NR5A2: a glucose metabolism regulator

Mª ÁNGELES QUILES ROMAGOSA, JOSÉ CARLOS RODRÍGUEZ-REY[1]

[1]Departament of Molecular Biology, School of Medicine, University of Cantabria, Santander 39000, Spain

INTRODUCTION

NR5A2 also known as α1 liver receptor homolog-1 (LRH-1) is a nuclear receptor which, in adult mammals, is predominantly expressed in endodermic tissues as liver, exocrine pancreas and intestine [5]. It plays a major role in cholesterol and bile acids metabolism, by regulating the expression of genes coding for enzymes (CYP7A1; CYP8B1) or transporters (APOA1) [1,3]. Also it is involved in the pluripotency control and the estrogen synthesis and regulation (2).

This evidence led our group to study the possible association between NR5A2 gene and different estrogen-related phenotypes. The results showed association between several NR5A2 SNPs with obesity and diabetes. For a better understanding of NR5A2 implication in these diseases our group examined the transcriptome variations due to the silencing of NR5A2 in HepG2 cells. The analysis was done by RNA sequencing of RNA samples prepared from cultures treated with NR5A2- specific siRNA. We found differences in a battery of genes associated with glucose homeostasis, suggesting that, apart from its role in cholesterol and bile acid metabolism, NR5A2 participates in the regulation of glucose metabolism. This would provide a possible relationship between the NR5A2 variants and diabetes.

Here we show the validation of the RNA sequencing results in cells overexpressing NR5A2, which support the previous dates in a NR5A2 KO cell line. Both of them seem to sustain that the NR5A2 association to diabetes could be understood by its role in the regulation of the expression of gene coding glycolysis and gluconeogenesis enzymes.

MATERIALS AND METHODS

Cell culture

The hepatocarcinoma cell line HepG2 was cultured in DMEM medium supplemented 10% of FBS and peniciline and streptomycin 1%

Construction of stable cells overexpressing NR5A2

NR5A2 cDNA was cloned in the expression vector pCEFL. HepG2 cells were transfected with the resulting plasmid using a nucleofector. Stable clones clones were selected with G418 (750 mg/l). NR5A2 overexpression was tested both by qPCR and inmunofluorescence.

Inmunofluorescence

Cells grown to 50% confluence in a cover glass were fixed and permeabilizated with cold methanol for ten minutes. An anti-NR5A2 antibody (Santa Cruz) and an anti-rabbit IgG conjugated to a green fluorophore were used as a primary and second antibodies respectively and DAPI was used for nuclear staining. Results were analyzed and quantified by fluorescence microscopy.

Expression assays

For PCR studies RNA was extracted from P100 disks at 80% confluence using a commercial kit (QIAGEN). The RNA quality was tested by spectroscopy and RNA agarose gel. 0,5 -1µg RNA was used in the rt-PCR.

For qPCR 1µl of cDNA in a 12,5 1µl of total reaction volume was employed. The paired primers were used at 200nm total concentration and for the analysis the S14 was used to normalizing gen expression.

