GFAP expression in the liver as an early marker of stellate cells activation

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SUMMARY

The activation of hepatic stellate cells is the conversion of quiescent cells into proliferative, contractile, and fibrogenic myofibroblasts. The alpha-smooth muscle actin is a well known marker of hepatic stellate cells activation. On the other hand, the Glial Fibrillary Acidic Protein expression is a key step in the astrocytes differentiation and constitutes the hallmark response of astrocytes to injury. Glial Fibrillary Acidic Protein expression was reported in quiescent stellate cells in vivo, with an increased expression in the acute response to injury in the rat, and a down-regulation in the chronic one. This study aims to evaluate the morphologic characteristics, distribution and percentage of Glial Fibrillary Acidic Protein expressing cells in normal and injured human livers.

Human liver biopsies (n=32) were divided in three groups: 1) donors liver; 2) chronic hepatitis C-virus related; 3) post-viral hepatitis cirrhosis. Samples were immunostained with anti- alpha-smooth muscle actin and anti- Glial Fibrillary Acidic Protein antibody. Subsequently they were semi-quantitatively evaluated. Liver fibrosis was quantified by morphometric analysis.

In chronic hepatitis livers and in the cirrhotic ones, hepatic stellate cells became larger with long cytoplasmic processes. The alpha-smooth muscle actin -positive stellate cells increase and expand from areas of piecemeal necrosis to the entire residual parenchyma with the progression of the fibrosis. By contrast Glial Fibrillary Acidic Protein-positive stellate cells are more evenly distributed in the earlier stages and confined to the periphery of the hepatic lobule in the advanced ones. Also some endothelial cells were Glial Fibrillary Acidic Protein positive in vessels of the expanding septa and at the edges of cirrhotic nodules.

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Glial Fibrillary Acidic Protein expression in the liver is probably linked to the fine modulation of the cytoskeleton during the formation of cytoplasmic processes in both stellate cells and endothelial cells. In the latter Glial Fibrillary Acidic Protein expression might be related to the remodelling of the vascular bed that occurs during the response to injuries. Otherwise in hepatic stellate cells it seems to be a marker related with the acquisition of contractile properties in a subpopulation more closely associated with precocious stages of the fibrosis.

INTRODUCTION

Fibrosis leading to cirrhosis can accompany virtually any chronic liver disease that is characterized by the presence of hepatobiliary distortion or inflammation. Fibrosis and cirrhosis represent the consequences of a sustained wound healing response to chronic liver injury from a variety of causes including viral, autoimmune, drug induced, cholestatic and metabolic diseases.

The hepatic stellate cells (HSCs) and their myofibroblastic counterparts, as main producers of an array of mediators, matrix molecules, proteases and their inhibitors, regulate the reparative processes in the liver (Friedman, 1993). The conversion of quiescent HSC into proliferative, fibrogenic, and contractile myofibroblasts, is the dominant event in fibrogenesis and proceeds along a continuum that involves progressive changes in cellular function, so that at any moment following injury, there are subpopulations of stellate cells with discrete cytoskeletal and phenotypic profiles.

The phenotypic modulation of HSC is characterized by the different expression of a number of intracellular markers. In addition to classical mesenchymal HSCs markers as desmin for rat HSCs, alpha-smooth muscle actin (SMA) for activated human and rat HSCs and vimentin for human and rat HSCs, HSCs were shown to express also neural/neuroendocrine features, including neural-cell adhesion molecule (N-CAM) and glial fibrillary acidic protein (GFAP) (Hautekeete and Geerts, 1997; Carpino et al., 2004, 2005; Geerts, 2001; Cassiman et al., 2002; Niki et al., 1996).

The alpha isotype of actin (a phenotypic marker of smooth muscle cells) is the best known marker reflecting the activation of HSC to myofibroblast-like cells with functions of matrix production and degradation, contractility and directed migration.

Moreover, GFAP immunoreactivity was detected in perisinusoidal cells of rat liver (Gard et al., 1985). GFAP is an intermediate filament first identified and characterized in astroglial cells in the central nervous system, where GFAP expression increases several-fold under conditions of brain injury (Brock and Callaghan, 1987), aging (Landfield et al., 1981) and neurodegenerative disease (Morrison et al., 1987).

In the rat GFAP was reported to be expressed in vivo in a subpopulation of quiescent HSCs, with an increased expression in the acute response to injury; however, GFAP decreased in the chronic one (Niki et al., 1996) as well as in primary culture in parallel to Ito cell activation (Neubauer et al., 1996).