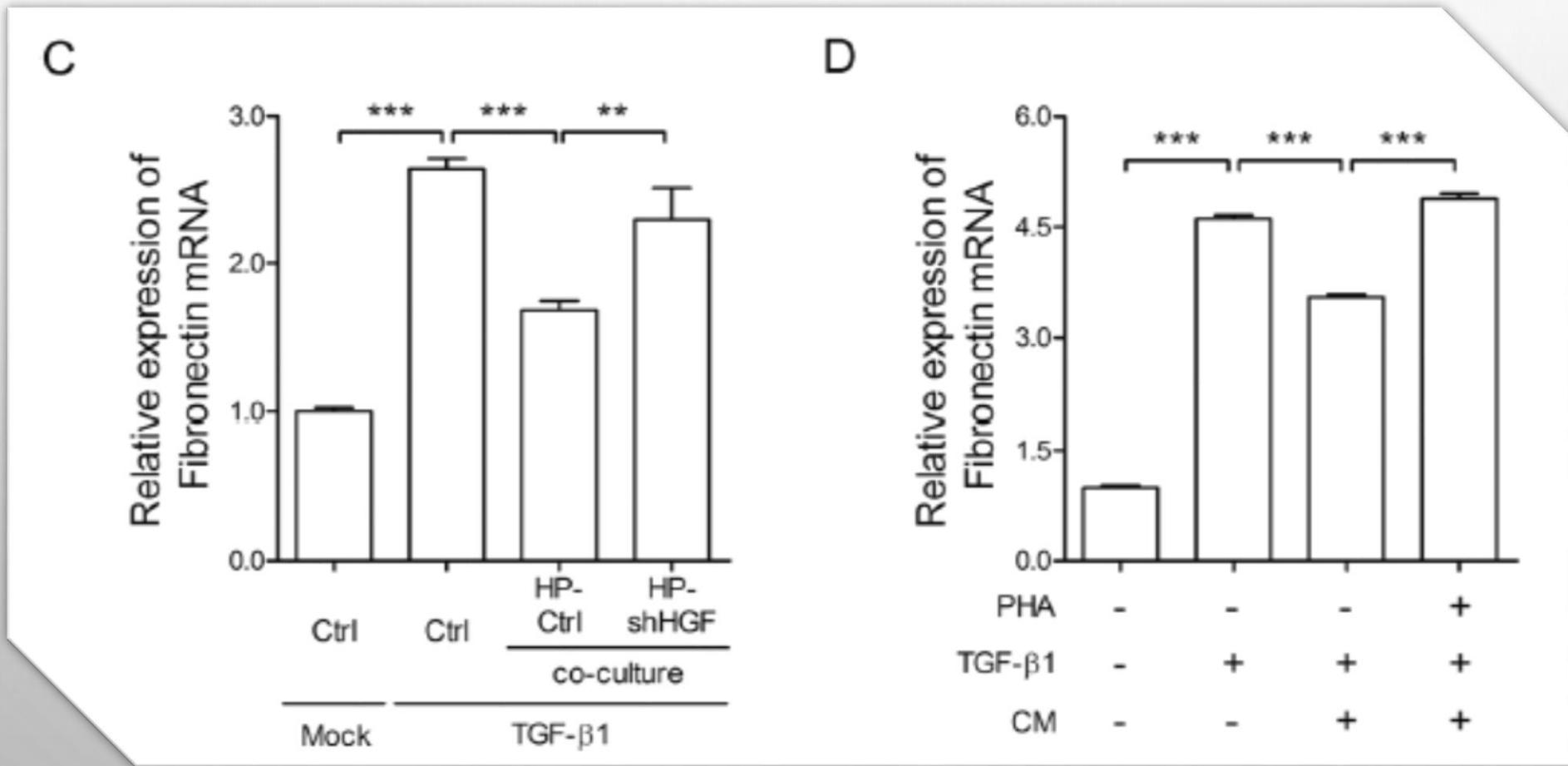


ECM production through
TGF- β 1-mediated Akt signaling





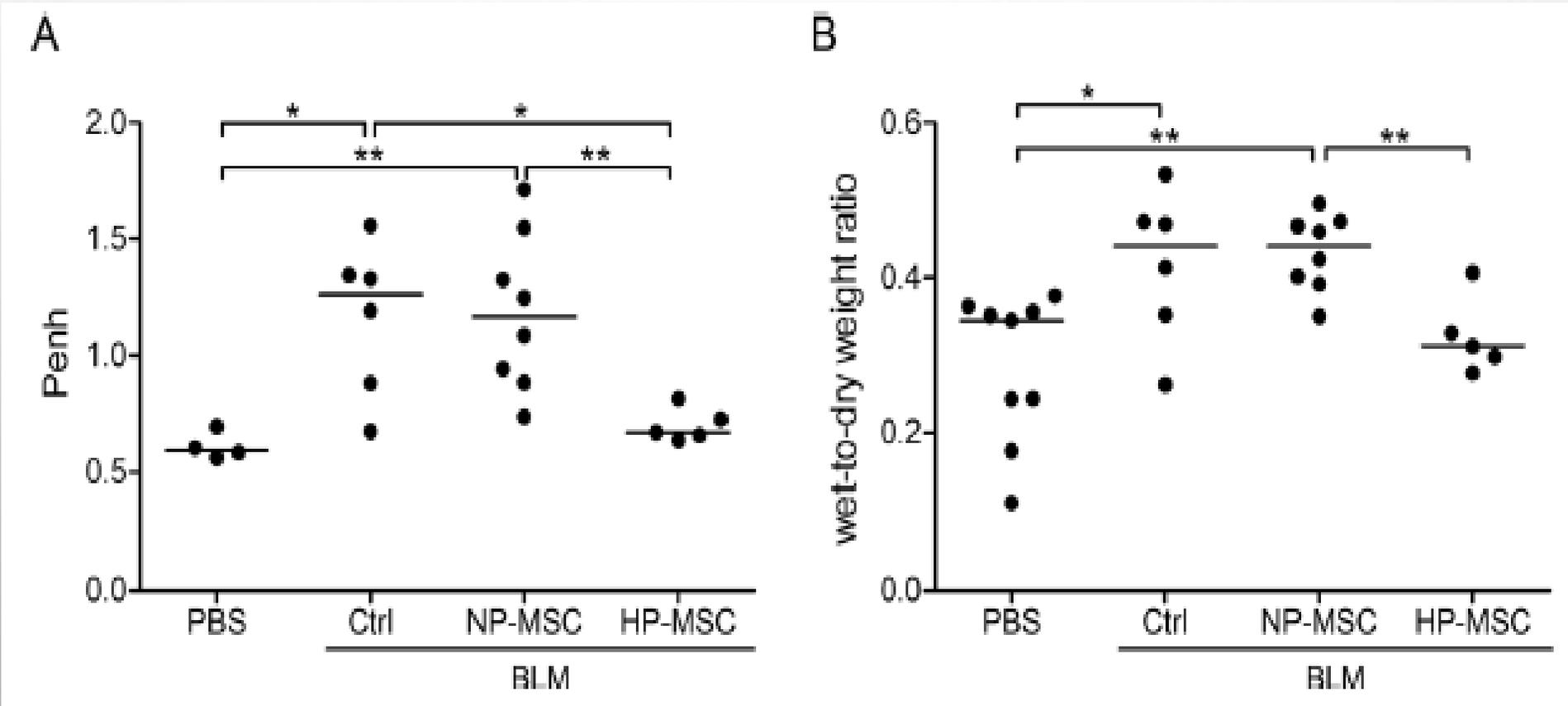
Are HP-MSCs Reduce ECM production through HGF?



