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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

PFKFB4 Is a Metabolic Driver of HCC Progression and Chemoresistance Through ROS Mitigation

Hepatocellular carcinoma (HCC) is one of the most frequent malignancies and the third leading cause of cancer-related death worldwide, representing a major social and health problem.[1] The heterogeneity of HCCs at genomic, metabolic, and immune levels, together with other features, compromise the success of therapies.[2,3] In particular, metabolic reprogramming is a well-stablished hallmark of cancer. To meet the high demand of energy and biomaterials to support uncontrolled growth, tumor cells acquire metabolic adaptations in response to a wide variety of extrinsic and intrinsic cell signals and remodel their nutrient absorption, energy production, and biomolecule synthetic mechanisms.[4] These metabolic changes promote malignant cell transformation, as well as cancer cell growth and resistance to drugs.[4]

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Kam et al[5] investigated the potential role of phosphofructokinase-fructose bisphosphatase 4 (PFKFB4), an activator of the key regulatory enzyme of glycolysis phosphofructokinase 1 (PFK1), in the pathogenesis of HCC. The investigators found that *PFKFB4* expression was upregulated in HCC tumors from 3 independent cohorts of patients compared to non-malignant liver tissues, being associated with larger tumor size, advanced tumor stage or invasion, and reduced patient overall survival. Of note, the expression of *PFKFB4* in HCC was also associated with the presence of *TP53* gene mutations. These findings were validated further using Clustered regularly interspaced short palindromic repeats - CRISPR-associated protein 9 (CRISPR-Cas9) engineered *TP53* knockout (KO) HCC cells. The authors also observed that the hyperactivation of Hypoxia inducible factor 1 subunit alpha under hypoxic stress promotes *PFKFB4* expression. Hypoxia is known to activate multiple pro-angiogenic pathways and PFKFB4 has been shown to induce angiogenesis in certain solid tumors,[6] but its role in HCC remains to be explored. Importantly, the experimental depletion (CRISPR-Cas9) of *PFKFB4* in HCC cells reduces their tumorigenicity and metastatic capacity in subcutaneous and orthotopic mouse models of HCC. Nevertheless, those findings were not observed in vitro. Such discrepancy could be attributed to; i) in vitro culture media, which might not recapitulate the nutrient composition of the liver microenvironment during HCC development and progression, or ii) hypoxia achievement, since in vivo is more likely to be a gradual process rather than acute hypoxia, which was assayed in culture. On the other hand, PFKFB4-mediated glycolytic reprogramming has been shown to promote fibrosis,[7] highlighting the need to explore its role in models of chronic liver injury and related HCC.

To elucidate the impact of PFKFB4 in the metabolic rewiring of HCC, metabolomic analyses were performed in the *PFKFB4* KO HCC cells, revealing that PFKFB4 generally acts as a phosphatase mediating the dephosphorylation of fructose-2,6-bisphosphate into fructose-6-phosphate, which is the substrate of PFK1. Thus, *PFKFB4* depletion in hypoxic conditions lead to the accumulation of downstream metabolites in glycolysis and the pentose phosphate pathway. Next, RNA sequencing analyses of the *PFKFB4* KO and control xenografts were conducted, identifying 63 differentially expressed genes, 16 of which were found to be upregulated in the *PFKFB4*-depleted xenografts and 47 were found to be downregulated. Among the upregulated genes, half were hypoxia-responsive, of which 25% were involved in oxidative stress alleviation and the other 25% were involved in glycolysis. With the aim to unravel the potential effect of *PFKFB4* loss on intracellular reactive oxygen species (ROS) production and tumorigenesis, the investigators evaluated ROS levels in *PFKFB4* KO xenografts by flow cytometry, observing a 2- to 3-fold increase of ROS, which did not result in apoptosis. In this regard, sorafenib-induced cell death in HCC has been reported to be dependent on ROS generation.[8] Because PFKFB4 appears to be involved in ROS mitigation through glucose metabolic reprogramming, the investigators evaluated the potential role of PFKFB4 in sorafenib cell resistance, demonstrating that *PFKFB4*-overexpressing cells and xenografts displayed higher tolerance to sorafenib and no reduction in tumor volume.

This study introduces PFKFB4 as a novel driver of hepatocarcinogenesis. The relevance of PKFKB4 in modulating oncometabolic processes in different cancers already has been reported, being PFKFB4 particularly associated with cancer cell survival and metastasis.[9,10] The current study describes an original pro-oncogenic role of PFKFB4 in HCC through the reduction of ROS levels and as a promoter of sorafenib resistance. Importantly, this work complements previous findings reporting the influence of genomic mutations in *TP53* and microenvironmental stress in *PFKFB4* expression,[11] and also reveals the clinical association between *PFKFB4* overexpression and tumor aggressiveness in a subgroup of patients with HCC carrying loss-of-function *TP53* mutations. Therefore, pharmacologic targeting of PFKFB4, alone or in combination with sorafenib, may represent a novel therapeutic avenue for patients with HCC, particularly for those harboring *TP53* mutations, which deserves further investigation. Indeed, considering that the phosphatase function of PFKFB4 is predominant in the context of HCC, administration of the first-in-class PFKFB4 selective inhibitor 5MPN, which inhibits the fructose-6-phosphate binding site of the PFKFB4 kinase domain,[12] could enhance the pro-oncogenic activity of PFKFB4. Hence, exploring and developing alternative PFKFB4

