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RAPID COMMUNICATION

Interruption of bile acid enterohepatic circulation inhibits glycogen synthesis and promotes hepatocellular carcinoma progression

In recent years, increasing attention has been given to public health. Cancer and obesity are hot topics, in which glucose and lipid metabolism play an important role. Glycogen synthase GYS2 has been found to regulate glycogen metabolism in hepatocellular carcinoma (HCC) and is a potential prognostic factor and therapeutic target.[1] Excessive bile acids induce intestinal injury and inflammatory bowel disease, and targeting bile acid receptors (FXRs) can regulate inflammation and repair damaged intestinal epithelial barriers.[2] However, the research model of metabolic pathways focuses on the key enzymes, while ignoring the overall relevance. Here, we targeted the glycogen anabolism pathway and screened the upstream factor, bile acid transporter SLC10A1. SLC10A1 participates in the enterohepatic circulation of bile acids and regulates the transcriptional activity of the bile acid receptor FXR. Besides, we found that diazinon could bind NTCP and inhibit glycogen synthesis by mimicking the disordered bile acid enterohepatic circulation. Collectively, enterohepatic circulation of bile acids coordinates lipid metabolism and glucose metabolism and draws the metabolic crosstalk network, which provides a new perspective for the clinical treatment of HCC.

We found that glycogen metabolism enzymes were highly expressed in liver tissue (Fig. S1A), indicating that active glycogen metabolism was carried out. However, the mutation frequency of these metabolic enzymes is not high in HCC cells (Fig. S1B). Further analysis showed that the expression of these metabolic enzymes was down-regulated in HCC, especially at the protein level (Fig. 1A; Fig. S1C). More importantly, the expression of glycogen-metabolizing enzymes was significantly associated with tumor grade, survival, and diagnosis (Fig. S1D–F). These results indicate that glycogen metabolism has clinical significance in the diagnosis and treatment of HCC.

The above results have shown that glycogen metabolism is blocked due to the down-regulation of metabolic enzyme expression, and we wanted to explore the mechanism of the down-regulation of these genes. We selected the top 100 genes (Table S1) associated with the expression of glycogen metabolism enzymes in HCC and identified the only candidate gene SLC10A1 (Fig. S2A). The expression of SLC10A1 was also suppressed in HCC and had a significant correlation with the grade and overall survival of HCC patients (Fig. 1B; Fig. S2B–D). We also found that there was a positive expression correlation between glycogen metabolism enzymes and SLC10Al (Fig. 1C; Fig. S2E). These findings suggest that SLC10A1 manages the course of HCC patients by regulating the expression of glycogen metabolism enzymes.

In fact, NTCP (encoded by SLC10A1) is not only a bile acid transport receptor but also a functional receptor of hepatitis virus. We wanted to determine whether the effect of NTCP on glycogen metabolism is dependent on bile acid receptor activity or hepatitis virus receptor activity. We did not observe altered expression of SLC10A1 and glycogen metabolizing enzymes in liver tissues or hepatoma cells infected with hepatitis virus (Fig. S2F). In contrast, the bile acid synthesis rate-limiting enzyme CYP7A1 and the bile acid export protein BSEP (encoded by ABCB11) were expressed in association with glycogen metabolism enzymes (Fig. S3A, B). Consistent with this, low expression of CYP7A1 and ABCB11 was significantly associated with HCC patient grade and survival. Based on pathway analysis, bile acid-related processes such as synthesis, metabolism, and recycling are disrupted in HCC compared to normal individuals (Fig. S3F). Among the three bile acid-related genes, CYP7A1

