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Aberrant Regulation of Key Enzymes in Hepatocellular Carcinoma: From Metabolic Reprogramming to Diagnostic Markers

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Abstract. Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide, with complex pathogenesis and limited treatment options. In recent years, the central role of enzymes in the proliferation of HCC has become increasingly prominent, beyond their traditional catalytic function. This review systematically describes the role of enzymes in HCC and their therapeutic potential. Firstly, review outlines some enzymes that drive HCC proliferation, invasion and metastasis, including metabolic enzymes (e.g. HK2, PKM2) in mediating Warburg effect; oxidative stress and detoxification enzymes (e.g. CYP450 family, SOD, PKM2) in accumulating of ROS and inducing of oncogenes; epigenetically modifying enzymes in silencing of tumor suppressor genes. Secondly, review discusses the potential of the enzyme as HCC diagnosis marker, emphasized the emerging markers (such as GP73) in make up for the traditional markers (AFP) lack of sensitivity and specificity of potential. Furthermore, we summarize the therapeutic strategies targeting enzymes, including approved multikinase inhibitors (sorafenib, lenvatinib), immunometabolism targets in clinical trials (IDO1 inhibitors), and preclinical hotspots (such as PKM2 inhibitors, HDAC inhibitors, and PARP inhibitors). Finally, we highlight the key challenges in the field, including the differences in enzyme expression profiles caused by tumor heterogeneity and the compensatory resistance mechanisms, then look forward to the future directions of combination therapy strategies integrated by new technologies such as single-cell sequencing and artificial intelligence. This review aims to highlight the great value and broad prospects of targeted enzyme systems as a precise and innovative strategy to overcome the clinical challenges of HCC.

Keywords: Hepatocellular Carcinoma, enzyme, metabolic reprogramming, Epigenetic modification, Biomarkers

1. Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor with the sixth highest incidence and the third leading cause of cancer-related death worldwide, with a particularly significant incidence in developing countries [1]. The incidence of HCC is closely related to a variety of risk factors, including viral hepatitis (hepatitis B and C), alcoholic liver disease, and non-alcoholic fatty liver

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disease (NAFLD) [1,2]. Viral hepatitis and alcohol abuse have long been the main pathogenic factors of HCC, but in recent years, with the prevalence of metabolic syndrome, NAFLD is rapidly becoming an important cause of HCC [1]. In addition, studies have shown that men have a higher risk of developing HCC than women, and the prognosis is usually worse [3].

For patients who have been diagnosed with HCC, there are several clinical treatment options: Surgical intervention: Liver resection and liver transplantation are common treatments for early-stage HCC. However, early HCC usually has no obvious symptoms, leading to delayed diagnosis. Only 23% of patients with early-stage HCC will have their liver cancer cells removed, and there is a risk of 8% of overtreatment [4]. Most patients have been diagnosed at an advanced stage and lose the opportunity for surgery.

Systemic therapy: Systemic therapy is the main treatment for advanced hepatocellular carcinoma, which usually uses targeted drugs such as sorafenib, lenvatinib, atezolizumab combined with bevacizumab [1]. However, problems such as high metastasis, high recurrence and high drug resistance still need to be solved [5].

In summary, in the face of the severe epidemiological status and clinical diagnosis challenges of HCC, it is increasingly urgent to explore the molecular mechanism of HCC in order to achieve more individualized treatment, such as more accurate risk stratification and new combined strategies. In recent years, more and more evidence has shown that enzymes are not only catalysts of basal metabolism, but also play a central role in cell signal transduction and tumor microenvironment remodeling. They play a key role in the proliferation, invasion and metastasis of HCC by regulating the metabolic reprogramming of cells, the balance between proliferation and apoptosis signals, and the composition of immune microenvironment [6-9]. Elucidating the function of enzymes is undoubtedly an important part of solving the mystery of HCC occurrence and development.