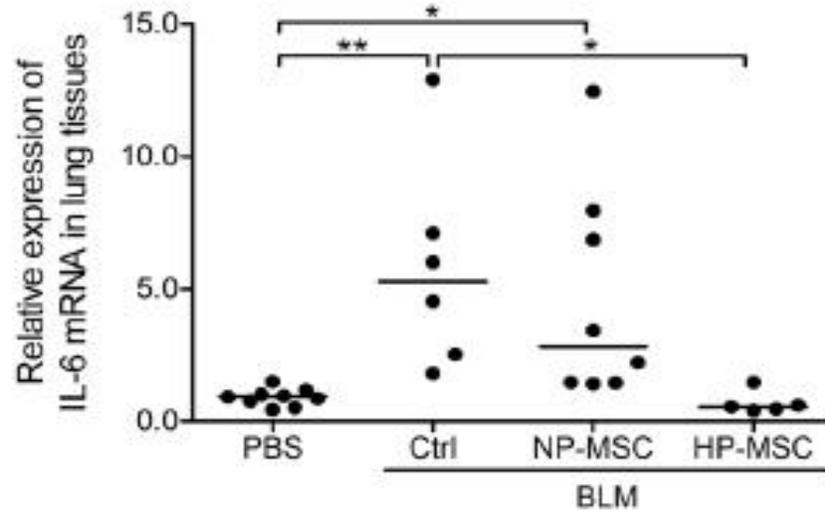


Improvement of pulmonary respiratory functions of HP-MSCs treatment

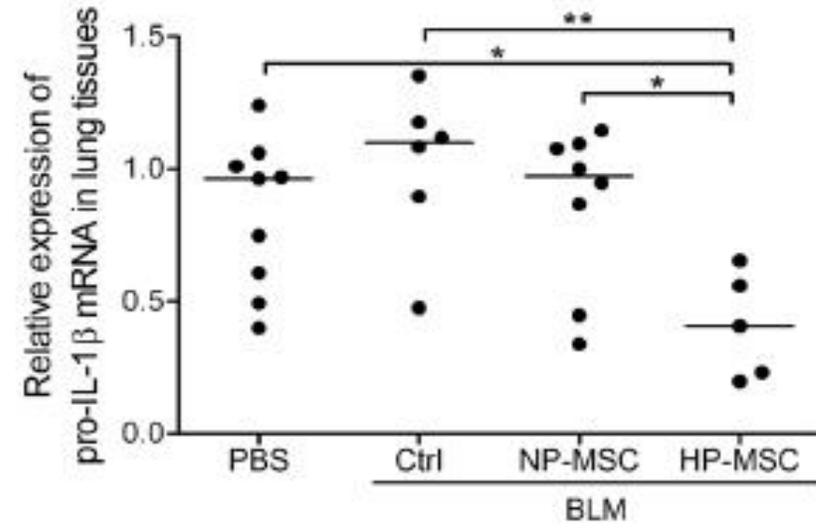
in the bleomycin-induced pulmonary fibrosis mouse model



