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Review Article

Metabolites involvement in the growth and spread of liver cancer

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ABSTRACT

Hepatocellular carcinoma (HCC), commonly known as primary liver cancer, is a leading cause of cancer-related mortality worldwide, primarily attributed to changing lifestyles and dietary habits. HCC arises from liver cirrhosis, hepatic fibrosis, or hepatitis B virus infection, and is caused by disruptions in protein and lipid metabolism. These metabolic alterations, recognized as a hallmark of cancer, are pivotal in the progression of chronic liver disease to HCC. Due to its asymptomatic nature in early stages, HCC is often diagnosed at advanced stages when treatment options are limited. Despite being a potentially curative option, liver transplantation remains hindered by high costs and donor scarcity, further compounded by suboptimal long-term success rates. This review examines the critical metabolites that play a part in developing HCC, focusing on their roles as possible biomarkers for disease progression and therapeutic targets. Additionally, the influence of the gut microbiome on HCC development is discussed, highlighting its interplay with metabolic pathways. Understanding the roles of metabolites and the gut microbiome in HCC progression underscores the importance of their potential use in early detection and the development of targeted therapies, offering new avenues for improving patient outcomes.

1. Introduction

Hepatocellular carcinoma (HCC) is the leading cause of cancer-related deaths,[1] with the global fatality rate expected to exceed 1 million by 2030.[2] It is the fifth most common cancer in men and the seventh in women.[3] Over 700,000 people have been diagnosed with HCC, with over 600,000 people having passed away.[4] The male-to-female ratio ranges from 2:1 to 4:1.[5] Those with chronic liver disease are more prone to developing HCC.[6] The main risk factors are viral infections (hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV)), liver cirrhosis, eating aflatoxin-tainted foods on a regular basis, consuming large amounts of alcohol over an extended period, being overweight, smoking, etc.[7] One of the characteristics of cancer is metabolic alteration. Due to their unregulated proliferation, cancer cells modify their metabolism to facilitate the incorporation of nutrients like amino acids for protein production, nucleic acids for DNA replication, and lipids for cell biomembrane synthesis into the biomass required for cell division. The Warburg effect is a prominent metabolic alteration in which cancer cells rely on aerobic glycolysis instead of mitochondrial oxidative phosphorylation to obtain energy, increasing lactate buildup.[8] Cancer cells are well-known to have several altered metabolic pathways, which include glycolysis and glucose metabolism. Since the discovery of the Warburg effect, subsequent studies have revealed that several other pathways, including glutamine metabolism, fatty acid oxidation, lipid synthesis, branched-chain amino acid metabolism, and one-carbon metabolism, are changed in cancer.[9] Adenosine, for example, is a metabolic regulator of glucose and lipids in the liver and adipose tissue. It has several physiological effects in various tissues, including the central nervous and cardiovascular

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systems, where it modulates the immunological response and works as a metabolic regulator.[10] In modern medicine, the treatment of HCC includes local ablation, surgical removal, targeted therapy, liver transplantation, and systemic chemotherapy. Over the past few years, clinical trials have tested several molecularly targeted drugs for advanced HCC, such as lenvatinib plus pembrolizumab.[7,11] Therefore, the medical sciences community needs to develop various biomarkers, such as metabolites, to assess the status of liver diseases. Analyzing metabolites in recognized tumors gives important insights into the underlying metabolic changes that drive cancer genesis and progression. This information can be used to enhance diagnosis, therapy selection, and patient care, leading to better outcomes for cancer patients. More than 90% of people with HCC have chronic liver disease. No matter where it begins, cirrhosis is a significant risk factor for HCC.[12,13] HCC is the leading cause of death in this population, with a 1%–6% annual risk rise in cirrhotic individuals. Additionally, chronic alcohol use, metabolic dysfunction-associated steatohepatitis (MASH) due to diabetes or obesity, and infection with either HBV or HCV greatly increase HCC risk.[12] Less common risk factors for HCC include primary biliary cholangitis, hemochromatosis-induced cirrhosis, and a deficiency in alpha 1 antitrypsin. Up to 45% of those with hemochromatosis who develop cirrhosis in their lifetime are at an increased risk of getting HCC.[14] HCC involves significant metabolic reprogramming, making metabolite-based diagnostic and therapeutic targets central to current research. Nanomaterials are mentioned here only as supportive tools with emerging potential to enhance these metabolite-focused strategies through improved delivery and targeting. However, their clinical application remains limited, and they should be viewed as adjunct approaches rather than primary therapeutic options. Therefore, the main emphasis of this review remains on metabolite targets, with nanotechnology considered only as a future complementary aid to improve specificity and therapeutic outcomes in HCC.