2. Abnormal function of key enzymes in HCC

2.1. Metabolic-related enzymes

The Carbohydrate metabolism enzyme hexokinase (HK2) and pyruvate kinase M2 (PKM2) are the core regulators of Warburg effect (aerobic glycolysis), and they act synergistically to promote the proliferation and metastasis of hepatocellular carcinoma (HCC). HK2 catalyzes the phosphorylation of glucose to glucose-6-phosphate (G-6-P), which "traps" glucose inside the cell so that it cannot escape. At the same time, HK2 binds to the voltage-dependent anion channel (VDAC) on the mitochondrial outer membrane, blocking the interaction of pro-apoptotic proteins (such as Bax) with VDAC and inhibiting mitochondrial pathway apoptosis. In addition to its catalytic role in pyruvate production, PKM2 can also act as a transcriptional coactivator in the nucleus and bind to oncogenic transcription factors such as hypoxia-inducible factor (HIF-1α) and c-Myc to up-regulate the expression of a variety of tumor proliferation and glycolysis related genes (such as GLUT1 and LDHA), forming a positive feedback loop. Studies have also shown that ZEB1 (an epithelial-mesenchymal transition transcription factor) can promote the metastasis and recurrence of HCC by regulating the expression of glycolytic enzymes [6]

In summary, carbohydrate-metabolizing enzymes not only provide energy and biological macromolecules for the rapid proliferation of HCC by mediating the Warburg effect, but also play an important role in avoiding programmed death and strengthening carcinogenic signaling pathways in the process of cancer proliferation. Glucometabolic is not an isolated process; The resulting metabolites also happen to provide important raw materials for other anabolic pathways. Among them, the lipid synthesis and glutamine metabolism pathways take advantage of the "outputs" of

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glucose metabolism to further drive the hyperactive biosynthetic process in HCC. For example, the up-regulation of fatty acid synthase (FASN) and acetyl-coa carboxylase (ACC) leads to lipid accumulation and drives tumor growth [7]; glutaminase (GLS) promotes HCC progression by supporting nucleotide synthesis.

Such metabolic reprogramming is the basis for malignant proliferation of HCC cells, which also makes them a promising target for anticancer therapy.

2.2. Oxidative stress and detoxification enzymes

Oxidative Stress refers to the imbalance between the production and removal of Reactive Oxygen Species (ROS) in the body's cells, leading to excessive accumulation of ROS. Low concentration of ROS is an important signal molecule, which participates in mitochondrial metabolism, inflammatory response and other mechanisms. However, high concentrations of ROS can cause serious damage to cells, especially to DNA, protein and lipid membranes. Cytochrome P450 enzymes are important phase I detoxification enzymes in the liver [8]. Their function is to add polar groups to fat-soluble foreign substances, making them more easily bound and excreted by phase II enzymes, such as glutathione-S-transferase GST. In chronic liver diseases such as NAFLD, CYP2E1 activity is significantly increased [8], and a large amount of ROS is "leaked". The imbalance of antioxidant enzyme (SOD, CAT) system makes cells unable to effectively remove these excessive ROS, which directly aggravates oxidative stress.

2.3. Epigenetic modification of enzymes

The dysfunction of epigenetic modifying enzymes leads to the turning on of a large number of oncogenes and the turning off of tumor suppressor genes, thereby driving tumor proliferation. For example, DNMTs are key enzymes that catalyze DNA methylation. Over-activity of DNMTS can lead to Hypermethylation in the promoter regions of Tumor Suppressor Genes that should remain open. It physically hinders transcription factors (TFS) from binding to DNA, and together with other inhibitory proteins, leads to gene Transcriptional Silencing [9].

3. Enzyme as a diagnostic marker for HCC

Early diagnosis of HCC is the key to improve the prognosis of patients. An ideal diagnostic marker should have high sensitivity and specificity. Traditional serum markers have obvious shortcomings in these two aspects, so the search for new markers, especially enzymes and their related proteins, has been stimulated.

Although traditional serum enzyme markers such as alpha-fetoprotein (AFP) are widely used, they still have problems such as low detection rate [10] and difficulty in distinguishing other liver diseases. Additionally, another marker ALT/AST (transaminase) is used to evaluate the inflammatory state of the liver. The level of Alt/AST is also not directly related to tumor burden and malignant degree, and it is non-specific. In order to overcome the limitations of traditional markers, many more promising new markers have been found: Golgi protein 73 (GP73), a transmembrane glycoprotein mainly expressed in bile duct epithelial cells, is involved in directly binding to AFP and increasing AFP secretion, and has higher sensitivity and specificity [10], which effectively makes up for the deficiency of AFP. Aldolase A (ALDOA) and exosome enzymes such as CD73 are also being explored as liquid biopsy targets.

