Suppression of insulin target gene expression by SecinH3 quantified by real-time PCR

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Method Article

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Abstract

Introduction

SecinH3 causes insulin resistance in mouse liver and in cell culture by inhibiting cytohesins that are effectors of the insulin signalling pathway. Stimulation with insulin leads to increased transcription of glycolytic genes, to suppressed transcription of gluconeogenetic genes and IGFBP1 in liver and in HepG2 cells. These insulin effects can be reverted by SecinH3. We quantified gene expression levels by real-time PCR.

Reagents

10 mg mouse liver \(or ca. 10^6-10^7 HepG2 cells grown to 75% confluence) SecinH3 \(100 mM in DMSO) D5 \(10 mM in DMSO) DMSO insulin \(Sigma) standard cell culture material and media RNA-extraction kit \(Absolutely RNA, Stratagene) RT-kit \(cDNA-Archive kit, Applied Biosystems) TaqMan probes for assayed genes \(Applied Biosystems) TaqMan MasterMix, 2x \(Applied Biosystems)

Equipment

Standard cell culture equipment Real-time PCR machine \(BioRad)

Procedure

A: HepG2 cells 1. ca. 10^5 HepG2 cells are seeded into wells of a 12-well culture plate and incubated overnight 2. after washing twice with PBS, the cells are serum starved for 24 hrs 3. compounds are added in desired concentrations \(SecinH3, D5 and Wortmannin or DMSO alone); final concentration of DMSO was 0.2% in our experiment 4. cells stimulated with 10 nM insulin for 12 hrs 5. Total RNA extracted with Absolutely RNA kit \(Stratagene) according to instructions from manufacturer; elution volume 50 µl B: Mouse liver 1. 10 mg liver homogenized in lysis buffer and RNA extracted with Absolutely RNA kit \(Stratagene) according to instructions of manufacturer; elution volume 50 µl RT and real-time PCR 1. RNA concentration determined by photometry 2. 2.5 µg RNA used for reverse transcription with cDNA Archive kit \(Applied Biosystems) according to instructions from manufacturer; RT volume: 50 µl 3. resulting cDNA diluted 1:3 with H_2O 4. quantitative PCR performed in 10 µl scale with gene-specific TaqMan probes \(Applied Biosystems). All expression-levels were normalized to the expression level of β-2-microglobulin.