2. Nanomaterials for HCC

The metabolic reprogramming observed in HCC underscores the need for exploring novel therapeutic and diagnostic strategies.[15] Recently, advancements in nanotechnology have opened new ways for addressing metabolic changes, making liver diseases such as HCC more manageable. In 2024, Huseynov *et al.*[16] discussed the potential of new nanomaterials for treating hepatobiliary diseases, emphasizing their role in targeted drug delivery and controlled drug release. Their findings suggest that nanomaterials could revolutionize liver cancer by enhancing specificity and reducing the risk of systemic toxicity. Additionally, Hajiyeva *et al.*[17] conducted ultrastructural studies to demonstrate the accumulation of iron oxide nanoparticles and their effects on liver tissues. Their work provides valuable insights into the interactions between nanoparticles and liver cells, laying the groundwork for their use in diagnostic imaging and therapeutic interventions for liver diseases. Such studies are crucial for understanding how nanoparticles function within complex biological systems, ultimately improving the effectiveness of liver cancer targeting.[17] Additionally, Eftekhari *et al.*[18] explored the application of magnetic nanoparticles for theranostics in liver fibrosis, indicating their potential for combined diagnosis and treatment in HCC as well.

Building on the critical role of metabolic alterations in HCC, nanotechnology provides innovative means for precisely detecting, tracking, and modifying these changes. The combination of metabolomic insights with nanomaterial-based techniques may enable the development of tailored therapies for liver cancer. Numerous monotherapeutic strategies have been developed to treat liver damage, reduce inflammation and oxidative stress, and reverse fibrosis, wherein nanoparticles can serve as versatile therapeutic agents or carriers. Recent improvements in nanoparticle delivery technologies have enhanced the targeting and distribution of drugs to diseased livers.[19] Nanoparticles are a potential approach for HCC therapy because they enable targeted drug delivery, enhance intracellular uptake, and reduce systemic toxicity through both passive (enhanced permeability and retention, EPR) and active ligand-mediated processes.[20] In particular, modified nanoparticles with specific target ligands may even improve particular cell uptake and penetration, lowering off-target toxicity and adverse effects linked to undesirable organ distribution.[21] Recent advancements in lipid-based, polymeric, and silica nanocarriers with stimuli-responsive and multifunctional characteristics show tremendous promise to overcome existing limitations and improve therapeutic outcomes in HCC.[22,23] Accordingly, nanoparticles should be viewed as emerging adjunct platforms that require precise molecular and metabolic targets to achieve meaningful therapeutic outcomes. Their future success depends on ongoing efforts to map metabolic vulnerabilities in HCC and align these with rational nanomaterial design.

3. Liver diseases

The various liver diseases that predispose individuals to HCC are summarized in Table 1,[24–32] while Fig. 1 illustrates their associated risks. The following subsections will discuss viral hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease in detail to highlight their significance in the development and progression of HCC.

3.1. HBV infection

HBV-associated chronic liver disease accounts for about 60% of HCC cases in African/Asian countries and 20% in Western countries.[33] HBV is a DNA virus that has the potential to integrate itself into the host genome at multiple sites. This process is known as insertional mutagenesis.[34] While most individuals with HBV-related liver cancer already have cirrhosis at diagnosis, HBV can still increase the risk of liver cancer, even in the absence of cirrhosis. Because of a higher prevalence of HBV-related diseases in East Asian countries, the risk of HCC is elevated in men (>40 years) and women (>50 years), which is higher than what is considered cost-effective, necessitating regular monitoring programs.[35] In African nations, people in their early 30s or 40s may develop HCC.[36] Aflatoxin B1 exposure, in conjunction with HBV, further increases the risk of HCC.[37] In some parts of Asia, HBV vaccination programs have made HCC less common, but many countries still lack adequate vaccination programs that reach everyone.[38]

3.2. HCV infection

Among many countries in Europe, North America, and Japan, people with HCC are most likely to have chronic HCV-associated chronic liver disease, which is frequently linked to HCC.[4] A growing number of people with HCV infection have been cured by direct-acting antiviral (DAA) drugs, which have cut the risk of HCC progression by about 50%–80%.[39] Racial and ethnic communities, as well as those from low socioeconomic backgrounds, remain untested for HCV and are therefore unaware of their infections.[40] Moreover, even after achieving sustained virologic response (SVR),[41] people with HCV have a greater chance of developing HCC (>2% each year) and should be continually followed.[42] HCV

Table 1
Role of liver diseases in HCC.

