Influence of extracellular matrix environment on gene expression in liver myofibroblast (summary)

Hepatic stellate cells (HSC) and liver myofibroblasts (MF) are two cell populations most likely responsible for the synthesis of majority of connective tissue components in fibrotic liver. They differ in their origin and location in the liver, and in the spectrum of genes they express. HSC are located in Disse spaces of normal rat liver around the sinusoids, in fibrotic liver they become activated, proliferate and they undergo transdifferentiation into myofibroblast-like cells. Myofibroblasts are heterogenous cell population that consists at least of portal pMF, septal sMF and interface iMF. pMF, which are adjacent to bile duct epithelia, may be a mediator of billiary type fibrosis. sMF are located within and along the collagenous septum in cirrhotic liver. Little is known about the expression of genes involved in connective tissue metabolism in MF cultured in fibrin or collagen gels that more closely resemble natural cell environment. Fibrin is deposited in liver at sites of injury and collagen type I forms a substantial part of fibrotic septa.

In our study oligo cDNA array analysis was used to determine gene expression in quiescent HSC, activated HSC and MF isolated from both normal and CCl_4-cirrhotic liver. The expression of genes coding for connective tissue proteins, proteoglycans, metalloproteinases and their inhibitors, growth factors and cellular markers was determined. We investigated the influence of extracellular matrix environment on gene expression in MF.

HSC were prepared by perfusion of rat normal and cirrhotic liver with pronase and collagenase solutions, followed by centrifugation of the cell suspension on a density gradient. HSC from normal liver were quiescent 2 days after plating on plastic but they became activated after another 5 days in culture. When the culture was passaged 5times, its character changed profoundly as HSC were replaced by MF. Both cell subpopulation HSC and MF were stained for expression of α-smooth muscle actin (α-SMA) and intermediate filament proteins vimentin, desmin and glial fibrillary acidic protein (GFAP) using immunocytochemisty. Expression of genes was studied oligo cDNA array, rt RT-PCR. Expression of some proteins was studied by immunofluorescence. The extent of gel digestion by the cells was measured.

The pattern of gene expression changed during HSC activation, there were distinct differences between HSC and MF. Activated HSC express more collagen type I, III, IV, perlecan, fibronectin, ostepontin than freshly isolated HSC. Expression of metalloproteinases (MMP) decreases with activation. Gene expression of HSC isolated from cirrhotic liver resemble expression of HSC activated in vitro by cultivation on plastic dishes. There was no difference between normal MF and those isolated from the cirrhotic liver. The spectrum of cytoskeletal proteins was changing during cultivation of HSC. HSC differ from MF by presence of desmin. GFAP could be detected only in normal cells 2 days after their isolation. In contrast, α-SMA was absent from normal cells at this time but its expression was pronounced later. Embedding MF in collagen gel resulted in pronounced morphological changes of the cells. mRNA expression of a group of metalloproteinase (MMP-2, -3, -9, -13, -14) increased, while that of plasminogen activator inhibitor decreased. Significant changes in the expression of cytokines IL-6, TGF-β1, -β2, -β3 and CTGF were found. The effects of fibrin gels on MF were milder than those of collagen. Protein expression paralleled changes in MMP mRNA. MF have the ability to solubilise about half of collagen gel body.

We conclude that MF and HSC are distinct cell populations. There was no difference between normal MF and those isolated from the cirrhotic liver. We found that embedding of cells in fibrin and collagen gel affected cell morphology and gene expression. Expression of matricellular proteins and cytokines important in scar tissue formation changed. Elevated expression of MMPs resulted in solubilization of gelous matrix.