Glycogen overload transforms liver

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Glycogen overload transforms the liver

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Running title: Accumulated glycogen drives liver tumorigenesis

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Glycogen, a fast and accessible form of energy, can provide organisms with glucose on demand. It is mainly present in the liver and skeletal muscles, with small amounts in the brain, heart, kidney, adipose tissue, and red blood cells [1]. Numerous enzymes are involved in glycogen synthesis, including glycogen synthase (GS), glycogen branching enzyme, and protein phosphatase 1. Many enzymes are linked to glycogen degradation, including glycogen phosphorylase (GP), glycogen debranching enzyme,
and glucose-6-phosphatase (G6PC). Defective glycogen metabolism-related enzymes result in glycogen accumulation.

Glycogen accumulation is associated with several physiological processes. In the human endometrium, accumulated glycogen serves as an important source of glucose for embryonic growth during gestation [2]. Moreover, it is vital for memory formation and maintenance of \( \text{CD}8^+ \) memory T cells [3]. Notably, glycogen accumulation is also reported in beige and brown adipocytes and is necessary for long-term cold adaptation [4].

However, aberrant glycogen accumulation is frequently implicated in the pathogenesis and progression of various tumors, including cervical cancer, breast cancer, ovarian clear cell carcinoma (OCCC), non-small cell lung cancer (NSCLC), clear cell renal cell carcinoma (CCRCC), and hepatocellular carcinoma (HCC) (Figure 1). The stored glycogen may act as a nutritional source or regulate signaling pathways to promote tumor growth. Ciclopirox olamine (CPX), a synthetic antifungal agent, is an anticancer agent. Recently, Fan et al. [5] reported that CPX induces glycogen accumulation and glycophagy in cervical cancer cells by inhibiting the activation of YAP1. Moreover, glycogen accumulation, due to the upregulation of protein phosphatase 1 regulatory subunit 3C (PPP1R3C), contributes to breast cancer cell invasion under hypoxia [6]. Similar observations were made toward anticancer drug resistance in OCCC under hypoxia [7]. Interestingly, the decrease in E3 ubiquitin ligase (Malin) activity promotes NSCLC progression, which can be attributed to intranuclear glycogen accumulation. This occurs due to a block in GP translocation from the cytoplasm to the nucleus, further leading to the decrease of nuclear histone acetylation [8]. GS1-induced glycogen accumulation promotes CCRCC progression by activating the NF-\( \kappa \)B signaling pathway [9].

Adult hepatocytes do not show glycogen accumulation [10], and glycogen accumulation is known to improve the survival of multiple cancer types. However, the specific regulatory mechanisms underlying the role of this accumulation in liver tumorigenesis remain unclear. Bone morphogenetic protein 4 (BMP4), belonging to the transforming growth factor \( \beta \) superfamily, facilitates hepatocellular carcinoma cell proliferation and migration by activating the MEK/ERK signaling pathway [11]. It promotes hepatic glycogen accumulation by activating the mTORC2 signaling pathway, thus inhibiting the progression of nonalcoholic fatty liver disease [12]. Hence, whether BMP4 promotes HCC development by increasing hepatic glycogen...
accumulation is worth exploring. YAP and TAZ transcriptional coactivators are downstream effectors of the Hippo signaling pathway and are drivers of tumor growth in experimental mouse models. Interestingly, Liu et al. [13] revealed that glycogen accumulation promotes liver tumor initiation by inhibiting the Hippo signaling pathway. They first found that glycogen accumulation occurs during the preliminary stage of mouse and human liver tumors. To gain a better understanding of the mechanisms, they performed RNA sequencing and verified that downregulation of G6PC might be responsible for the increased glycogen accumulation at the early stage of the tumor. Thereafter, a liver-specific G6pc-knockout (G6pc^△Alb) mouse model was established. As expected, glycogen accumulation was higher in the livers of G6pc^△Alb mice than in the livers of their wild-type littermates G6pc^{fl/fl} mice. The Hippo/Mst1/2 pathway suppresses tumor development by inactivating the oncoprotein YAP. G6pc^△Alb mice exhibited a phenotype similar to Hippo-signaling-deficient mice, with increased liver/body weight ratio and elevated percentage of Ki67^+ proliferating liver cells. A Pygl-knockout (Pygl^△Alb) mouse model was also established to explore whether glycogen accumulation induces liver enlargement and Hippo signaling inactivation but not metabolic disorders due to G6PC deficiency. The results hinted that glycogen accumulation inhibits Hippo signaling activities and consequently activates YAP, which induces liver cancer development.