Liver diseases	Role of different liver diseases in HCC	References
HBV infection	Through genetic and epigenetic modifications, the HBx encourages the development of hepatocarcinogenesis. Prolonged inflammation caused by HBV raises the risk of HCC and causes liver fibrosis.	24
HCV infection	The HCV may interfere with cell cycle checkpoints that typically stop unchecked growth and inhibit tumor suppressor genes. Additionally, it may trigger signaling pathways that encourage cell division and proliferation, which might eventually result in the formation of HCC.	25
HDV infection	The risk of HCC is increased by HDV infection via activating STAT-3 and NF-kappa B. HDV antigen controls the cell activity.	26
Alcoholic liver disease	HCC and liver cirrhosis are associated with heavy alcohol intake. Alcohol raises HCC and cirrhosis risk linearly. Alcohol use (30–50 g/day) increases liver cirrhosis risk, while >60–100 g/day increases HCC risk.	27
MASLD	The incidence of HCC in MASLD patients is projected to increase by 45%–130% by 2030. Diabetes is the main risk factor for HCC in MASLD, while obesity, arterial hypertension, and dyslipidemia increase it.	28
MASH	MASLD worsens into MASH. Fat in the liver causes MASLD. A buildup that causes inflammation and damage is called MASH and can harm the liver.	29
Hepatic fibrosis	There are molecular pathways that have been shown to relate fibrosis to HCC, such as TGF-β-induced EMT and Wnt/β-catenin signaling; however, the whole range of processes is still not fully understood.	30
Liver cirrhosis	Excessive immune cells generate chronic inflammation that damages DNA. Genomic and epigenomic alterations disrupt cell metabolism and promote cancer growth.	31,32

Abbreviations: EMT, epithelial-mesenchymal transition; HBV, hepatitis B virus; HCV, hepatitis C virus; HBx, hepatitis B virus X protein; STAT-3, signal transducer and activator of transcription-3; NF-kappa B, nuclear factor kappa B; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis.

proteins core, NS3, and NS5A alter host signaling by modifying MAPK, nuclear factor-kappa B (NF-kB), and phosphatidylinositol 3-kinase (PI3K)/protein kinase (AKT) pathways, resulting in oxidative stress and mitochondrial dysfunction. They suppress innate immunity by inhibiting interferon signaling and antigen presentation, while continuous activation of inflammatory cascades promotes immunological evasion and hepatocellular damage.[43,44]

3.3. HDV infection

HDV is an RNA virus that needs the HBV surface antigen for infection production. About 20 to 40 million people around the world are thought to have HDV.[45] People with HDV have a higher risk of extensive liver damage, including a high risk of cirrhosis and fibrosis, than people with chronic HBV. Many research articles showed that the coinfection of HBV and HDV leads to a higher risk of HCC in comparison to HBV disease alone.[46,47] By intensifying immune-mediated hepatocyte destruction, encouraging chronic inflammation, and raising fibrogenesis, HDV hastens liver deterioration. Malignant transformation is further facilitated by HBV-HDV coinfection, which increases viral replication stress, causes oxidative stress, and interferes with hepatocyte signaling pathways, including NF-κB and JAK/STAT.[48]

3.4. Alcoholic liver disease

According to patient population studies, drinking-related cirrhosis has a prevalence rate of 1%–3% and a tertiary medical care service provider referral center incidence of around 2%–3%, causing 15%–30% of HCC cases, depending on location.[49] Drinking habits may also raise the risk of HCC from additional causes, such as HBV carriers with chronic liver disease. When these patients consume alcohol, they are more likely to develop HCC than those who do not.[50] Whether HCC starts in chronically alcoholic individuals or those with MASH, the diseases share many pathophysiological features such as oxidative stress, chronic hepatic inflammation, fibrogenesis, and dysregulation of lipid metabolism. However, each person has unique protumorigenic signatures.