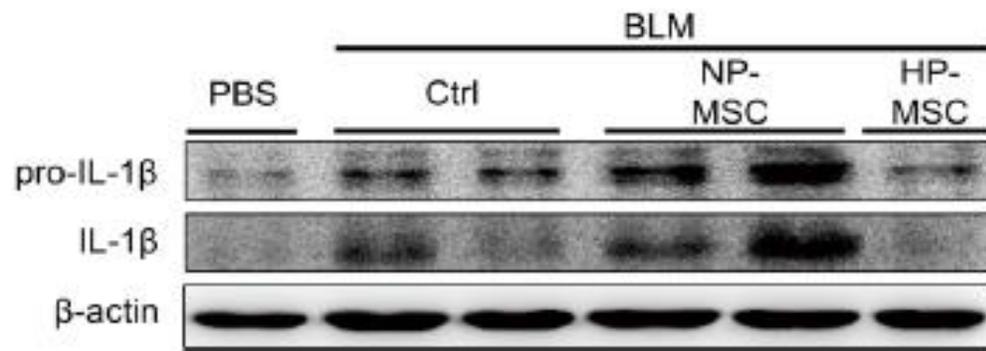
A



B

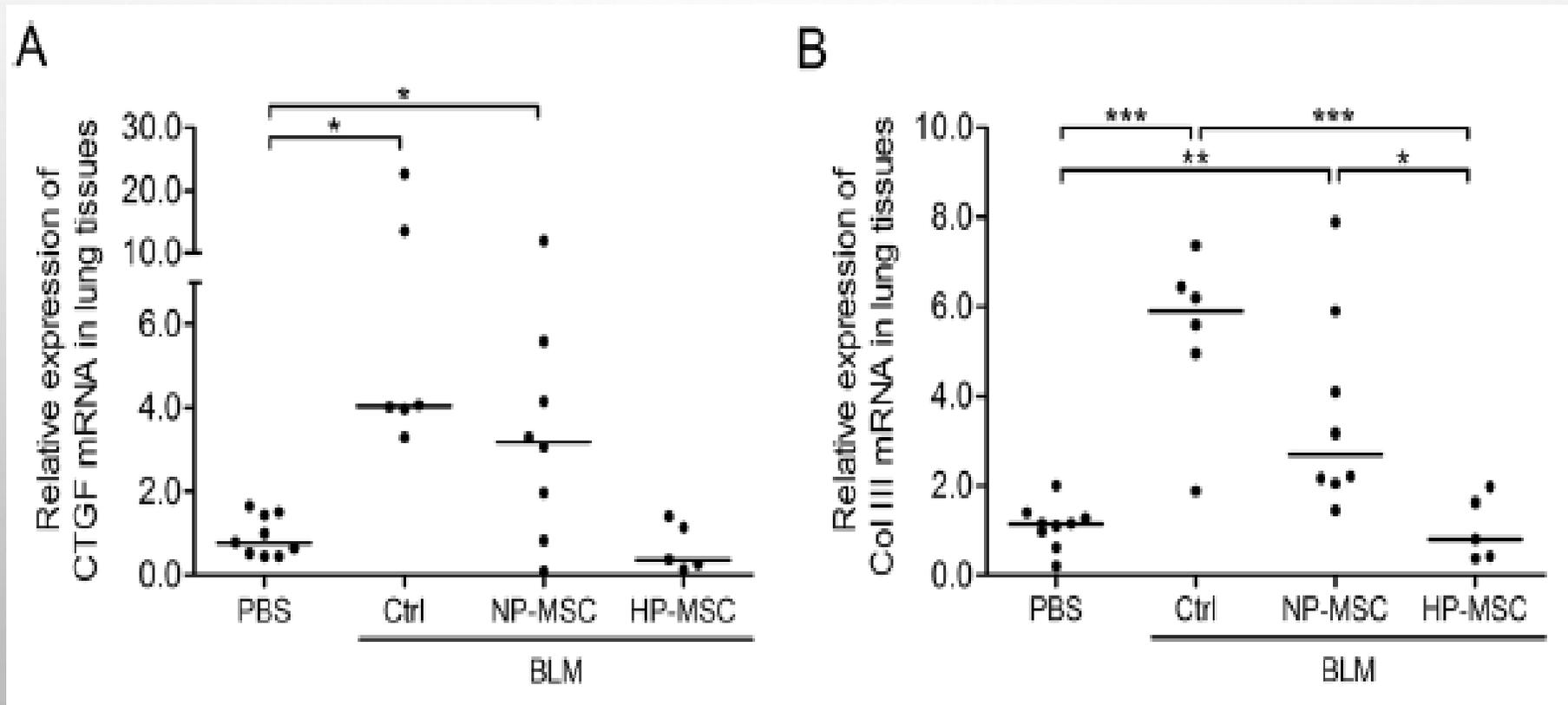


C

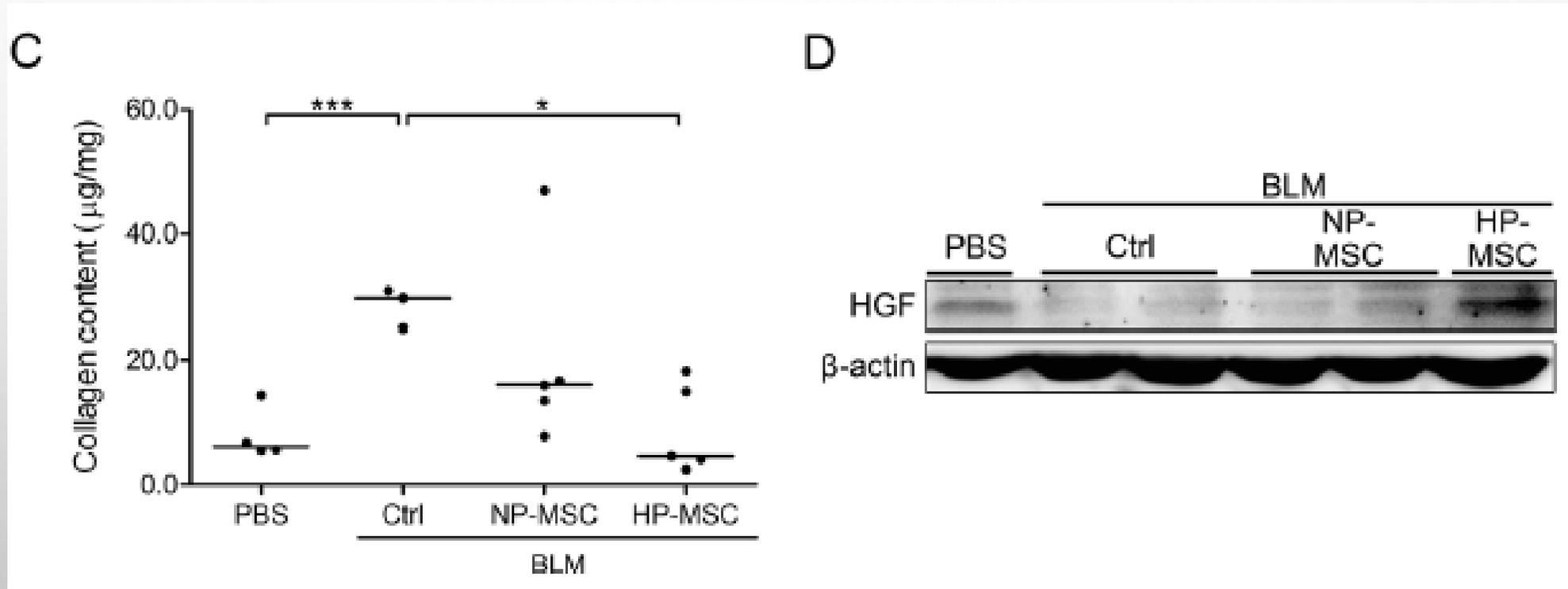




Expression of 2 factors mediating fibrosis namely collagen type III and connective tissue growth factor (CTGF)