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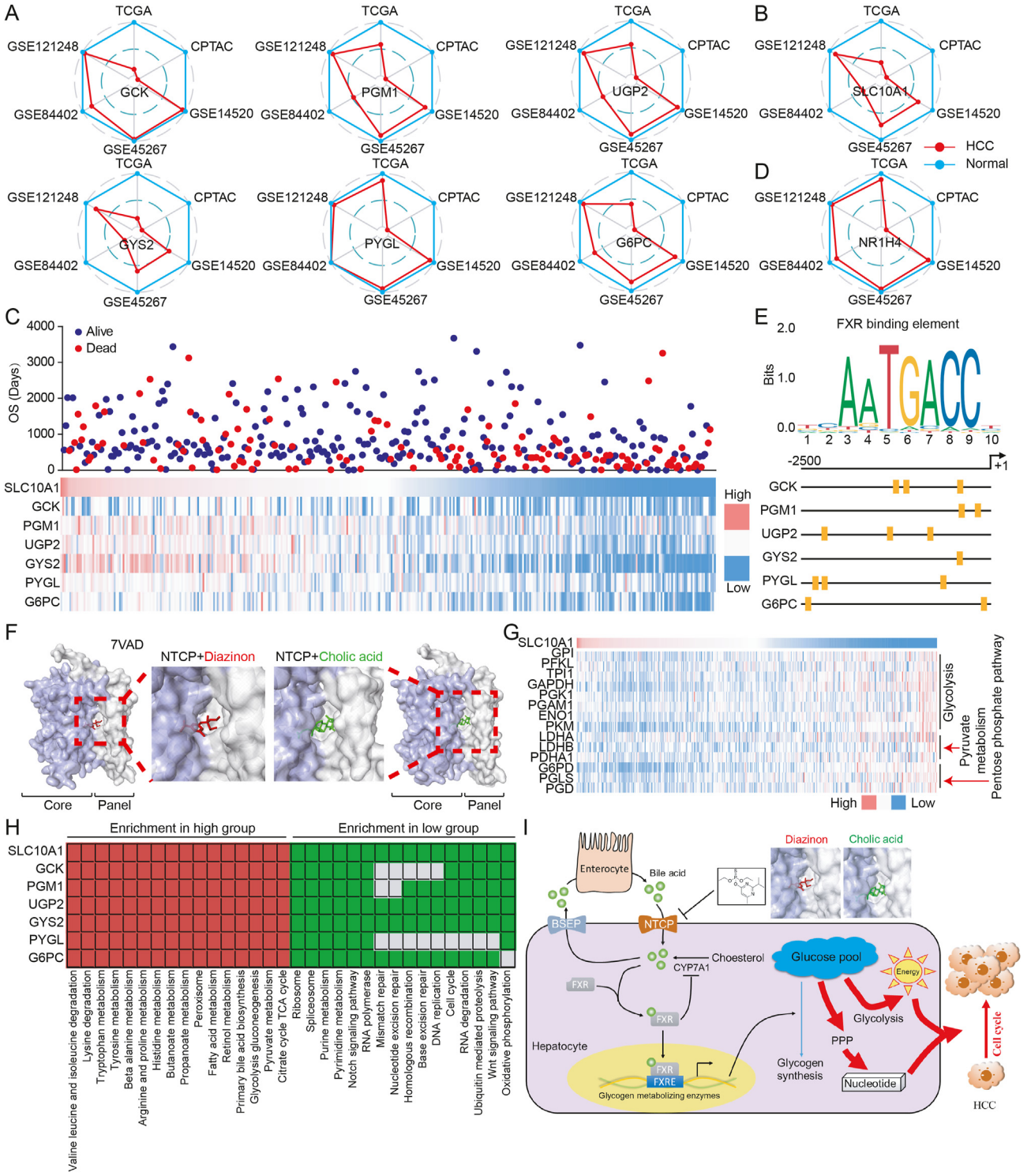

Figure 1 Bile acid cycle regulates glycogen synthesis and promotes tumor progression. **(A, B, D)** Differential analysis of glycogen metabolism enzymes SLC10A1 and NR1H4 at mRNA and protein levels in hepatocellular carcinoma patients and normal individuals from different databases (including TCGA database, CPTAC database, and GEO database). **(C)** The correlation between expression of SLC10A1 and glycogen metabolizing enzymes in the TCGA-LIHC dataset and its effect on patient survival. **(E)** The predictive analysis of binding elements for the transcription factor FXR at glycogen metabolizing enzyme promoters. **(F)** Molecular docking analysis between NTCP proteins (PDB ID: 7VAD) and different small molecules (diazinon or cholic acid). **(G)** The heatmap showed the expression of metabolic enzymes in the glycolysis, pyruvate metabolism, and pentose phosphate pathways according to the expression level of SLC10A1. **(H)** GSEA was used to analyze the enrichment of biological processes according to the expression level of specific genes. **(I)** Regulation of glucose metabolism and cell cycle by enterohepatic circulation of bile acids.