3.5. MASH

Another prominent etiology of cirrhosis in humans is MASH, which is often associated with diabetes mellitus, obesity, and may eventually progress to HCC.[51] MASH has been the most prevalent trigger of cirrhosis in many regions of the globe due to increased obesity rates.[52] From 2000 to 2016, MASLD-HCC increased from 2.1% to 16.2%, making it the fastest increasing cause of HCC and the fastest growing indication for orthotopic liver transplantation. According to dynamic Markov modelling, the incidence of MASLD-HCC is expected to increase by 122% by 2030 in eight different nations.[52]

Because metabolic syndrome and MASH often co-occur in people with various liver problems, it is expected that more than 20% of those with metabolic disease and MASH will have both conditions.[53] Even though the annual rate of HCC in cirrhosis caused by MASH is lower than that in cirrhosis caused by viruses (3%–5%), it is still higher than 1.1 per 100 people, which suggests that monitoring is a good idea and should not be skipped.[54] The use of present surveillance programs, which primarily focus on persons with cirrhosis, has been challenged by the discovery that 25%–30% of MASH-mediated HCC occurs without cirrhosis.[55]

3.6. Hepatic fibrosis

Hepatic fibrosis is a reversible process that causes the gathering of extracellular matrix (ECM) components, leading to fibrous scar formation. Extensive depositions of ECM consist of glycoproteins, collagens, matrix proteins, proteoglycans, and matrix-bound growth factors. Hepatic fibrosis can cause serious damage to the liver, and if not treated properly, may result in liver cirrhosis.[56,57] Hepatic stellate cells (HSCs) contribute centrally to hepatic fibrogenesis, undergo activation, and transdifferentiate into myofibroblasts, which are characterized by enhanced cell expansion, loss of vitamin A storage capacity, ECM overproduction, α-smooth muscle actin (α-SMA) expression, and type I collagen production.[58] The most prevalent causes of liver fibrosis include excessive alcohol intake, irrational medication use, consumption of fast food, and exposure to various environmental pollutants.[45] The pathogenesis of liver fibrosis involves inflammatory pathways, lipid signaling, and growth factor signaling.[59]

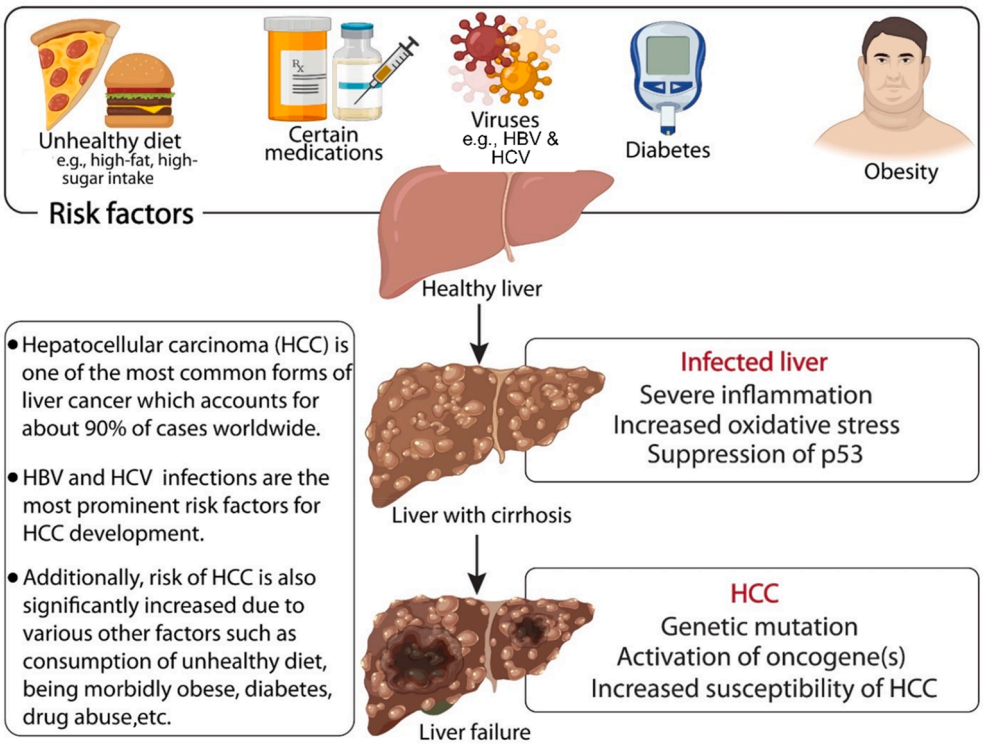

Fig. 1. Progression of liver disease to HCC. The diagram illustrates the sequential stages from a healthy liver to HCC, such as chronic liver disease and cirrhosis. The diagram also depicts the pathological progression and contributing factors associated with HCC. Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

