



Expression of Hepatoma-Derived Growth Factor during Post-Pneumonectomy Compensatory Lung Growth

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Abstract

RATIONALE. Following pneumonectomy, rapid compensatory growth occurs in the remaining lung resulting in restoration of total lung volume, mass and alveolar number. Although the mechanisms involved are not understood, it is becoming apparent that a variety of growth factors contribute to the complex signaling pathways which regulate this "regenerative" growth. One such growth factor may be hepatoma-derived growth factor (HDGF), which has recently been shown to exert angiogenic and mitogenic effects on several cell types in the lung including endothelial, smooth muscle, and epithelial cells. We hypothesized that expression of HDGF is upregulated in the lung during compensatory growth. **METHODS.** Adult C57BL6 male mice underwent a left pneumonectomy (PNX) or left thoracotomy (Sham). The right lungs were harvested at 1, 3, 7 and 14 days after surgery and either frozen for protein isolation or fixed with 4% paraformaldehyde via intratracheal instillation (n=5/group). Expression of HDGF was assessed by Western analysis of lung protein homogenate and by immunohistochemistry of paraffin sections. **RESULTS.** Western blot revealed a 1.5-fold increase in HDGF expression at 7 and 14 days post-pneumonectomy (p<0.05). Immunohistochemistry confirmed an increase in the number of HDGF-positive peripheral epithelial cells with expression also noted in occasional endothelial cells. At 7 and 14 days after pneumonectomy a shift to increased expression in bronchial epithelial cells was observed. Sham lungs showed no increase in HDGF. **CONCLUSIONS.** This study documents an increased expression of HDGF in the lung after pneumonectomy. These results suggests that HDGF may play an important role in the regulation of compensatory lung growth through its mitogenic and angiogenic properties and could serve as a potential therapeutic target in lung disease.

Background

Hepatoma-derived growth factor (HDGF) exerts mitogenic effects on several types of cells including fibroblasts¹, endothelial cells², vascular smooth muscle cells³, hepatoma cells¹ and lung epithelial cells⁴. Translocation to the nucleus is essential for its mitogenic activity, indicating that HDGF is a unique factor belonging to a nuclear/growth factor group.

Pneumonectomy (removal of a lung) results in rapid, hyperplastic, compensatory growth of the remaining lung. The molecular mechanisms that regulate this regenerative growth are not well known. An understanding of these mechanisms and the role of angiogenic growth factors in this growth could lead to therapies for lung injury, pulmonary hypertension, respiratory failure, transplantation for endstage lung disease, and even stimulation of regenerative growth in patients with minimal pulmonary tissues left after lung resection.

We hypothesized that expression of HDGF is upregulated in the lung during compensatory growth after pneumonectomy.

Methods

Animals: C57BL6 adult male mice were used (n=5/group).

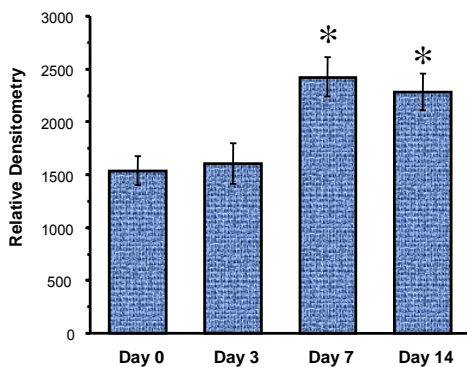
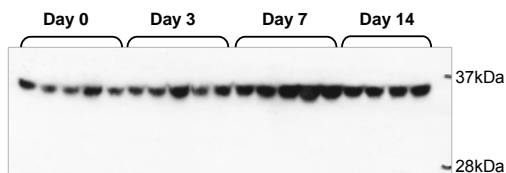
Surgery: Mice were anesthetized with inhaled halothane followed by tracheotomy and ventilation with room air. Left pneumonectomy was performed through a small left anterior thoracotomy. The left hilum was isolated and ligated with a titanium clip and the left lung excised. Sham surgery consisted of left thoracotomy only. The chest was closed in layers and animals recovered under normoxic conditions.

Harvest: At designated days after surgery, mice were anesthetized and the lungs were either frozen or inflation fixed with 4% paraformaldehyde at 20 cm H₂O.

Results

HDGF Expression - Western Blot

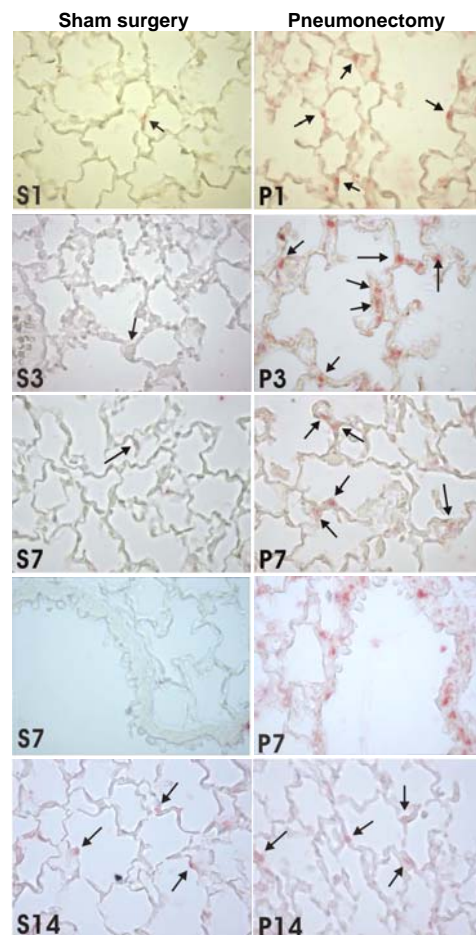
Total lung protein (50 µg) was fractionated on a 10% SDS polyacrylamide gel. The blot was transferred to nitrocellulose, blocked with 5% milk and incubated with anti-HDGF antibody² (1:2000). Protein bands were visualized by chemiluminescence and quantitated by computerized densitometry (Alpha Innotech Corp.).



Results

HDGF Expression – Immunohistochemistry

Lungs were inflation-fixed with 4% paraformaldehyde. Paraffin sections were immunostained using an anti-HDGF antibody³ (1:1000), commercial ABC AP kit (Vector laboratories) and Vector Red as a Substrate. S=sham, P=pneumonectomy, numbers=days post surgery.

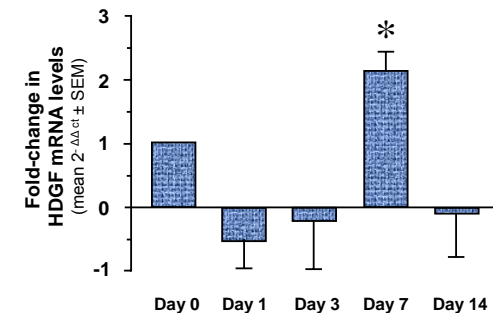


*p < 0.05 vs. Day 0

Results

HDGF Expression – Real-Time PCR

1µg total RNA was reverse transcribed and amplified by PCR using primers to amplify a specific 120 bp mouse HDGF sequence for 40 cycles using BioRad SYBR green Taq DNA polymerase. Amplification of the mouse TATA box binding protein was used as an internal control. Lungs from 0, 1, 3, 7 and 14 days post-pneumonectomy were evaluated.



Conclusions

This study documents an increased expression of HDGF in the lung after pneumonectomy, predominantly at 7 days post-pneumonectomy.

These results suggests that HDGF may play an important role in the regulation of compensatory lung growth through its mitogenic and angiogenic properties and could serve as a potential therapeutic target in lung disease.

References

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