showed a different expression profile from SLC10A1/ABCB11. We believe that this may be due to a feedback mechanism. Cholesterol in hepatoma cells is still being broken down into bile acids when SLC10A1 and ABCB11 are down-regulated, resulting in the inability of bile acids to be transported. When the accumulation of bile acids in hepatoma cells reaches a threshold, the activated FXR will inhibit the expression of CYP7A1, thereby blocking the metabolism of cholesterol. This is evidenced by different expression profiles with the same prognostic impact.

To elucidate the molecular mechanism by which SLC10A1 regulates glycogen metabolism enzymes by affecting the biomass of bile acid, we analyzed the bile acid receptor FXR (encoded by NR1H4) and found that NR1H4 is down-regulated in HCC (Fig. 1D). Surprisingly, NR1H4 and glycogen metabolizing enzymes have a positive expression correlation (Fig. S4A). Therefore, we postulate that FXR regulates glycogen metabolism in response to bile acids. The ChIP-seq of FXR and the existence of the FXR binding element in the promoter of the glycogen metabolism enzyme gene further confirm our previous speculation (Fig. 1E; Fig. S5). These results indicate that SLC10A1 synergizes with CYP7A1 and ABCB11 to inhibit bile acid synthesis and recycling in hepatoma cells, decrease the transcriptional activity of FXR, and regulate the expression of glycogen metabolism enzymes.

Based on the effect of drug treatment on the expression of glycogen metabolism enzymes (Fig. S4B), we identified diazinon as a candidate compound. Diazinon has been reported not only to affect glucose metabolism (depletion of glycogen and up-regulation of glucose concentration), but also to up-regulate cholesterol levels.[3,4] Given that diazinon did not alter SLC10A1 expression (data not shown), we speculated that there might be an interaction between SLC10A1 and diazinon. Based on the structure of NTCP resolved by different laboratories, we performed molecular docking between NTCP and diazinon, and found that diazinon could bind in the pocket of NTCP (Fig. 1F; Fig. S4C, D). Therefore, we believe that diazinon inhibits glycogen synthesis by binding to NTCP and blocking bile acid transport. However, it should be noted that diazinon has potential toxic side effects.

Tumor cells reprogram the metabolic process mainly to obtain more energy and raw materials to meet their rapid proliferation needs. Thus, the use of this glucose by liver cancer cells will be explored in the next step of our research. In addition to glycogen, glucose also flows to glycolysis and pentose phosphate pathways to produce energy and macromolecular synthetic raw materials, respectively. The metabolic enzymes of glycolysis, pyruvate metabolism, and pentose phosphate pathway all showed increased expression when SLC10A1 was underexpressed (Fig. 1G). Metabolic enzymes of these pathways were also shown to be up-regulated in HCC compared to normal individuals (Fig. S6A). These results strongly suggest that down-regulated SLC10A1 in HCC inhibits glucose flow to glycogen and instead enters the glycolysis and pentose phosphate pathways. Based on multi-gene GSEA analysis, cell cycle, DNA replication and multiple DNA damage repair pathways were enriched (Fig. 1H). In addition, bile acid-associated SLC10A1, CYP7A1, and ABCB11 were strongly associated with the S4 subtype among the reported proteome-differentiated pan-cancer subtypes (Fig. S6B).[5] The S4 subtype is thought to be involved in fatty acid metabolism, glycolysis, and the pentose phosphate pathway, which supports our analysis. In summary, we found that decreased SLC10A1 disrupts bile acid cycling across organs, reroutes glucose metabolism, activates DNA replication and cell cycle, and promotes HCC progression (Fig. 1I).

Many diseases, including cancer, are driven by genetic abnormalities, but previous research paradigms often focus on individual genes or regulatory pathways. In this study, we found that the enterohepatic circulation of bile acids plays an important role in nutrient uptake and disease development, and is a promising target for cross-organ therapy. This work not only broadens the communication between metabolic networks in physiological processes, but also provides new ideas for the treatment of cancer and other diseases. However, we have to admit that these results require further experimental verification and corroboration of clinical results.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.026.

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