Establishment of Hepatic Stellate Cells Model that Superexpress EGFP-Caveolin-1

Ilha.M¹; Biehl,H.B²; Martins,L.A.M¹; Vasconcelos,M¹; Schein,V.¹; Barbé-Tuana, F¹; Guma,F.C.R¹

¹Department of Biochemistry, ICBS-UFRGS. Porto Alegre/RS Brazil, ²Electron Microscopy Center, UFRGS, Porto Alegre/RS, Brazil

INTRODUCTION Caveolins are a class of structural proteins that stabilize cells membrane. Among these, caveolin-1 is the most important member responsible for maintain protein residing in caveolae: small invaginations on the plasma membrane characterized as membrane microdomains rich in glycosphingolipids, cholesterol and GPI-anchored proteins also denominated lipid rafts. These structures are related to many cellular processes such as cholesterol homeostasis, signal transduction pathways, and endocytosis. In cirrhotic livers, it was found an increased expression of caveolin-1 in sinusoidal endothelial cells and hepatic stellate cells (HSC), suggesting this protein relation with the portal hypertension and liver fibrosis. Here, we aimed to establish a permanent cell line from GRX, a HSC line, which express EGFP-fluorescent caveolin-1. MATERIALS & METHODS It was firstly established a protocol for inserting the caveolin-1 with EGFP-conjugated gene by transfecting a DNA plasmid into GRX, further denominated GRX\textsuperscript{GFP-Cav}. The cell morphology was observed through optical microscopy. Immunoblotting and immunocytochemistry for caveolin-1 conjugate, respectively, to peroxidase and alexafluor 555 secondary antibodies were performed to ensure the technique success. Confocal images were acquired for quantifying the colocalization between transfected green caveolin-1 and the red-conjugated caveolin-1 antibody. RESULTS AND DISCUSSION By optical microscopy, it was observed that GRX\textsuperscript{GFP-Cav} cells exhibit an altered morphology compared to the GRX wild type. As expected, the immunoblotting data showed that there was an increased expression of caveolin-1 in GRX\textsuperscript{GFP-Cav}. The calculation of Pearson’s and Meander’s coefficients showed a very positive relation between green transfected caveolin-1 and its red fluorescent-conjugated antibody, thus revealing the successful of the protocol. CONCLUSION Since caveolin-1 plays an important role on the activation state of HSC, the GRX\textsuperscript{GFP-Cav} will be an excellent tool for further studies in confocal microscopy that investigate the cellular processes involving this protein.

Keywords: Caveolin-1, DNA transfection, hepatic stellate cells, Liver fibrosis

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