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4. Challenges and future directions

At present, multikinase inhibitors, such as sorafenib and lenvatinib, exert anti-angiogenesis and anti-proliferation effects by inhibiting VEGFR, PDGFR and other kinases to regulate the downstream enzyme network indirectly, and become the first-line treatment options [11]. Inhibitors, such as Epacadostat, aim to regulate the immunosuppressive environment mediated by tryptophan metabolism and restore T cell function. Although the preliminary clinical results were not as expected [12], they still suggest the therapeutic potential of immunometabolic regulation. In preclinical studies, a variety of specific enzyme inhibitors have shown great potential, PKM2 allosteric activators, such as TEPP-46, inhibit tumor growth by blocking the glycolytic pathway; HDAC inhibitors, such as Panobinostat, restore tumor suppressor gene expression. PARP inhibitors, such as Olaparib, use synthetic lethality to target DNA repair-deficient HCC. Together, these strategies highlight the potential of enzyme-targeted therapy.

5. Challenges and future directions

Despite the promising prospect of the neighborhood of targeted enzymes, its clinical application faces many challenges. Tumor heterogeneity and drug resistance mechanisms are two of the major challenges.

HCC is a highly heterogeneous cancer. This means that tumors from different patients, or even different cells within the same patient's tumor, can differ significantly in molecular features, behavioral patterns, and response to therapy. For example, the differences in enzyme profiles between metabolic type (dependent on glycolysis) and inflammatory type (driven by immune microenvironment) HCC need to be solved by personalized treatment strategies [13]. Cancer cells have strong compensatory and adaptive capabilities. Initially effective targeted enzyme therapy, after extensive use, almost always eventually develops resistance and leads to disease progression. This is the greatest challenge of current targeted therapy. For example, when a key pathway (such as glycolysis) is inhibited, cancer cells will rapidly activate another alternative pathway (such as up-regulation of oxidative phosphorylation) to maintain survival and proliferation [14].

To address the challenges of heterogeneity and drug resistance, emerging technologies have given cancer research greater depth and precision. Single-cell sequencing can analyze the gene expression profile of every cell in the tumor microenvironment (TME), reveal the heterogeneity of enzyme expression in the tumor and the mechanism of immune cell interaction. AI algorithms can integrate complex data to predict enzyme-drug interactions and guide combination therapy.

6. Conclusion

The occurrence and development of HCC is a complex process driven by multi-molecule involvement. This review systematically illustrates the important role of enzymes in this process: abnormal enzyme activity is involved in almost every step of the cancer process, from metabolic reprogramming (e.g. HK2, PKM2 in mediating Warburg effect), oxidative stress (e.g. CYP450 family, SOD, PKM2 in accumulating of ROS and inducing of oncogenes) to epigenetic regulation (e.g., DNMTs, HDACs in silencing of tumor suppressor genes). The diversity of enzyme functions highlights the complexity of HCC pathological mechanisms on the one hand, and also provides a wealth of therapeutic targets on the other hand. The detection strategy based on new enzyme markers such as GP73 and exosomal CD73 is expected to improve the early diagnosis rate. The development of inhibitors for IDO1, PKM2, PARP and other enzymes has also expanded from

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traditional multi-kinase inhibition to more specific immunometabolism, epigenetic and synthetic lethality strategies.

However, the heterogeneity and drug resistance of HCC are still the core challenges. Future research needs to use new technologies such as single-cell sequencing and artificial intelligence to analyze the complex data of enzyme expression in the tumor microenvironment. Through reasonable combined treatment strategies (such as the combination of enzyme inhibitors with immunotherapy and targeted therapy), the compensatory activation pathway can be inhibited from multiple dimensions to break through the bottleneck of drug resistance. Targeted enzyme systems are expected to provide clinically meaningful solutions to improve the prognosis of HCC patients and provide promising directions for the design of future clinical trials.

References

[1] Vogel, A., Meyer, T., Sapisochin, G., Salem, R., & Saborowski, A. (2022). Hepatocellular carcinoma. Lancet (London, England), 400 (10360), 1345-1362. https: //doi.org/10.1016/S0140-6736 (22) 01200-4

[2] Lewis, S., Dawson, L., Barry, A., Stanescu, T., Mohamad, I., & Hosni, A. (2022). Stereotactic body radiation therapy for hepatocellular carcinoma: From infancy to ongoing maturity. JHEP reports : Innovation in hepatology, 4 (8), 100498. https: //doi.org/10.1016/j.jhepr.2022.100498

[3] Xu, Z. Q., Luo, S. Q., Wu, Z. J., & Liao, R. (2025). Current status and perspectives of molecular mechanisms of gender difference in hepatocellular carcinoma: The tip of the iceberg? The Bioscience trends, 19 (3), 266-280. https: //doi.org/10.5582/bst.2025.01103