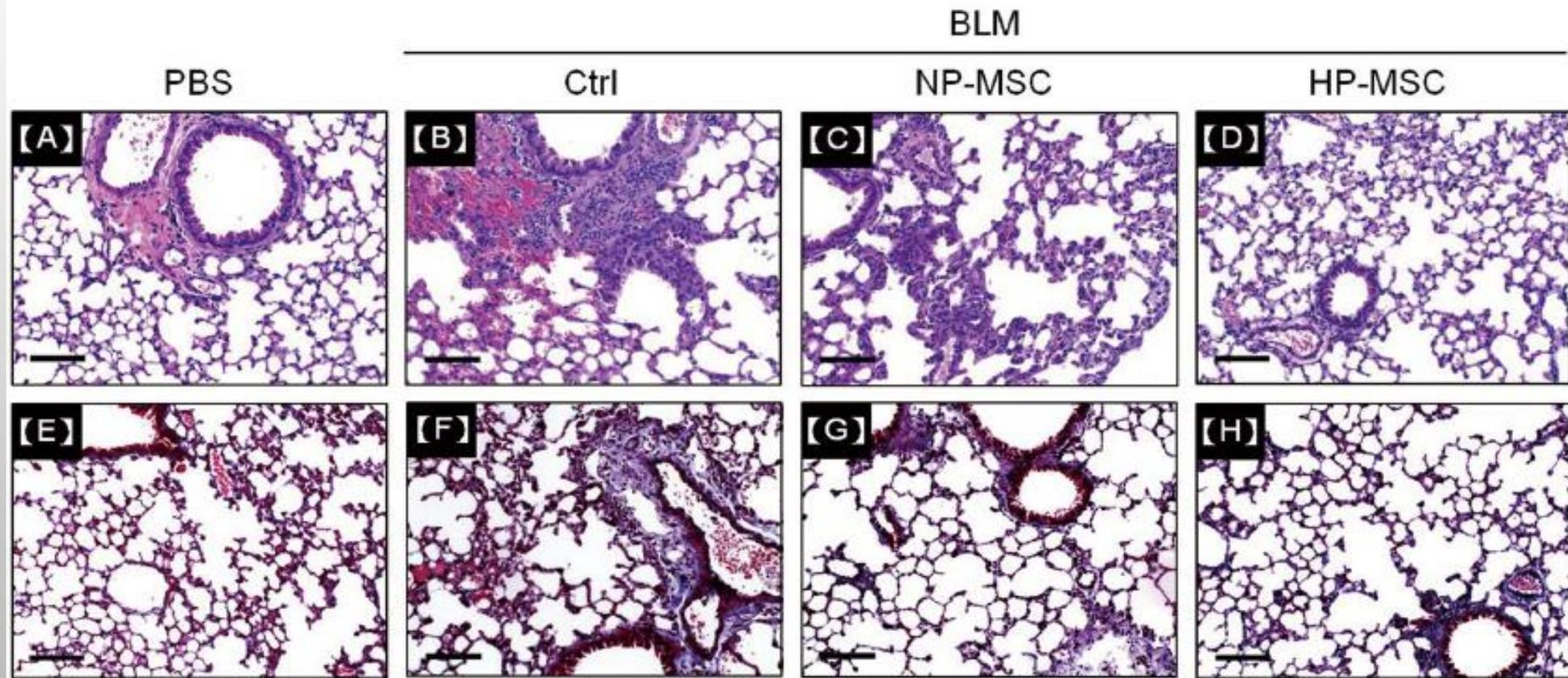


Collagen assay

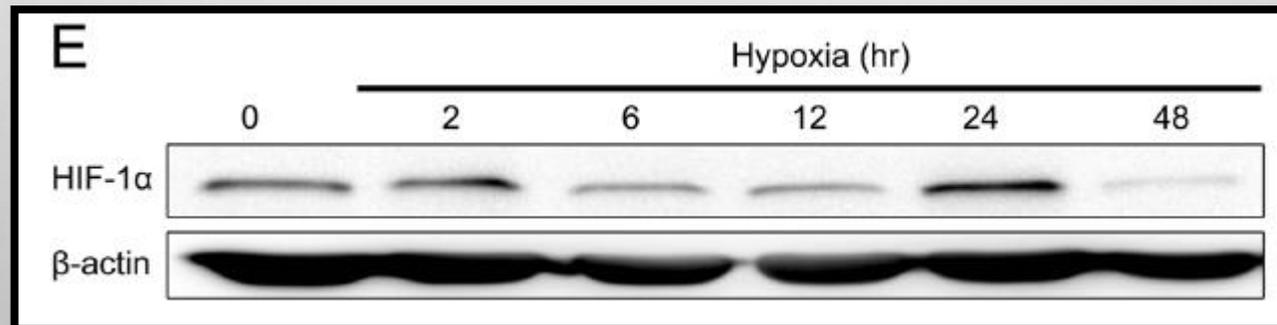
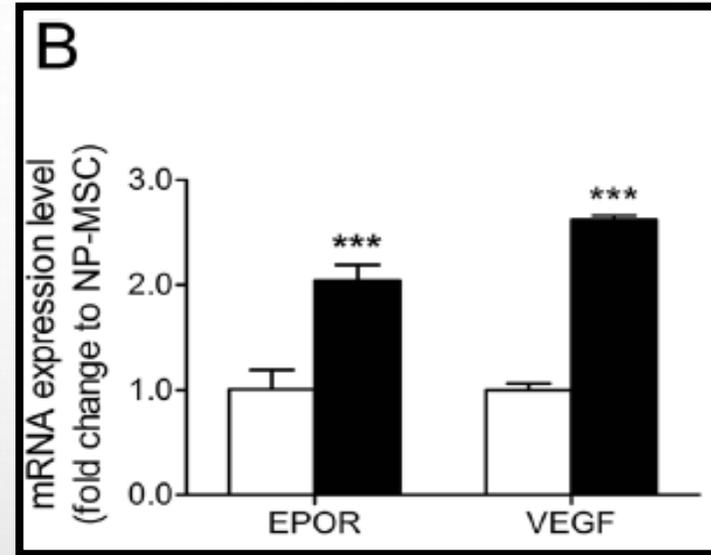
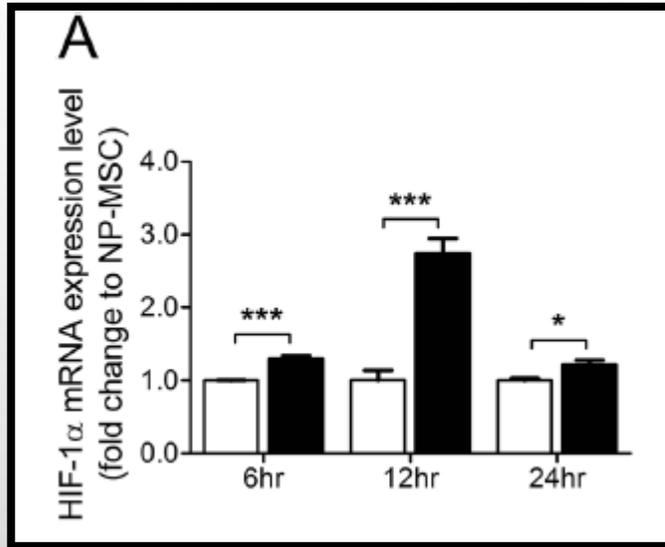


versamax spectrophotometry OD at 550 nm

HP-MSCs transplantation alleviates histological changes in the lungs of the bleomycin-induced pulmonary fibrosis mouse model

