針對肝細胞癌的糖酵解體和代謝異常調節開發新的抗癌策略

Targeting Glycolytic Body and Metabolism Dysregulation in Hepatocellular Carcinoma

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Metabolic reprogramming is a hallmark of cancer. Tumor cells use the Warburg effect to obtain necessary energy for cell proliferation and metastasis. Previously, we demonstrated that upregulation of ribose 5-phosphate isomerase (RPIA), a key enzyme in the pentose phosphate pathway (PPP), triggers hepatocarcinogenesis via activation of phosphorylated extracellular signal-regulated kinase (pERK) [1]. RPIA promotes colorectal cancer formation by stabilizing and activating β-catenin [2]. RPIA increased pERK and AMP-activated protein kinase (pAMPK) and β-catenin during liver cancer formation in zebrafish model [3]. However, the underlined molecular mechanism for the coordination of glycolysis, PPP pathway, lipid metabolism and hepatocarcinogenesis is still ambiguous. In this study, we reveal the coordination between glycolysis and PPP pathway. We discover PFKL (a key enzyme in glycolysis converting fructose-6-phosphate to fructose-1,6-bisphosphate) stabilizes RPIA (a key enzyme in the PPP) protein levels by inhibiting the ubiquitination/proteasome. The pro-inflammatory and tumor cytokine interleukin 6 induces PFKL expression through dual pathways: pAMPK stabilizes the PFKL protein; pSTAT3 increases the transcription of AMPK and PFKL. PFKL is the major component in Glycolytic body (G-body) which is a glycolytic center associated with tumorigenesis Using human liver specimens array, we discover multienzyme metabolic complex G-body containing PFKL, AMPK, RPIA and PKM2 is the center for coordinating glucose metabolism, PPP pathway and cancer formation in human liver diseases. G-body which starts forming at chronic hepatitis, dramatically increases during active hepatitis, and the size of G-bodies becomes bigger from cirrhosis to hepatocellular carcinoma. Targeting AMPK and STAT3 with specific inhibitors block the IL6-STAT3-AMPK/PFKL pathway and reduce the hepatoma cell viability and HCC formation in zebrafish model. This study provided a potential therapeutic strategy against HCC. Targeting AMPK or STAT3 might be a promising therapeutic direction for the treatment of liver cancer. This manuscript is under revision in Cell Reports.

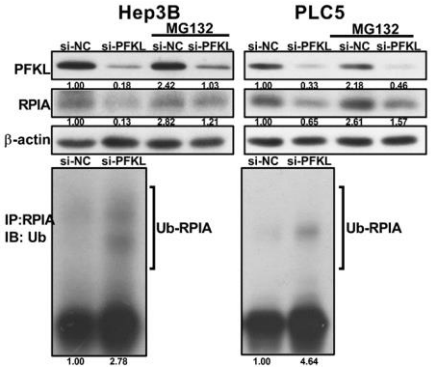

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Figure 1. PFKL stabilizes RPIA protein levels by inhibiting the ubiquitination/proteasome. MG132 (protease inhibitor) treatment rescues the PFKL knockdown mediated downregulation of RPIA in

hepatoma cells. Knockdown of PFKL increases the RPIA ubiquitination levels.

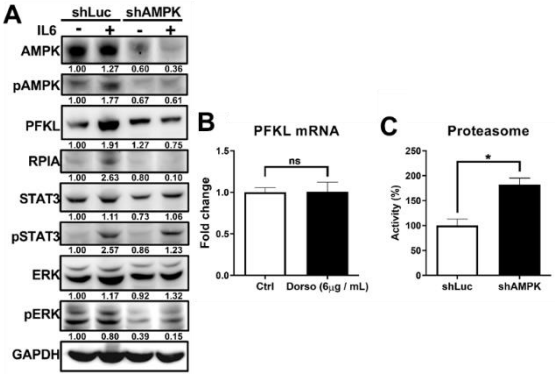


Figure 2. IL6 induces PFKL through AMPK activation to stabilize PFKL protein level.
(A) Knockdown of AMPK by shRNA diminishes IL6-increase PFKL expression in PLC5 cells resulting in reductions of RPIA and ERK levels, but had no effect on phosphorylation of STAT3. (B) AMPK knockdown doesn't affect the mRNA expression of PFKL. (C) AMPK knockdown increase proteasome activity in PLC5 cells, suggesting that AMPK stabilizes PFKL through inhibiting proteasome activity.