3.7. Liver cirrhosis

Liver cirrhosis is the most common end-stage pathological manifestation of chronic liver diseases. It is caused by various factors, including excessive alcohol consumption, chronic HCV infection, and metabolic dysfunction-associated steatotic liver disease (MASLD, formerly called NAFLD). The highest rates of HCC incidence are observed in East Asia and sub-Saharan Africa.[60] Other aetiologies include hereditary illnesses such as Wilson's disease, hemochromatosis, primary biliary cirrhosis, autoimmune hepatitis, and primary sclerosing cholangitis.[61–63]

4. Differences in metabolomics based on etiology

The metabolic signature in chronic hepatitis B and C infections has been studied, but this approach overlooks variations in the HCC metabolomics patterns associated with other etiologies. According to metabolomics expression patterns in HCV and HBV cirrhosis-associated HCC progression, lysophosphatidylcholine (LPC) levels were significantly lower in HBV cirrhosis-associated HCC patients compared to HCV-associated HCC patients.[64] HCC is associated with multiple etiologies, including toxic environmental factors, MASLD, alcoholic cirrhosis, and viral hepatitis. Most metabolomics investigations have focused on single-etiology signatures, thereby overlooking variations in metabolomic expression patterns when different etiologies coexist in HCC. LPC expression was shown to be lower in HBV cirrhosis-associated HCC patients than in HCV-associated HCC patients, according to the HCV metabolomic expression patterns of cirrhosis-associated HCC and HBV cirrhosis-associated HCC.[65] This difference persists despite both patient groups having developed HCC. The cirrhotic stage itself is characterized by a large rise in bile acids, bilirubin, biliverdin, and acylcarnitines, along with significant downregulation of glycerophospholipids. These metabolic changes may serve as indicators of the liver injury underlying cirrhosis.[66] A significant amount of cell death that happens during cirrhosis may be shown by the pattern of LPCs dropping precipitously from viral hepatitis and cirrhosis, reaching a minimum with the onset of HCC.[67] This leads to decreased LPC levels, which are then monitored by a large utilization of leftover LPCs by the expanding HCC, additionally depleting the LPC reservoir. In HCC, the downregulation of LPCs at the proteomic and genomic levels may be due to their antitumor activities, capacity to cause apoptosis, effects against invasion, and sensitivity of the cancerous cells to different anticancer medications.[68]

5. Role of metabolites in HCC

In advanced HCC cases, therapeutic options that can significantly improve patient survival remain limited; however, early diagnosis offers the best opportunity for favorable therapeutic outcomes. Understanding the fundamental molecular mechanisms driving hepatocarcinogenesis is crucial for developing drugs that can inhibit or reduce HCC progression. The field of lipidomics is an invaluable resource for analyzing the intricate metabolic alterations that may precede the development of liver cancer. This approach provides critical insights into these pathological changes.[69] Several biomarkers/metabolites in Table 2 are arranged by their origins and their distinct functions in HCC.[70–83] This categorization emphasises their importance in diagnosis, prognosis, and treatment, offering valuable information about the course of the illness and possible clinical uses for better HCC management. The following sections detail relevant biomarker categories (Table 2).

5.1. Levo-carnitine (L-carnitine)

The essential amino acids, methionine and lysine, are converted into L-carnitine in the human liver, kidneys, and brain. It is also

Table 2
Role of different biomarkers/metabolites in HCC.