Nevertheless, the exact regulatory mechanisms by which glycogen accumulation modulates the Hippo signaling pathway warrant further elucidation. Liu et al. [13] found that Mst1/2-glycogen forms aggregate foci in glycogen-accumulated liver tissues and tumor nodules treated with DEN, as well as in patients with HCC. To observe the formation of Mst1/2-glycogen aggregate foci in real time in live cells, they constructed a plasmid encoding the GFP-conjugated carbohydrate-binding domain of Stbd1 (CBM20-GFP) and transfected it into HepG2^GW2 cells. Interestingly, the accumulated glycogen induced the formation of glycogen-Mst1/2 aggregate foci, blocking the Hippo signaling in cells. Furthermore, polymer blends can undergo phase separation [14]. As expected, the accumulated glycogen spontaneously underwent liquid-liquid phase separation both in vivo and in vitro. Laforin, a dual-specificity phosphatase, binds to glycogen and mediates the recruitment of Mst1/2 into glycogen droplets, thereby forming Mst1/2-glycogen aggregation foci. Normally, Mst1/2 combines with WW45 in the Hippo pathway, inhibiting YAP
activation in healthy liver cells. Importantly, due to the dynamicity of glycogen aggregates, Mst1/2 are inclined to the shuttle between the glycogen droplet and the cytoplasm, where Mst1/2 can interact with WW45, leading to competition between WW45 and glycogen aggregates for interaction with Mst1/2. However, the retention of Mst1/2 in the glycogen droplets disrupted the assembly of the WW45-Mst1/2 complex, subsequently blocking the Hippo pathway. Thus, glycogen accumulation inhibits Hippo signaling via glycogen phase separation, thereby contributing to liver tumor incidence.

Therefore, alleviation of excessive hepatic glycogen accumulation is a potential strategy for the treatment of liver tumorigenesis. Fortunately, many natural products that reduce glycogen accumulation have been identified, including corosolic acid (CA), quercetin, and resveratrol. CA isolated from Eriobotrya japonica leaves can stimulate glucose consumption and decrease glycogen accumulation by inhibiting PEPCK mRNA expression in human hepatocellular carcinoma cells [15]. Quercetin is a major flavonoid that is ubiquitous in fruits and vegetables. It may be the most effective targeted drug for early hepatocarcinogenesis by decreasing glycogen accumulation and facilitating glycogen redistribution [16]. Similarly, resveratrol, a natural phytoalexin, increases glycolysis and inhibits gluconeogenesis, thereby alleviating the accumulation of hepatic glycogen [17].

Besides natural organic compounds, antagonists of glycogen synthesis are also promising candidates for restraining glycogen accumulation. GW6471, a small-molecule synthetic PPARα antagonist, can inhibit glycogen accumulation by blocking glycogen synthesis and increasing glycogen decomposition under hypoxic conditions [18]. Rapamycin, an mTOR inhibitor, can prominently reduce glycogen accumulation by inhibiting GS expression and glucose uptake in cells [19]. Moreover, glucocorticoid regulated kinase 1 (SGK1) is an enzyme that regulates cellular glucose uptake by activating glucose transporters. Geldanamycin, a pharmacological antagonist of SGK1, may be an effective therapeutic agent for suppressing glycogen accumulation [20]. These compounds may open new avenues for therapeutic intervention in liver tumors.

In summary, these findings indicate that accumulated glycogen in the liver drives tumorigenesis by blocking Hippo signaling via glycogen phase separation, where the Hippo pathway is involved in decreasing glycogen accumulation, thereby abolishing
liver cancer incidence. Glycophagy is involved in glycogen accumulation in cervical cancer cells. Whether glycophagy contributes to liver tumor incidence is a topic for future research.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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Figure legend

Figure 1. Glycogen accumulation plays important roles in various tumors and ciclopirox olamine induces glycogen accumulation and glycosphagy in cervical cancer cells by inhibiting the activation of YAP1. Glycogen accumulation induced by hypoxia inducible factor 1 (HIF1)-mediated PPP1R3C expression contributes to breast cancer invasion. The expression of HIF1 activates glycogen synthase, resulting in anti-cancer drug resistance in ovarian clear cell carcinoma via glycogen accumulation. Decreased malin activity inhibits glycogen phosphorylase expression and promotes glycogen accumulation, thereby reducing nuclear histone acetylation, which augments non-small cell lung cancer tumorigenesis. GYS1-induced glycogen accumulation promotes clear cell renal carcinoma progression by activating the NF-κB signaling pathway. Accumulated glycogen caused by G6PC deficiency suppresses Hippo signaling and drives liver tumor initiation. CPX: Ciclopirox olamine; YAP1: Yes1 associated transcriptional regulator; HIF1: hypoxia-inducible factors 1; PPP1R3C: protein phosphatase 1 regulatory subunit 3C; GS: glycogen synthase; OCCC: ovarian clear cell carcinoma; Malin: an E3 ubiquitin ligase; GP: glycogen phosphorylase; NSCLC: non-small cell lung cancer; GYS1: glycogen synthase 1; NF-κB: nuclear factor-kappa B pathway; CCRCC: clear cell renal carcinoma; G6PC: glucose-6-phosphatase; Hippo: Hippo signaling pathway; HCC: hepatocellular carcinoma.
Figure 1. The role of glycogen accumulation in multiple tumors