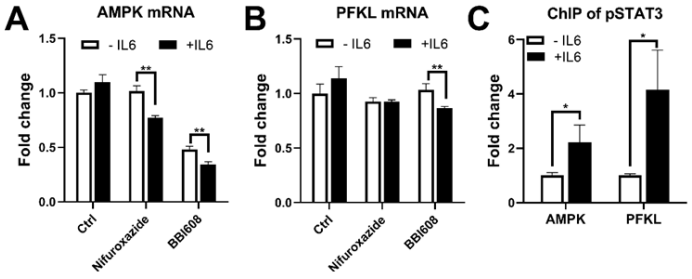


Figure 3. IL6 activates STAT3 phosphorylation to directly bind to the promoter of AMPK and PFKL and increase AMPK and PFKL mRNA levels. (A) Targeting STAT3 by nifuroxazide and BBI608 reduce IL6-mediated transcriptions of AMPK and (B) PFKL in PLC5. (C) Chromatin immunoprecipitation assay reveals pSTAT3 directly binds to the promoter of AMPK and PFKL in IL6-treated PLC5.

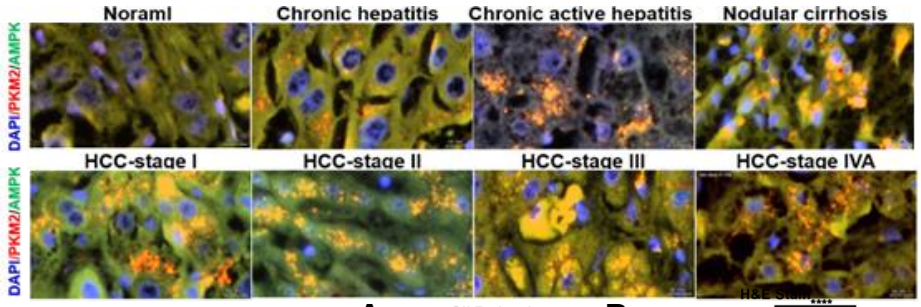


Figure 4. Co-localization of PFKL, AMPK, RPIA and PKM2 in the G-body correlates to development of hepatitis to HCC.

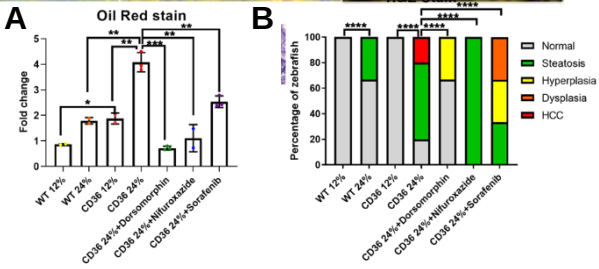
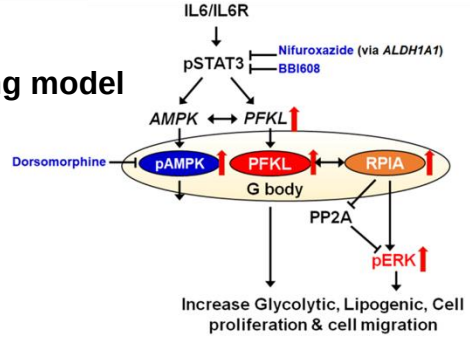


Figure 5. Pharmacological inhibition of AMPK or STAT3 dramatically reduces lipid accumulation, tumor cell proliferation and HCC formation in the CD36-HFD transgenic fish. (A) The anti-lipid accumulation effect in 15 day old fish. (B) The anti-HCC effect in a 1 month old fish.

Working model



References

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[2] Chou YT et al and Wang HD*, Yuh CH*, *PLoS Biology*, 2018 Jan 16;16(1):e2003714.
[3] Chou YT et al and Wang HD*, Yuh CH*, *Carcinogenesis*, 2019 May 14;40(3):46