Association of Fibulin-3 with liver fibrosis, a new regulator of hepatic stellate cell homeostasis?

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Résumé

Chronic liver diseases are associated with the development of liver fibrosis, characterized by excessive accumulation of extracellular matrix (ECM). This microenvironment affects tissue homeostasis and favors initiation and progression of hepatocellular carcinoma (HCC). During liver injury, hepatic stellate cells (HSCs) are activated and differentiate into contractile myofibroblasts that secrete ECM components, therefore contributing to fibrosis. We previously conducted proteomic analyses of adjacent tissues from patients with HCC using ECM-enriched samples. Based on the matrisome signature, we identified 106 deregulated proteins in at least one grade of fibrosis. Among them, three members of the fibulin family, Fibulin-2, -3 and -5, were associated with fibrosis progression and tumor aggressiveness (Thomas et al., MBE, Florence, 2022). The aim of the present study is to compare functional implications of these fibulins in liver fibrosis.

Analyses of available single cell RNAseq data indicated that these fibulins are mainly expressed by fibroblasts and HSCs in healthy liver. Using the human HSC-derived cell line, LX-2, we showed that mRNA levels of these three fibulins are differentially regulated and that only Fibulin-2 and -3 proteins are detected in basal condition. RNA interference experiments led to different changes in morphology. Fibulin-3 depleted cells, specifically, were smaller, less spread-out with shorter cell body extensions. Staining of actin fibers revealed a default of cytoskeleton organization in Fibulin-3 depleted cells. Together, these results suggest that Fibulin-3 may have a role in HSC shape and adhesion. We tested this hypothesis by focusing on integrin signalling pathways and observed a decreased activation of the integrin-associated protein kinase, FAK, in Fibulin-3 depleted cells. Consistently, in silico analyses of protein-protein interaction (PPI) networks identified protein partners of integrins in the Fibulin-3 PPI network.

While all these fibulins are associated with liver fibrosis and HSC activation, our data therefore demonstrate the differential regulations of Fibulin-2, -3 and -5 in HSC-derived LX-2 cells. Silencing approaches suggest that Fibulin-3 is involved in HSC adhesion through integrin signalling pathways.

Mots-Clés: chronic liver diseases, extracellular matrix, fibrosis, hepatic stellate cells, fibulins

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