Sources	Biomarkers/Metabolites	Role in HCC	References
Enzymes	Pyruvate carboxylase (PC)	PC is necessary for cancer growth and spread because it regulates several energy pathways that are beneficial during the metastatic phase.	70
	Protein tyrosine phosphatase (PTP)	PTP in HCC may promote or inhibit tumor growth.	71
Fats	Glycocholic acid (GCA)	GCA demonstrates superior diagnostic performance for liver diseases compared to conventional blood enzymology and metabolomics approaches.	72
	Arachidonic acid (AA)	AA increased reactive oxygen species (ROS) and transglutaminase 2 (TG2) activation, hindering hepatic cell growth.	73
Proteins	Serine	Liver cancer-specific serine protease inhibitor kazal (LC-SPIK) is detected in increased quantities in HCC patients.	74
	Alpha-fetoprotein (AFP)	AFP is the core biomarker for HCC diagnosis and progression.	75
Amino acids	Levo-carnitine (L-carnitine)	L-carnitine supplementation may reduce liver alanine aminotransferase levels and hepatic steatosis.	76,77
	Glutamine and glutamate	This biosynthesizes liver cancer-causing basic materials.	78,79
	Branched-chain amino acids (BCAAs)	BCAAs may prevent obesity-related hepatocarcinogenesis by reducing visfatin activation.	80
	Proline	In HCC models, the proline-making enzyme pyrroline-5-carboxylate reductase 1 (PYCR1) was elevated.	81
	Methionine	Methionine reduces liver cancer cell proliferation and activates adenosine 5'-monophosphate activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) pathways.	82
	Lysine	During liver disease, lysine acetylation by enzyme is essential for the development of metabolic pathway.	83

known as 3-hydroxy-4-N-trimethylaminobutyrate. Conversely, most individuals obtain their L-carnitine from the foods they consume. L-carnitine exerts its optimal effect and facilitates the oxidative release of energy by acting as a stimulant for the transport of long-chain fatty acids across the mitochondrial membrane.[84] A deficiency of L-carnitine in the diet, poor absorption, and insufficient endogenous production in the liver can lead to liver cirrhosis. All of these factors combined cause cirrhosis.[85] Numerous studies have indicated the potential protective effect of L-carnitine against hepatotoxicity.[86–88] Patients with MASH experienced improvements in their liver functions and histological patterns after taking an oral L-carnitine supplement.[89] L-carnitine supplementation essentially enhanced the quality of life in cirrhotic patients with moderate hepatic encephalopathy.[90] A combination of L-carnitine and branched-chain amino acid (BCAA) medicine may be utilized as a novel liver preventive regimen for individuals with HCC.[91]

5.2. Pyruvate carboxylase (PC)

PC catalyses the carboxylation of pyruvate to oxaloacetate (OAA), producing OAA, which is used in fat metabolism, gluconeogenesis, and amino acid biosynthesis.[70]

The precursor for several C4 intermediates is OAA. The PC is a tetramer composed of four identical subunits, each of which weighs around 130 kDa. It belongs to the family of biotin-dependent carboxylases. Numerous creatures, including bacteria, fungi, plants, and mammals, contain PC. However, PC is only found in the cytoplasm in certain filamentous fungi, including *Aspergillus terreus*, *Aspergillus nidulans*, and *Rhizopus oryzae*, as well as in *Saccharomyces cerevisiae*. These organisms lack the peptide necessary for mitochondrial targeting.[70] The increasing amount of knowledge on PC's role in cancer over the last ten years suggests that PC is necessary for cancer development and metastasis due to its regulatory effects on several energy pathways. In addition to providing the metabolic adaptability that cells require to endure changing and unfavorable conditions throughout the metastatic phase, PC activity may be required to establish a relationship between the consumption of energy substrates and cancer growth.[92]

5.3. Serine

Serine synthesized endogenously (*de novo*) may serve different functions compared to exogenously derived serine, highlighting the need to investigate potential differences in their utilization. Another intermediate of the serine synthesis pathway (SSP) may be important. In contrast to the phosphoserine aminotransferase (PSAT) reaction that produces phosphoserine, the phosphoglycerate dehydrogenase (PHGDH) reaction yields NADH and phosphohydroxypyruvate (PHP). The NAD^+/NADH ratio in cancerous cells is extraordinarily high, and even in cells with high SSP flow, approximately 10% of glycolytic carbon is diverted to serine synthesis.[93] Therefore, PHGDH levels in cancer cells are unlikely to constrain NADH generation. Furthermore, if SSP flow affects the NAD^+/NADH ratio, boosting NADH production from SSP could worsen the NAD^+ deficit in cancer cells.[93] While the biosynthetic or regulatory functions of phosphoserine or PHP remain unclear, they represent interesting possibilities. Other metabolic processes, such as nucleotide biosynthesis, are similarly regulated by *de novo* serine synthesis; this function cannot be achieved by merely increasing extracellular serine levels.[94]