1) MODEL VALIDATION

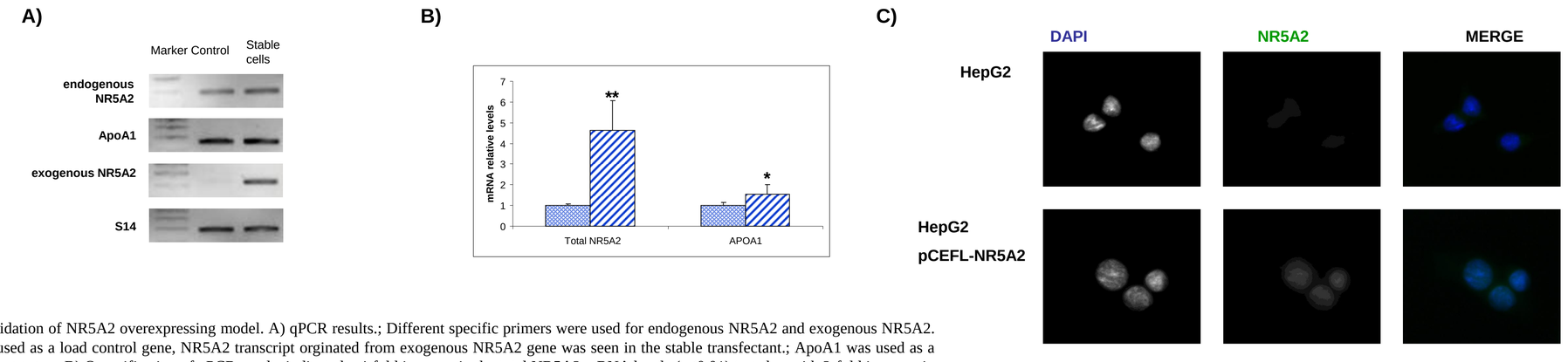

Fig.1. Validation of NR5A2 overexpressing model. A) qPCR results.; Different specific primers were used for endogenous NR5A2 and exogenous NR5A2. S14 was used as a load control gene, NR5A2 transcript orginated from exogenous NR5A2 gene was seen in the stable transfectant.; ApoA1 was used as a NR5A2 target gene B) Quantification of qPCR results indicated a 4-fold increase in the total NR5A2 mRNA levels ($p<0,01$) together with 2-fold increase in APOA1 mRNA levels. C) Inmunofluorescences in HepG2, against NR5A2. The specific nuclear localization is clearly observed in both cases. The higher fluorescence intensity in the pCEFL-NR5A2 cells, demonstrate the increase in NR5A2 protein levels.

2) EFFECTS ON GENE EXPRESSION AND ON GLUCOSE METABOLISM

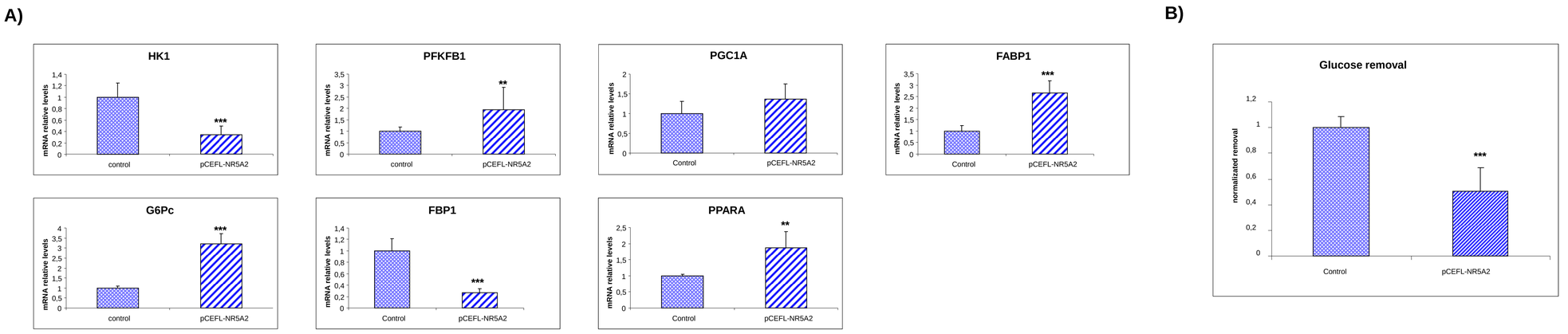

Fig. 2. A) Expression of exogenous NR5A2 results in changes in the expression of several genes related to glycolysis and gluconeogenesis enzymes (HK-1, G6Pc, PFKFB1, FBP1); transcription factors (PGC1A, PPARA) and fatty acids metabolism enzymes (FABP1). These data suggest changes in the glycolysis and in the gluconeogenesis. B) Removal of glucose from the medium in cells overexpressing NR5A2 (results normalized with the number of cells per well).

CONCLUSIONS

1. The previous NR5A2 silencing experiments treated with siRNA have been confirmed with the present results

2. In HepG2 cells, overexpression of NR5A2 modifies the expression of several genes involved in lipids metabolism, glycolysis and gluconeogenesis:

- NR5A2 seems to regulate negatively the glycolysis pathway by acting on genes encoding enzymes as hexokinase-1, and positively the gluconeogenesis pathway, by acting on the genes coding for enzymes as glucose 6 phosphatase and phosphofructokinase β1.

- Also NR5A2 works by increasing the transcription of nuclear receptors and coactivators: Peroxisome proliferator-activated receptor alpha (PPAR-alpha) gene codes for a transcription factor whose activation promotes uptake, utilization, and catabolism of fatty acids by upregulation of genes involved in fatty acid transport and peroxisomal and mitochondrial fatty acid β-oxidation. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha) is a coactivator involved in regulating cellular cholesterol homoeostasis and the development of obesity linked to external stimuli.

- Moreover, NR5A2 plays a positive role in the regulation of the transcription of fatty acid-binding protein, a protein related to fatty acid uptake, transport, and metabolism.

3. In addition, our experiments showed that changes in NR5A2 expression are associated with changes in the concentration of glucose in the medium. Taken together, these results suggest that this nuclear receptor participates in the regulation of glucose consumption in cells and thus provide a possible mechanism to the association of NR5A2 genotypes to diabetes and obesity.

FURTHER RESEARCH

Some research is needed to confirm NR5A2 as possible diabetogenic agent. In future experiments our group is going to study the effect of a DPLC, a recently- described ligand for this nuclear receptor [4] on the regulation of both lipid and carbohydrate pathways.

NR5A2 cell lines from another metabolism-related tissues as mice muscle (C2C12) or adipocytes cell lines (3T3-L1) might also be done.

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