Role of Adenylyl Cyclase-Associated Protein 1 (CAP1) in Mediating Resistin Actions in Mouse Liver Cells

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Abstract

Resistin is a cytokine produced mainly by the white adipose tissue (WAT) that plays a role in modulating insulin sensitivity of peripheral tissues. Adenylyl cyclase is believed to play a major role in the development of insulin resistance in the liver. Resistin is a pro-inflammator that plays a role in the development of insulin resistance in the liver. We further treated BNL CL.2 cells with resistin, both untransfected and CAP1 siRNA-transfected and performed a qRT-PCR to profile the expression of 84 genes involved in the insulin resistance pathways (Table 3 and Figure 2). Results from the analyses demonstrated that treatment affected the expression of 77 genes two- or more than two-fold (Figure 3). Our data indicated that when CAP1 was knocked-down, there was up-regulation of insulin resistance markers, and adipogenic pathway as well as chemoresistance of infiltrating leukocytes (Table 3 and Figure 3).

Introduction

Resistin is a cytokine produced mainly by the white adipose tissue (WAT) that plays a role in modulating insulin sensitivity of peripheral tissues. 1,2 Multiple clinical and in vivo studies involving genetic or diabietic obesity models found that WAT resistin levels correlate with HOMA insulin resistance levels in rats. 1,3,4,5,6 In considering the role of CAP1 in mediating insulin actions.

Materials & Methods

Reagents

Mouse recombinant resistin was purchased from Sigma-Aldrich. The lipopolysaccharide was recovred with endotoxin (E. coli O111:B4) with a final concentration of 100 ng/ml. per 50 ml of FBS and 50 μl of PBS, per 50 ml of cell culture media.

Cell line

Mouse hepatocellular carcinoma BNL CL.2 cells were purchased from ATCC. Cells were grown in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin solution.

Western blot analysis

Whole cell lysates were analysed by Western blotting. The membrane was incubated with primary antibodies at 1:1000 dilution for 1 h and detection was performed by using enhanced chemiluminescence reagents (ECL) with peroxidase-conjugated secondary antibodies (Thermo Scientific). Densitometry was calculated by using ImageJ software. The relative expression of CAP1 and β-actin was normalised to the corresponding internal controls. Quantitative real time PCR analyses of gene expression were performed by using PowerUp SYBR Green Master Mix (Applied Biosystems). Results were normalised to the corresponding internal controls. The fold change was calculated by using the following formula: fold change = 2^{- \Delta \Delta Ct}.

Results

Time- and concentration-response experiments for resistin treatment of BNL CL.2 cells

Initially, to utilise the most appropriate concentration of resistin for further treatments, we stimulated BNL CL.2 cells with 0.1, 0.5, and 5 μg/ml of resistin for 1 to 6 hours. qPCR analysis showed that a concentration of 12.5 ng/ml of resistin for 6 hours induced the highest expression of genes involved in adverse insulin signalling pathway. We further treated BNL CL.2 cells with resistin, both untransfected and CAP1 siRNA-transfected and performed a qRT-PCR to profile the expression of 84 genes involved in the insulin resistance pathways (Table 3 and Figure 2). Results from the analyses demonstrated that treatment affected the expression of 77 genes two- or more than two-fold (Figure 3). Our data indicated that when CAP1 was knocked-down, there was up-regulation of insulin resistance markers, and adipogenic pathway as well as chemoresistance of infiltrating leukocytes (Table 3 and Figure 3).

Conclusions

To the best of our knowledge, this is the first study to demonstrate the role of CAP1 in mediating resistin signaling pathway in mouse liver cells. Further studies aiming to clarify the mechanisms of interaction between resistin and CAP1 are ongoing.

Literature

1. Han S, Han KD, Han SK, Jeon HC, Lee M. Effects of insulin resistance in the liver, we performed all further experiments using concentration of 12.5 ng/ml for 6 hours.