5.4. Glutamine and glutamate

Both normal and cancerous cell growth rely on glutamine and glutamate as crucial energy sources. However, cancer cells have a higher demand for biofuels to support their energy supply, antioxidant functions, and biomass production, compared to healthy tissues. Mutations in metabolic genes further enhance cancer cells' dependency on glutamate, making it a vital component for their survival and proliferation.[95] The enzyme glutamine synthetase primarily produces glutamine from glutamate and ammonia in skeletal muscle, the lungs, adipose tissue, and the liver. Glutamate is a precursor to the metabolic processes required for cell division and development.[95,96]

However, the majority of glutamine is hydrolyzed by glutaminase and eventually serves as a substrate for the synthesis of urea and glucose in the liver, ammonia production in the kidney, and respiration of enterocyte and immune system cells.[97] Surprisingly, evidence is mounting that glutamate signaling, as mediated by receptors, is involved in human cancer.[98] Excess extracellular glutamate is also produced through dysregulation of glutamate transporters, which activates glutamate receptors on cancer cells and prompts the growth of malignancy, such as the excitatory amino acid transporter and the cystine/glutamate antiporter system. Further research is required to identify a tumor-specific step in a metabolic chain in order to target the glutamate metabolic chain in cancer without disrupting normal metabolism.[98] In the cancer metabolic network, many mutations may

occur, and these alterations can lead to the expansion of specific therapeutic targets for cancer therapy. Although the role of glutamate receptor-guided cell signaling and/or transporters in the development of cancer has been widely recognized, therapeutically accessible antagonists for cancer therapy remain unknown.[98] A proposed method for distinguishing tumor cells reliant on glutamine from those that do not involve metabolic imaging-guided identification of "glutamine-addicted" cancer cells. Since not all tumors or cells within a diagnosed cancer depend on glutamine, a more accurate evaluation of the extent of glutamine addiction would undoubtedly be beneficial.[99]

5.5. BCAAs

Three BCAAs, namely isoleucine, leucine, and valine, are present in mammals. Human cells are unable to produce BCAAs; instead, they must be obtained from food and recycled from scavenged proteins. The essential amino acids produced by gut bacteria include BCAAs, which are characterized by nonlinear aliphatic side chains.[100] Mammalian BCAAs account for approximately 63% of all hydrophobic amino acids found in mammalian proteins.[101] The principal BCAA transporter, the L-type amino acid transporter (LAT) 1 (SLC7A5), is a member of the SLC7 family of L-type amino acid transporters and is highly expressed in a wide range of malignancies, including lung, breast, and prostate cancer. Significantly, a recent study has established that LAT1 is a pH-dependent transporter, with maximal activity at neutral pH and markedly reduced activity at acidic pH.[102] LAT1 (SLC7A5) imports BCAAs and a variety of essential amino acids, including phenylalanine, isoleucine, tryptophan, leucine, and tyrosine, with high affinity in exchange for the efflux of intracellular tyrosine, histidine, and glutamine.[102] To maintain amino acid nutrition for tumor formation, the procarcinogenic transcription factors (*e.g.,* cellular myelocytomatosis (c-Myc), hypoxia-inducible factor (HIF) 2, and NOTCH), as well as the post-transcriptional regulator microRNA-126, regulate the expression of the LAT1 (SLC7A5) transporter. Recent advances in developing selective small-molecule inhibitors targeting LAT1 (SLC7A5) and LAT2 (SLC7A8) have facilitated the clinical translation of system L transporter research.[103,104] Furthermore, in culture systems, studying the direct effects of BCAAs on HCC is important. Higher BCAA concentrations have been observed to inhibit the growth of HCC cell lines in culture conditions. Moreover, it has been shown that all three BCAAs accelerated the post-transcriptional degradation of insulin-induced vascular endothelial growth factor messenger RNA, thus decreasing vascular endothelial growth factor synthesis as HCCs develop. Furthermore, it has been indicated that BCAAs inhibit insulin-induced PI3K/Akt and NF-kB pathways via mammalian target of rapamycin complex (mTORC) 1 and mTORC2 processes, leading to the induction of apoptosis in liver cancer cell lines.[105,106] By reducing the stimulatory impact of visfatin, a key adipokine in the formation of HCC, BCAAs may also protect against obesity-related hepatocarcinogenesis.[80]

