Osteopontin expression and potential roles in tissue-protection of the airways

Background
Osteopontin (OPN) is a multifunctional, highly anionic phosphoglycoprotein, initially discovered in bones where it is shown to be involved in the regulation of both physiological and pathological mineralization. Besides its expression in osteoclasts and osteoblasts, OPN has been detected in various types of cancer cells and tumor tissues, it has also been found in normal cells and in most tissues and body fluids, such as blood, urine, bile, cerebrospinal fluid and milk.

In the lungs, there is a constitutive expression of OPN which is increased upon injury or stimulation by many factors including cigarette smoke, the latter a major risk factor associated with the development of Chronic Obstructive Pulmonary Diseases (COPD). Moreover, OPN up-regulation is correlated with other inflammatory diseases such as asthma and cystic fibrosis. In the airways, multiple functions have been attributed to OPN including promotion of Th1-inflammation, matrix remodeling as well as recruitment of neutrophils and eosinophils through its integrin binding site. In addition, OPN plays key roles in various physiological and pathological processes like wound healing, inflammation, autoimmune disease, and cancer metastasis.

Methods and Results
In the current studies, our immunohistochemistry results showed that there is a comparable expression of OPN in the airway epithelium of healthy donors, smokers and individuals suffering from COPD GOLD stages I and IV, while in COPD GOLD stages II-III, the OPN expression was
significantly higher, which might reflect the activity of the disease. In COPD lung tissues, immunohistochemistry revealed that OPN co-localized with goblet and club cells but neither basal nor ciliated cells, an observation that may explain the increased OPN expression seen in severe COPD lung tissues, as goblet cell hyperplasia is a feature of the disease. These results were further confirmed, when we differentiated human bronchial epithelial cells towards goblet cell or ciliated cell lineages using air-liquid interface and subsequently stimulated the cells with cigarette smoke, where OPN production was much more pronounced in goblet cell enriched cultures compared to ciliated cell enriched cultures.

Moreover, we showed that OPN is upregulated in mouse models of histone-induced acute lung injury, a disease phenotype that is accompanied with elevated inflammatory responses, cell damage and death, and release of the highly cationic cytotoxic histones. In this model, the wild type mice survived while OPN knockout mice died within 24 hours. Interestingly, the latter group of mice were rescued after being co-instilled with recombinant OPN, indicating that OPN may bind and neutralize the toxic effect of histones.

**Conclusion**

Increased OPN expression in inflammatory airway diseases may be part of an inherent protective mechanism, as suggested by the fact that the OPN knockout mice died 24 hours after airway instillation of histones at a dose that was sublethal to wild type mice. Under stress conditions such as COPD or acute lung injury, the epithelium will sense the danger and trigger airway differentiation towards secretory cells such as goblet and club cells, thus increasing OPN production, which among other functions, will sequester histones and dampen its cytotoxic effect.
EXPRESSION OF OSTEOPONTIN IN THE AIRWAY EPITHELIAL LINING IS DEPENDENT ON DIFFERENTIATION AND CONFINED TO SUBSETS OF CELLS

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OPN has been extensively studied over the past decades, but the distribution and phenotype of cells responsible for producing this protein in the airway epithelium have not been characterized.

\textbf{Aim:} In this study we sought to investigate the main source of OPN in the airways and whether cellular remodeling affect its production in the lungs.

\textbf{Results:} In COPD lung tissues, our investigation revealed that OPN is expressed in Goblet and Club cells but not in ciliated and basal cells. Moreover, cigarette smoke extract which is one of the major causes of COPD, has shown to be a potent inducer for OPN expression and production. Goblet cell hyperplasia is a feature of COPD, for this reason we differentiated the human bronchial epithelial cells using air-liquid interface cultures to either a goblet cell enriched phenotype or a ciliated cell enriched phenotype. After exposing the cells to cigarette smoke, OPN production was significantly higher in goblet cell enriched cultures when compared to ciliated cell enriched culture.

\textbf{Conclusion:} Taken together, OPN-production in small airways is confined to goblet and club cells. Alteration of epithelial cell subsets, for example goblet cell hyperplasia, is likely to alter the number of OPN-producing cells, for example in response to cigarette smoke.
OSTEOPONTIN PROTECTS AGAINST LUNG INJURY CAUSED BY EXTRACELLULAR HISTONES

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During acute lung injury (ALI) there are series of events that are important to initiate inflammation including danger-associated molecular patterns (DAMPs) being released from dead cells, as exemplified by extracellular histones. Extracellular histones promote inflammation and cause tissue damage because of their cationic nature. Thus, mechanisms dampening the cytotoxic properties of these molecules are important to prevent lung injury.

Aim: This study set out to investigate whether the OPN that is expressed at high levels in the airways during inflammation, may protect the airways against the pro-inflammatory and cytotoxic properties of extracellular histones.

Results: In a model of histone-induced acute lung injury, OPN⁻/⁻ mice showed increased inflammation, tissue injury, and died within 24 hours compared with wild type mice that showed lower degrees of inflammation and also survived. In lipopolysaccharide-induced acute lung injury, wild type mice displayed less inflammation and tissue injury compared to OPN⁻/⁻ mice. In healthy humans, OPN and markers of tissue-damage increased in parallel after exposure to swine dust.

Conclusion: These findings demonstrate that OPN plays a key role in modulating the pro-inflammatory and cytotoxic properties of free histones. Increasing OPN expression or adding the molecule exogenously could prevent tissue-damage in the airways.