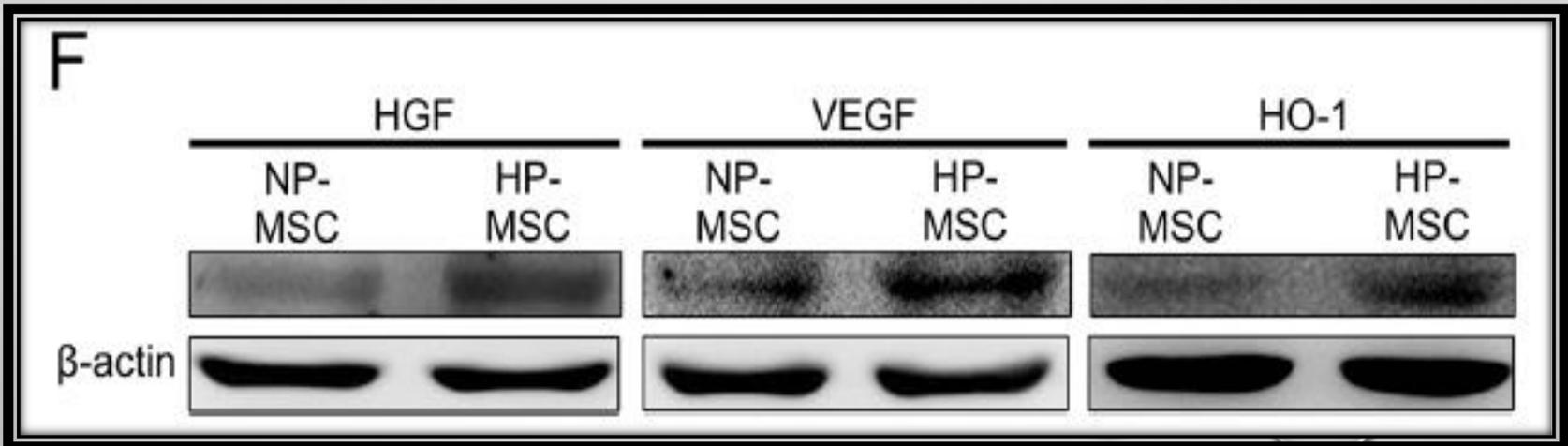
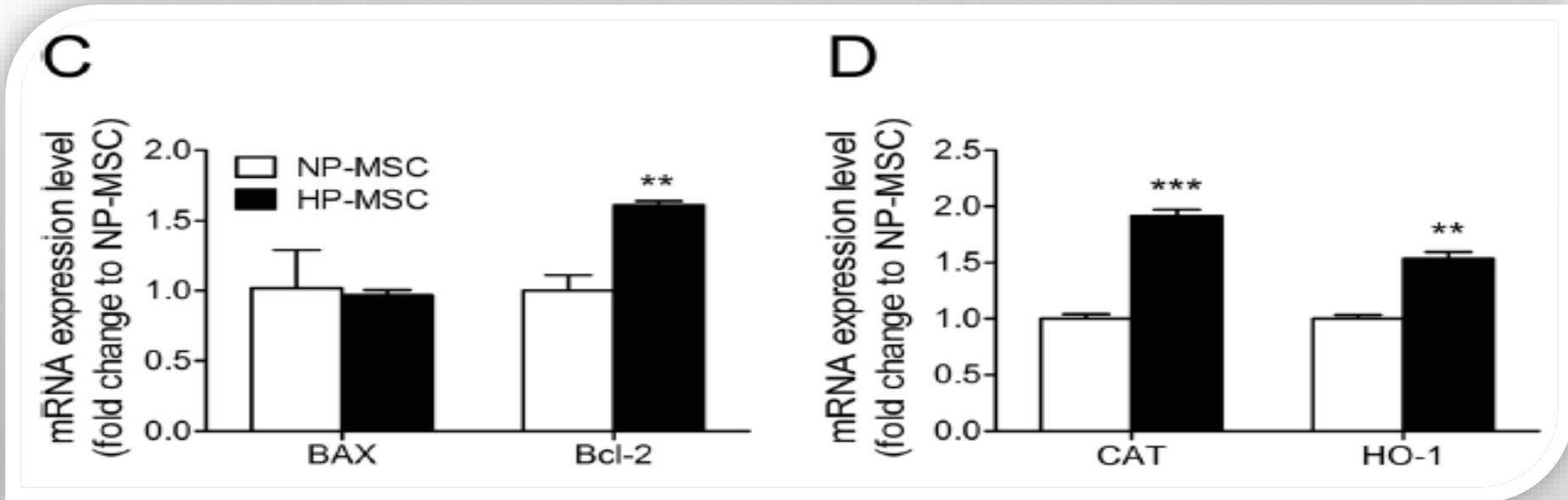


Result



□ NP-MSC
■ HP-MSC

Result





Result

- The pulmonary respiratory functions Improved for up to 18 days of hypoxia-preconditioned MSC treatment.
- ↓ Expression of inflammatory factors and fibrotic factor in the lung tissues of the hypoxia-preconditioned MSC-treated mice.
- ↑ (HGF) played an important role in Mediating the therapeutic effects of transplanted hypoxia-preconditioned MSCS
- Several **lacz-labeled MSCs** were observed within the Lungs in the (HP-MSc) treatment groups at **day 21**, but no signals were detected in the normoxic MSC Group.



Discussion

Engraftment of HP-MSCs

therapeutic effects



untreated MSCs in a BLM-induced pulmonary fibrosis animal model.