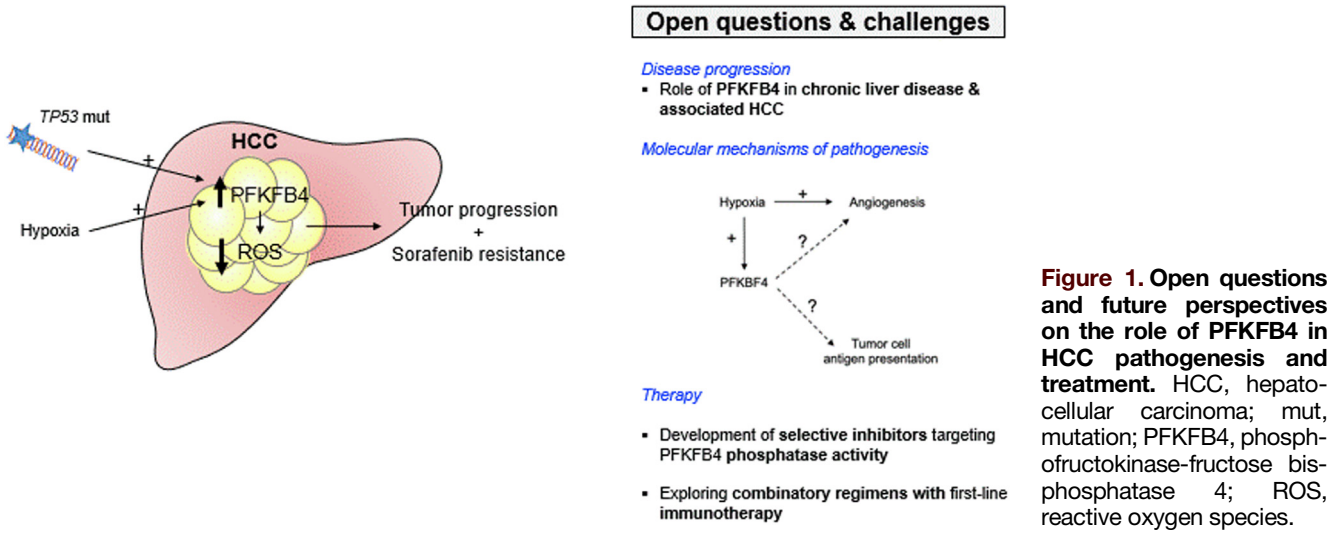

Figure 1. Open questions and future perspectives on the role of PFKFB4 in HCC pathogenesis and treatment. HCC, hepatocellular carcinoma; mut, mutation; PFKFB4, phosphofructokinase-fructose bisphosphatase 4; ROS, reactive oxygen species.

inhibitors to selectively block the phosphatase activity might provide novel therapeutic opportunities for patients with HCC. Furthermore, future studies should explore the role of PFKFB4 in the promotion of antigen cell presentation and response to immunotherapy (Figure 1).

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References

1. Vogel A, Meyer T, Sapisochin G, et al. Hepatocellular carcinoma. Lancet 2022;400:1345–1362.
2. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7(1):6.
3. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008; 359:378–390.
4. Faubert B, Solmonson A, DeBerardinis RJ. Metabolic reprogramming and cancer progression. Science 2020; 368(6487):eaaw5473.
5. Kam CS, Wai-Hung Ho D, Sheung-In Ming V, et al. PFKFB4 drives the oncogenicity in *TP53*-mutated hepatocellular carcinoma in a phosphatase-dependent manner. Cell Mol Gastroenterol Hepatol 2023;15: 1325–1350.
6. Li D, Tang J, Gao R, et al. PFKFB4 promotes angiogenesis via IL-6/STAT5A/P-STAT5 signaling in breast cancer. J Cancer 2022;13:212–224.
7. Lee MO, You CH, Son MY, et al. Pro-fibrotic effects of PFKFB4-mediated glycolytic reprogramming in fibrous dysplasia. Biomaterials 2016;107:61–73.
8. Coriat R, Nicco C, Cheŕeau C, et al. Sorafenib-induced hepatocellular carcinoma cell death depends on reactive oxygen species production in vitro and in vivo. Mol Cancer Ther 2012;11:2284–2293.
9. Goidts V, Bageritz J, Puccio L, et al. RNAi screening in glioma stem-like cells identifies PFKFB4 as a key molecule important for cancer cell survival. Oncogene 2012; 31:3235–3243.
10. Ros S, Santos CR, Moco S, et al. Functional metabolic screen identifies 6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 4 as an important regulator of prostate cancer cell survival. Cancer Discov 2012;2:328–343.
11. Ros S, Flöter J, Kaymak I, et al. 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 is essential for p53-null cancer cells. Oncogene 2017;36:3287–3299.
12. Chesney J, Clark J, Lanceta L, et al. Targeting the sugar metabolism of tumors with a first-in-class 6-phosphofructo-2-kinase (PFKFB4) inhibitor. Oncotarget 2015;6:18001–18011.

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