**Decreased Extracellular Superoxide Dismutase Augments Hypoxia-Induced Loss of Hyaluronin in Pulmonary Vessels**

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**BACKGROUND & RATIONALE**

Pulmonary arterial hypertension (PAH) is a progressive lethal disease characterized by vasoconstriction and vascular remodeling. Oxidant/antioxidant imbalance in PAH: Pathogenesis of PAH includes altered redox signaling due to an increase in oxidative production (e.g. superoxide, O$_2^-$) and a decrease in antioxidant protection, including the key vascular antioxidant enzyme, extracellular superoxide dismutase (EC-SOD).

**Interaction between antioxidant EC-SOD and Hyaluronan**

Hyaluronan (HA), a glycosaminoglycan, is an important component of the extracellular matrix and functions in maintenance of structure; HA can be fragmented by hyaluronidases (Hyal) or reactive oxygen species, leading to inflammation and tissue remodeling. EC-SOD protects HA from oxidative fragmentation.

**Increased HA in PAH:** HA is increased in plasma, alveolar fluid, lung tissue and media of pulmonary artery smooth muscle cells isolated from PAH patients and in animal models of PAH.

This study impacts the field of anatomy by interrogating alterations in a key extracellular matrix component, known to regulate physical properties of blood vessels, skin, joints and other tissues in health and disease.

**Hypothesis:**

Increased vascular EC-SOD will result in decreased HA content around the pulmonary artery in a model of PAH due to increased oxidative fragmentation of HA.

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**RESULTS**

**Figure 1:** Mice with reduced expression of EC-SOD had decreased HA content in the lung and increased HA in the plasma after chronic hypoxic exposure.

**Figure 2:** Mice with total body and SMC specific decrease in EC-SOD expression were more susceptible to chronic hypoxia-induced loss of PA HA

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**CONCLUSIONS**

The data indicate that increased extracellular oxidative stress due to absence or lack of EC-SOD increases HA at baseline and augments fragmentation of HA in the PA with release into the plasma in the setting of PAH.

Loss of vascular EC-SOD due to decreased tissue binding or absent SMC expression both decreased PA HA, implicating vascular oxidative stress.

Further studies of the mechanisms and consequence in HA fragmentation in the setting of oxidative stress may not only elucidate its importance in vascular diseases, but also its role in inflammatory conditions such as rheumatoid arthritis or cancer.

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**REFERENCES & ACKNOWLEDGEMENTS**

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**Jiang D, Liang J, Noble PW. 2007. Increased hyaluronan content in idiopathic pulmonary arterial hypertension is associated with the expression of vascular EC-SOD. Eur J Pharmacol. 564(1-3):179-85.**

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**PROJECT APPROACH**

Mouse model of chronic hypoxic PAH: 4-6 week old mice were exposed to normobaric normoxia (Denver altitude) or hypobaric hypoxia (18,000 ft) for 35 days.

**Mouse Strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Change</th>
<th>EC-SOD content vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Normal</td>
<td>Normal Normal Normal Plasmap</td>
</tr>
<tr>
<td>EC-SOD KO</td>
<td>EC-SOD knock out (EC-SOD-KO)</td>
<td>Single nucleotide polymorphism in EC-SOD HDD decreases EC-SOD matrix binding affinity</td>
</tr>
<tr>
<td>EC-SOD R213G</td>
<td>HA content (ng/ml)</td>
<td>Normal Normal Normal Normal</td>
</tr>
</tbody>
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**EC-SOD**

EC-SOD KO: Mice lacking all EC-SOD

EC-SOD R213G: Inducible knock-down of EC-SOD in smooth muscle cells

EC-SOD control (EC-SOD-Lox): Control strain for EC-SOD SMC KO

**HA content:** HA content was measured by ELISA in lung (ng HA/mg protein) and plasma (ng HA/mL).

**EC-SOD analysis:** Vascular localization of HA was determined by staining for HA using a biotinylated HA binding protein (BHA/BP) and immunostaining with the smooth muscle cell marker, a-smooth muscle actin, followed by computerized analysis to determine the amount of HA in the PA in a uniform population of mid-sized (180-200 μm diameter) pulmonary arteries (total 5 pixels/microcircumference).

**HA fragmentation**

Fragmentation of HA was assessed in lung tissue by separating low-molecular-weight HAs and high-molecular weight HAs by gel electrophoresis, transfer to a hybridization membrane and detection with HAAPB.

**Statistical Analysis:** 2-way ANOVA in GraphPad Prism

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**RESULTS**

**Figure 3:** Optimization of method to study HA fragmentation

**Evaluation of HA in lungs from different strains**

Blue arrow: LMW HA, Red arrow: Discrete sized HA in untreated lung tissue

Hyaluronidase, Dnase, Rnase, proK: treatment to degrade DNA, RNA, protein

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