HP

Upregulated cytoprotective and regenerative genes

Stabilized mitochondrial membrane potentials

↑ cell proliferation

Acted against H₂O₂-induced cell death in the treated MSCs

↓ BLM induced cell apoptosis

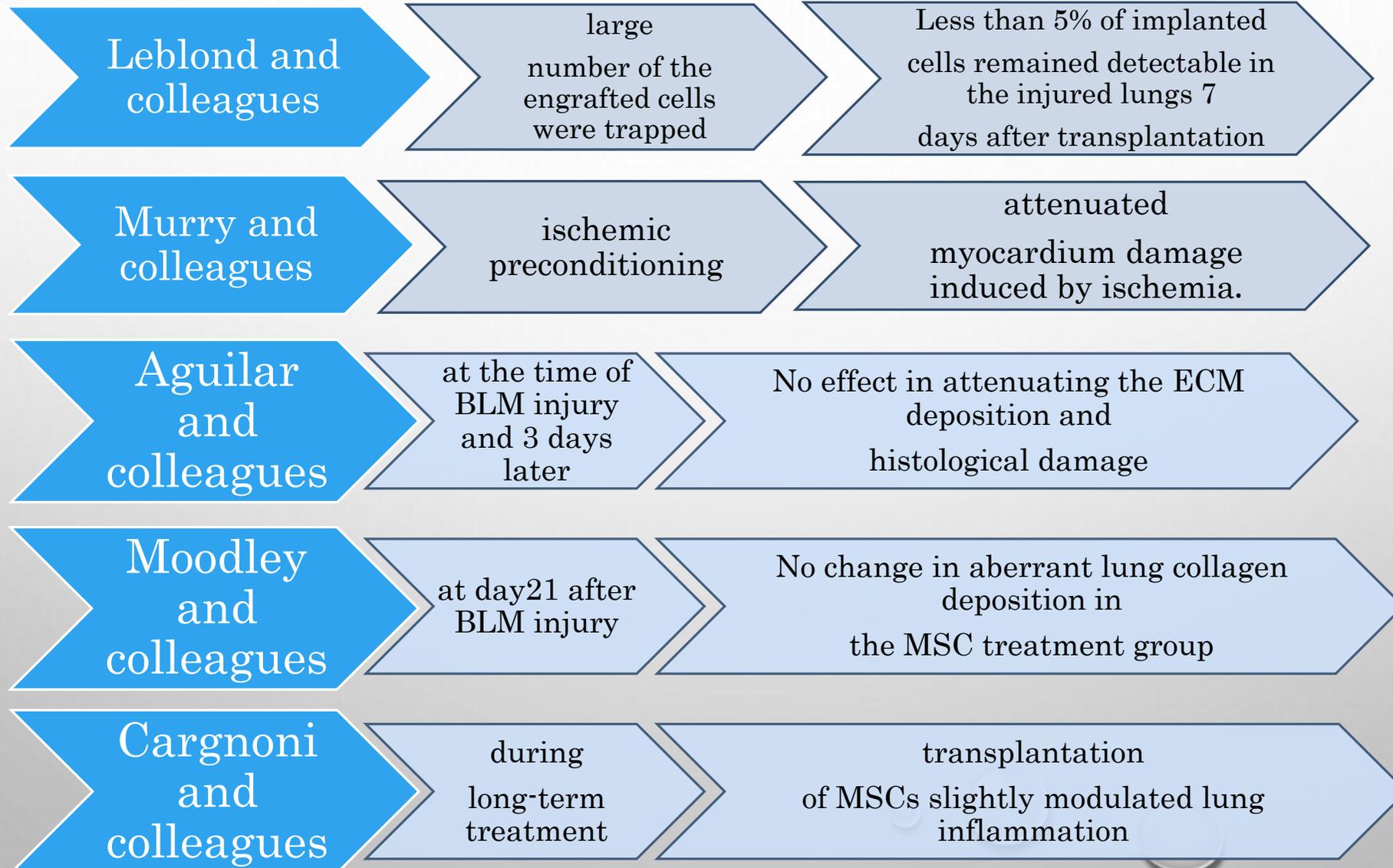
↓ ECM production through TGF-β₁-mediated Akt signaling via paracrine effects

Improved lung functions

↓ lung edema

↓ Levels of pro-inflammatory and fibrotic factors.

