

## **The Overexpression of Cav-1 Modulates the Actin Cytoskeleton and the Activation State of Hepatic Stellate Cells**

Ilha, M<sup>1</sup>; Moares, K.S<sup>1</sup>; Martins, L.A.M<sup>1</sup>; Barbé-Tuana, F<sup>1</sup>; Guma, F.C.R<sup>1</sup>

<sup>1</sup> Departamento de Bioquímica; Instituto de Ciências Básicas da Saúde UFRGS, Porto Alegre / RS, Brazil

**Introduction** The caveolae are small invaginations of plasma membrane, rich in glycosphingolipids, cholesterol, and GPI-anchored proteins. Caveolin-1 and cavin-1 (Polymerase-1 and release transcript factor, PTRF1) are the main structural proteins of caveolae. The hallmark of liver fibrosis is the activation of hepatic stellate cells (HSC), the primary source of the extracellular matrix (ECM) that is characterized by the expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA). In cirrhotic livers, it was described an increase on the expression of Cav-1 in HSC. In a previous work, we used the pCav1EGFP plasmid in GRX lineage to obtain a permanent cell strain that overexpresses Cav-1, named GRX<sup>EGFP-Cav</sup>. **Objectives** In the present study, the roles of overexpression of Cav-1 in the actin cytoskeleton, cellular adhesion and the activation state of GRX<sup>EGFP-Cav</sup> were investigated. **Materials and Methods** To evaluated this lineage, we measured mRNA over real time-PCR of  $\alpha$ SMA, Cav-1, PTRF1 and Col-I. The protein expression of Cav-1,  $\alpha$ SMA and Col-I was measured through immunocytochemistry and quantified fluorescence by Image J. Likewise, we also analyzed the actin cytoskeleton. For labeling of actin cytoskeleton, cells were incubated with the toxin phalloidin conjugated Rhodamine or Alexa Fluor 488 and view in confocal microscope. **Results and Discussion** We demonstrated by immunocytochemistry that Cav-1 overexpression induce actin cytoskeleton reorganization in GRX<sup>EGFP-Cav</sup>: the major stress fibers were reduced in length compared to GRX cells. Also, small cell clusters were formed, indicating that the cell–cell adhesion was stronger than the cell–substrate adhesion. In addition, we also showed through real time-PCR and immunocytochemistry that Cav-1 overexpression was associated to an increase of  $\alpha$ SMA mRNA and protein expression. **Conclusion** The significance of Cav-1 expression in normal and cirrhotic livers are little known. Thus, considering the role of HSC in liver diseases, we believe that the permanent GRX<sup>EGFP-Cav</sup> line can be a very interesting experimental tool for the study of these pathologies.

**Keywords:** Caveolin-1, Hepatic Stellate Cells, Liver Fibrosis

**Financial supports:** CNPq/CAPES and FAPERGS