[4] Fraquelli, M., Nadarevic, T., Colli, A., Manzotti, C., Giljaca, V., Miletic, D., Štimac, D., & Casazza, G. (2022). Contrast-enhanced ultrasound for the diagnosis of hepatocellular carcinoma in adults with chronic liver Diseases. The Cochrane database of systematic reviews, nine (9), CD013483. https: //doi.org/10.1002/14651858.CD013483.pub2

[5] Luo, X., He, X., Zhang, X., Zhao, X., Zhang, Y., Shi, Y., & Hua, S. (2024). Hepatocellular carcinoma: Signaling pathways, targeted therapy, and immunotherapy. MedComm, 5 (2), e474. https: //doi.org/10.1002/mco2.474

[6] Zhou, Y., Lin, F., Wan, T., Chen, A., Wang, H., Jiang, B., Zhao, W., Liao, S., Wang, S., Li, G., Xu, Z., Wang, J., Zhang, J., Ma, H., Lin, D., & Li, Q. (2021). ZEB1 enhances Warburg effect to facilitate tumorigenesis and metastasis of HCC by transcriptionally Activating PFKM Theranostics, 11 (12), 5926-5938. https: //doi.org/10.7150/thno.56490

[7] Wang, F., Ma, S., Chen, P., Han, Y., Liu, Z., Wang, X., Sun, C., & Yu, Z. (2023). Imaging the metabolic reprograming of fatty acid synthesis pathway enables new diagnostic and therapeutic Opportunity for breast cancer. The cancer cell international, 23 (1), 83. https: //doi.org/10.1186/s12935-023-02908-8

[8] Massart, J., Begriche, K., Hartman, J. H., & Fromenty, B. (2022). Role of Mitochondrial Cytochrome P450 2E1 in Healthy and Diseased Liver. Cells, 11(2), 288. https: //doi.org/10.3390/cells11020288

[9] Chen, F., & Chen, L. (2025). CRISPR/Cas-mediated macromolecular DNA methylation editing: Precision targeting of DNA methyltransferases in cancer therapy. International journal of biological macromolecules, 308 (Pt 2), 142401. https: //doi.org/10.1016/j.ijbiomac.2025.142401

[10] Liu, Y., Wang, J., Yang, R., Cheng, Y., Zhou, Y., Li, H., Jiang, W., & Zhang, X. (2021). GP73-mediated secretion of AFP and GP73 promotes proliferation and metastasis of hepatocellular carcinoma Cells. The Oncogenesis, ten (10), 69. https: //doi.org/10.1038/s41389-021-00358-3

[11] Chen, R., Li, Q., Xu, S., Ye, C., Tian, T., Jiang, Q., Shan, J., & Ruan, J. (2022). Modulation of the tumour microenvironment in hepatocellular carcinoma by tyrosine kinase inhibitors: from modulation to combination therapy targeting the microenvironment. Cancer cell international, 22(1), 73. https: //doi.org/10.1186/s12935-021-02435-4

[12] Fujiwara, Y., Kato, S., Nesline, M. K., Conroy, J. M., DePietro, P., Pabla, S., & Kurzrock, R. (2022). Indoleamine 2, 3-dioxygenase (IDO) inhibitors and cancer immunotherapy. Cancer treatment reviews, 110, 102461. https: //doi.org/10.1016/j.ctrv.2022.102461

[13] Zhang, A., Miao, K., Sun, H., & Deng, C. X. (2022). Tumor heterogeneity reshapes the tumor microenvironment to influence drug resistance. International -- -- in sciences, 18 (7), 3019-3033. https: //doi.org/10.7150/ijbs.72534

[14] Snaebjornsson, M. T., Poeller, P., Komkova, D., Rohrig, F., Schlicker, L., Winkelkotte, A. M., Chaves-Filho, A. B., Al-Shami, K. M., Caballero, C. D., Koltsaki, I., Vogel, F. C. E., Frias-Soler, R. C., Rudalska, R., Schwarz, J. D., Wolf, E., Dauch, D., Steuer, R., & Schulze, A. (2025). Targeting aldolase A in hepatocellular carcinoma leads to

DOI: 10.54254/2753-8818/2025.AU26853

imbalanced glycolysis and energy stress due to Place uncontrolled FBP accumulation. Nature metabolism, 7 (2), 348-366. https: //doi.org/10.1038/s42255-024-01201-w