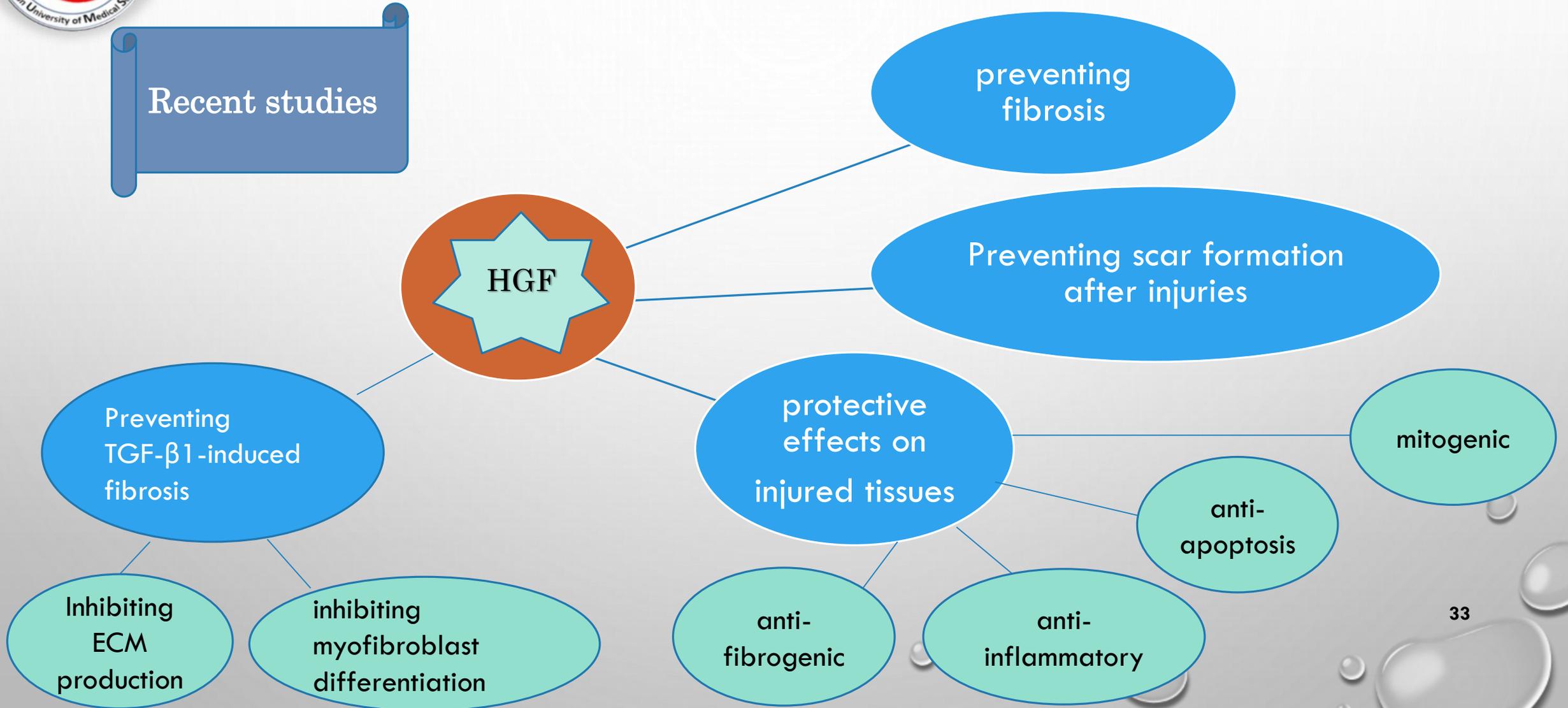
Discussion





Discussion

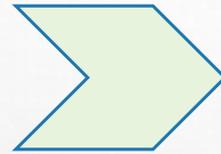
Recent studies





Local and systemic infusion

- Local (intratracheal)
- Systemic (IV or IP)



Side effect

Recipient requiring
Total body irradiation
to promote stem cell
engraftment



Cause of improving therapeutic effects

- Activation of HIF-1 α
 - upregulation of growth factors
 - downregulation of proinflammatory Cytokines and chemokines in hypoxic MSCs
- BLM or downstream TGF- β 1
 - Induce ECM synthesis
 - ECM deposition Through Akt signaling in fibroblasts
- **Dysregulation Of intercellular** between **alveolar epithelia** and **Fibroblasts** is a critical event in the fibrosis process.
