Glucokinase sensitizes hepatocarcinoma cells to the lipogenic activity of fructose and controls accumulation of lipid droplets and secretion of triglyceride-rich lipoproteins.

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INTRODUCTION

Human hepatocarcinoma cell lines are in vitro models for the study of lipid metabolism, hepatic steatosis and carcinogenesis. However, these cells have an unbalanced lipid metabolism that is strongly dependent on exogenous fatty acids to synthesize triglycerides and lipoproteins. This motivates the need for a physiologically relevant hepatocyte in vitro model allowing a deep analysis of the cellular mechanisms that regulate glycolysis, de novo lipogenesis and lipoprotein synthesis in normal and pathological situations

AIM

○Restore the hepatocyte-specific lipid metabolism in hepatoma cell lines by switching hexokinase (HK) isoenzymes.

○ Decipher the metabolic rewiring in hepatocytes expressing HK2 or GCK

METHOD

Like virtually all cancer cells, hepatocarcinoma cells express the “cancer-type” hexokinase isoenzyme HK2. Here, we restored the hepatic hexokinase isoenzyme (glucokinase, GCK) expression in the paragon hepatocarcinoma cell line Huh7 invalidated for HK2 by CRISPR-Cas9. Endo- and exo-metabolites have been analysed by a combined approach of biochemistry and high-field Nuclear Magnetic Resonance metabolomics.

RESULTS

Remodeling of metabolic profile in Huh7 GCK+HK2- cells

We measured the glucose consumption and lactate secretion by Huh7 and huh7 GCK+HK2- cells under standard culture conditions (i.e. DMEM 4.5g/L Glc, SVF 10%). Figure 1 shows that glucose consumption of both cell lines is identical.

Figure 1

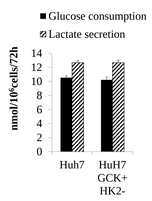
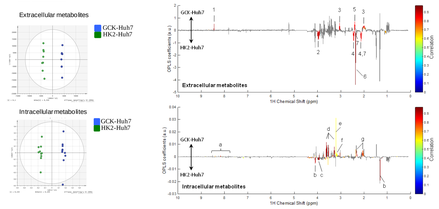


Figure 2

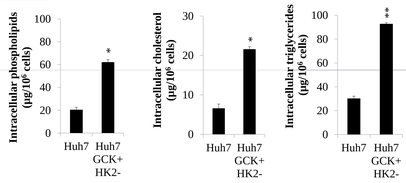


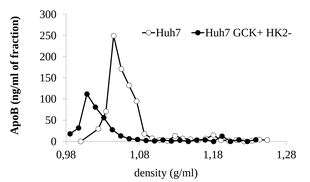
The intracellular and extracellular metabolites were extracted and analyzed by NMR. The 1H spectra obtained for each cell type were compared by multivariate analysis. Principal Component Analysis (PCA) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) revealed a specific endo- and exo-metabolome (respectively lower and upper panel, Figure 2) for Huh7 and Huh7 GCK+HK2- cells. In particular, significant variations were observed in intracellular metabolites (a: AXP, NAD, NADP; b: lactate; c: serine; d: myo-Inositol; e: o -phosphocholine; f: alanine; g: glutamate) and extracellular metabolites. (1: formate, 2: serine, 3: ornithine, 4: glutamine, 5: succinate, 6: pyruvate, 7: glutamate).

GCK induced lipids accumulation as well as secretion of triglyceride-rich ApoB+ lipoproteins

We analyzed the lipid content of huh7 and huh7 GCK+HK2- . (figure 3). Phospholipids, cholesterol and triglycerides were quantified in cells extracts.

Figure 3



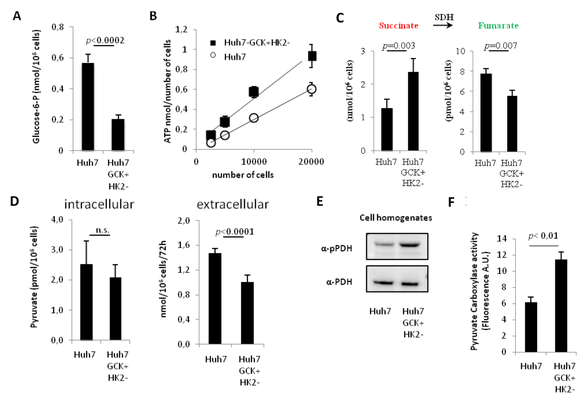


Quantification of ApoB in the different density fractions of cell supernatants: Huh7-GCK+HK2- cells were able to secrete triglyceride-enriched lipoproteins in the form of very low-density lipoproteins while Huh7, like all other hepatoma cells, secrete low-density lipoproteins.

TCA cycle is rewired at the level of pyruvate entry and succinate dehydrogenase

Huh7 and Huh7-GCK+HK2- cells differently use glucose-6-phosphate (G6P) and pyruvate (figure 4). In Huh7 cells, G6P is mainly oxidized in the glycolysis pathway and generates an excess of pyruvate that is excreted. In Huh7-GCK+HK2- cells, G6P can also actively fuel the pentose-phosphate pathway, resulting in an increase of intracellular nucleotides (figure 4A and B) and NADPH for lipid synthesis in addition to the classical glycolysis pathway.
Succinate is increased in huh7-GCK+HK2- cells whereas fumarate is decreased, suggesting inhibition of SDH activity (figure 4C).
Huh7 secrete more glucose-derived pyruvate than Huh7-GCK+HK2- (figure 4D). Normally pyruvate dehydrogenase (PDH) is the preferential route of entry for pyruvate in the TCA in hepatocyte. In Huh7-GCK+HK2- cells PDH is inhibited (i.e. PDH phosphorylation, figure 4E). Pyruvate fuels the TCA through the pyruvate carboxylase that is activated (figure 4E).

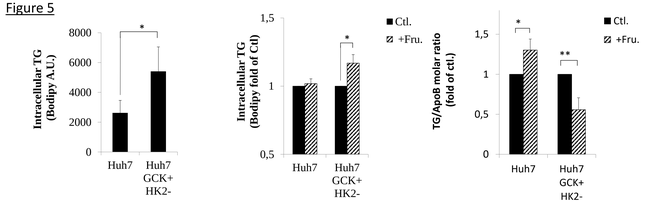
Figure 4



GCK-expressing cells become responsive to fructose by accumulating intracellular lipids as well as decreasing triglycerides load of ApoB+ lipoproteins.

Intracellular neutral lipids were stained with the specific probe Bodipy® and the fluorescence analyzed by flow cytometry (figure 5). Intracellular TG and secreted ApoB were quantified in cell supernatants and molar ratio determined.

Figure 5



In contrast to HK2-expressing hepatoma cells, Huh7-GCK4+HK2-cells respond to fructose by accumulating intracellular TG while triglyceride enrichment in secreted lipoprotein is decreased.

CONCLUSIONS

Restoring GCK expression in hepatoma cells has induced a large scale metabolic remodelling with a balanced lipid metabolism mimicking in vivo lipogenesis. Restoration of VLDL biosynthesis and secretion, activation of the reductive metabolism of pyruvate in the mitochondria and sensitivity to fructose are making Huh7-GCK+HK2- cells a unique model for the study of metabolic modifications triggered by nutritional stresses.This physiologically-relevant hepatocyte model will certainly provide improved clues to control liver inflammation, NAFLD and tumorigenesis.

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