Versican/PG-M assembles hyaluronan into extracellular matrix and inhibits CD44-mediated signaling toward premature senescence in embryonic fibroblasts

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Versican/PG-M is a large chondroitin sulfate proteoglycan of the extracellular matrix which interacts with hyaluronan at the N-terminal G1 domain, composed of A, B, and B' subdomains. Recently, we generated knockin mice Cspg2<sup>2<sup>3/3</sup></sup>, whose versican, without the A subdomain, has decreased HA-binding affinity, thereby exhibiting reduced deposition of versican in the extracellular matrix. Here, we show that the Cspg2<sup>2<sup>3/3</sup></sup> fibroblasts within 20 passages proliferate more slowly and acquire senescence. Whereas the extracellular matrix of the wild type fibroblasts exhibited a network structure of hyaluronan and versican, that of the Cspg2<sup>2<sup>3/3</sup></sup> fibroblasts exhibited ~35% and ~85% deposition of versican and HA, without such a structure. The Cspg2<sup>2<sup>3/3</sup></sup> fibroblasts showed a substantial increase of ERK1/2 phosphorylation and expression of senescence markers p53, p21, and p16. Treatment of wild type fibroblasts with hyaluronidase and exogenous hyaluronan enhanced ERK1/2 phosphorylation, and treatment with an anti-CD44 antibody that blocks HA-CD44 interaction inhibited the phosphorylation. These results demonstrate that versican is essential for matrix assembly involving hyaluronan, and that diminished versican deposition increases free hyaluronan fragments that interact with CD44 and increase phosphorylation of ERK1/2, leading to cellular senescence.

Ovalbumin-induced Airway Hyperresponsiveness is increased in SHAP-hyaluronan complex deficient Mice

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Background: Serum-derived hyaluronan-associated proteins (SHAP), the heavy chains of inter-α-trypsin inhibitor, covalently bind to hyaluronan (HA) to form the SHAP-hyaluronan (SHAP-HA) complex. The SHAP-HA complex was found to be involved in the pathophysiology of inflammatory diseases such as arthritis and hepatitis. Thus, we sought the possibility that the complex is also involved in airway allergy.

Methods: The SHAP-HA deficient (KO) mice and wild type (WT) mice were used. The mice were immunized twice by intraperitoneal injection of ovalbumin (OVA), and exposed to aerosol OVA for 30 minutes each day for 2 weeks. Twenty-four hours after the final OVA challenge, airway responsiveness to inhaled methacholine (Mch) was measured, and analysis of bronchoalveolar lavage fluid (BALF) and lung histological studies were performed.

Results: Compared to WT mice, KO mice showed higher airway hyperresponsiveness (AHR) to inhaled Mch and higher late phase response to OVA, but the early phase response was comparable. In KO mice, total number of inflammatory cells in BALF was high, due to the increased number of macrophages and neutrophils as revealed by differential cell count. Furthermore, decreased concentrations of soluble tumor necrosis factor receptor-1 (sTNFR1) and interleukin (IL)-12p40 were found in BALF from KO mice, although levels of Th1 and Th2 cytokines were not different from WT mice.

Conclusions: The findings suggest that in murine model of asthma, the SHAP-HA complex plays an inhibitory role in the development of AHR and allergic airway inflammation, at least in part via negative feedback mechanisms by sTNFR1.