- Net Balance $\xrightarrow{\hspace{10em}}$ die $\xrightarrow{\hspace{1em}}$ TGF- β 1 shift the balance
toward \uparrow fibroblast proliferation and ECM production



The therapeutic effects at day **7** or day **14** after BLM treatment , but only a few studies showed the longer period of treatment effects



Therapeutic Efficacy



Transplanted cell number



Cell ages

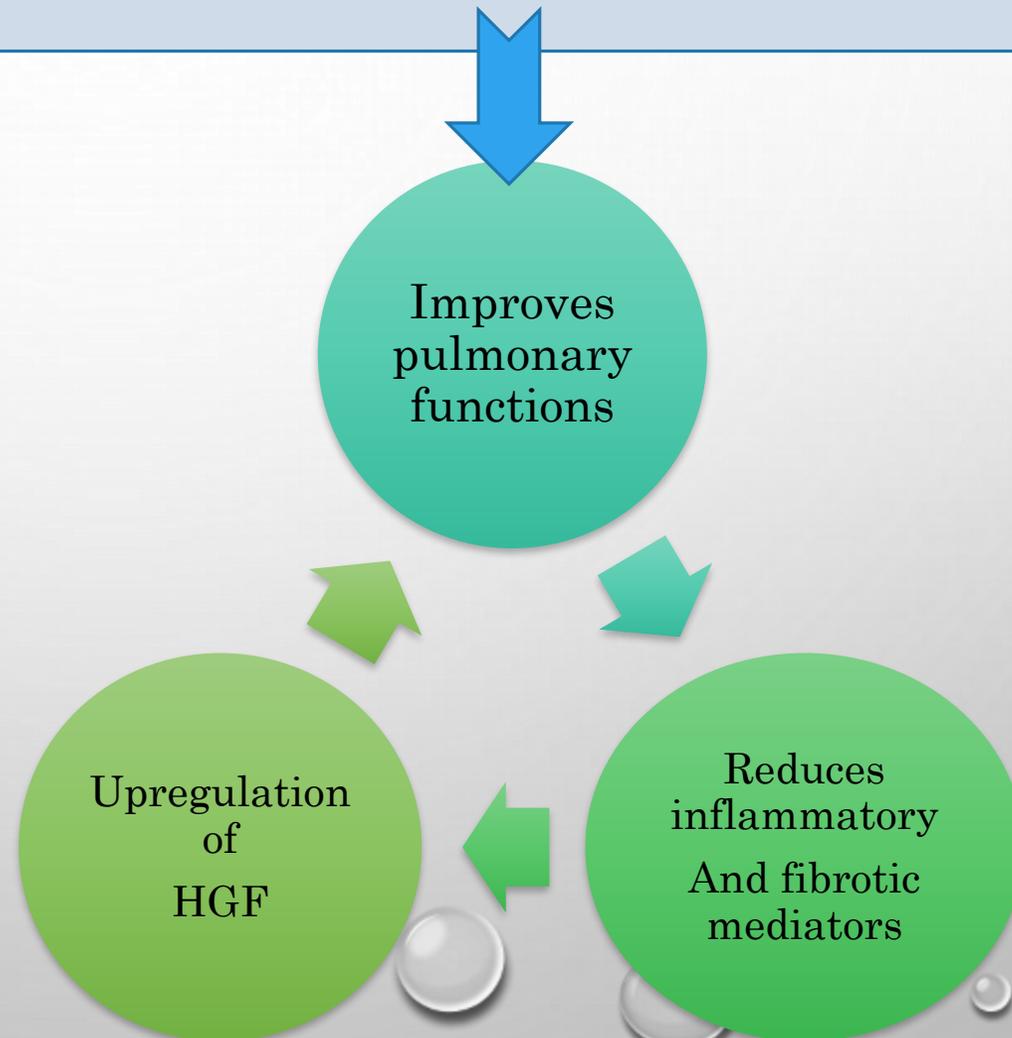


Timing of transplantation

Treatment period

Conclusions

Transplantation of HP-MSCs





***THANKS
FOR YOUR
ATTENTION***

Is there a question that I will
gladly answer ?