

**Extracellular S100A9-mediated secretion of MMP3 does not contribute to smoke-induced loss of lung function**

Rationale: S100 calcium-binding protein A9 (S100A9), is elevated in plasma and bronchoalveolar lavage fluid (BALF) of COPD patients and our group has demonstrated that inhibiting its signaling in mice prevents smoke-induced COPD. In addition, inhibition of S100A9 in mice results in a significant reduction in matrix metalloproteinase 3 (MMP3) levels within the BALF. MMP3 polymorphisms are associated with cancer development in COPD patients and MMP3 plays a significant role in lung remodeling in pulmonary fibrosis. However, little is known about the role of MMP3 in COPD. Here, we investigated the role of MMP3 in cigarette smoke-induced COPD and explored the mechanism of S100A9-induced MMP3. Methods: Male and female Mmp3 knockout mice and wild type control mice were exposed to cigarette smoke daily for 6-months and pulmonary function testing was performed using the Scireq Flexivent System. Since MMP3 is primarily expressed by fibroblasts within the lung, human primary lung fibroblasts were treated with the ERK inhibitor, LY3214996, or transfected with ERK siRNA prior to treatment with S100A9. MMP3 responses were quantified by qPCR and Luminex bead assays. Results: Mmp3 knockout mice were sensitive to cigarette smoke-induced inflammation, airspace enlargements and loss of lung function. Extracellular S100A9 regulated MMP3 gene expression and secretion in fibroblasts. Modulating ERK signaling, with a chemical inhibitor or with siRNA, reduced S100A9-mediated MMP3 production and secretion from fibroblasts. Both TLR4 and RAGE signaling contributed to S100A9-mediated ERK activation and MMP3 production and secretion. Conclusions: In conclusion, Mmp3 deficiency in mice did not protect the lungs from cigarette smoke-induced COPD. However, extracellular S100A9 triggers TLR4 and RAGE signaling to induce ERK-mediated MMP3 expression and secretion. Thus, MMP3 may still have a role in COPD exacerbation or comorbidities of COPD, such as lung cancer.