5.6. Methionine

Methionine is an essential amino acid crucial for cellular development and proliferation. It is mainly processed in the liver. However, its role in cancer prevention, particularly in the context of liver cancer, remains debated. In the presence of high methionine levels and adenosine 5'-monophosphate activated protein kinase (AMPK) inhibition, an integrative metabolomic and proteomic analysis reveals rewiring of the central carbon metabolism with an upregulation of the tricarboxylic acid (TCA) cycle and mitochondrial adenosine triphosphate (ATP) production.[107] In addition, methionine supplementation reduces liver cancer cell growth and promotes AMPK and mTOR pathway activity.[64]

5.7. Glycocholic acid (GCA)

GCA is a newly identified HCC biomarker. Due to their high specificity and sensitivity, the anti-GCA monoclonal antibody (mAb) may bind GCA, and the 50% inhibitory rate (IC50) was 77.09 ng/mL, indicating their promise as accurate analytical techniques for GCA detection and quantification.[72] Consequently, the research provides fresh perspectives on HCC and hepatobiliary disease research and treatment, indicating the potential of GCA for early HCC detection in clinical practice.[108] GCA is produced by the reaction of glycine and cholic acid. GCA present in the bile facilitates the digestion and absorption of fat.[109]

5.8. Alpha-fetoprotein (AFP)

In recent years, AFP has been the most widely utilized HCC biomarker. After delivery, serum AFP levels often drop rapidly and remain low in adulthood. Serum AFP levels in healthy persons usually vary between 5 and 10 ng/mL. However, HCC and other liver disorders are often linked to high blood levels of AFP. According to studies, an AFP level of 400 ng/mL or more is often regarded as a diagnostic indicator for HCC.[110]

Multiple studies have shown that HCV-infected individuals with elevated AFP levels face higher HCC risk.[111,112] Although not universally reliable, AFP testing is generally more objective than ultrasound or CT imaging, which is often dependent on the knowledge and judgment of medical professionals. Additionally, there is a financial argument for using AFP to identify HCC, especially in many developing countries where advanced imaging tools may be scarce or absent.[113]

5.9. Arachidonic acid (AA)

High-fat diets cause excessive accumulation of AA, a precursor to inflammation mediators. Of particular importance is its predominant accumulation within the hepatic phospholipids.[73] The changes in AA levels and the increased enzyme expression involved in producing eicosanoids and prostanoids are in line with the inflammation severity and development of MASLD.[114]

5.10. Fatty acid (FA)

Aberrant oncogenic signaling pathways alter the expression and activity of lipid-metabolizing enzymes, leading to dysregulated FA metabolism, a novel characteristic of cancer cells. This dysregulation may contribute to the genesis and HCC progression.[115] Tumors go through metabolic alterations to maintain uncontrolled growth, escape cell death, and seed in secondary organs. An increasing emphasis on cancer lipid metabolism has uncovered a variety of pathways that enhance tumor development and survival.[116]

5.11. Uric acid

In a previous study, ten purine metabolites were discovered in the serum of HCC patients. HCC patients exhibited significantly higher blood levels of guanine, xanthine, xanthosine, hypoxanthine, and guanosine compared to healthy controls. For instance, the HCC group had higher blood uric acid levels than chronic hepatitis B patients and healthy volunteers.[117] Uric acid is produced from xanthine and hypoxanthine under the action of xanthine oxidoreductase (XOR) in the purine metabolic pathway

in the human body. In both cells and living organisms, XOR is a vital rate-limiting factor in the breakdown of purine nucleic acids. It exists in two distinct forms: xanthine oxidase (XOD) and xanthine dehydrogenase (XDH).[118] Moreover, the relationship between blood uric acid levels and patient survival times remains unknown. Elevated blood uric acid levels suggest that individuals with advanced HCC may have a worse survival probability.[117] The urea cycle begins in the hepatocytes' mitochondria and ends in the cytoplasm.[119] Several studies have demonstrated that high blood uric acid suggests a poor survival risk in advanced HCC patients, and purine metabolism was significantly altered in diethylnitrosamine-induced HCC mice.[117,120] Prior research found ten purine metabolites in the serum of HCC patients. Additionally, it was shown that HCC patients' blood levels of guanine, xanthine, xanthosine, hypoxanthine, and guanosine were considerably different from those of healthy controls.[120]

5.12. Proline

In HCC tumor tissue, proline metabolism was significantly altered, with fast proline consumption and an accumulation of hydroxyproline. These changes were strongly associated with elevated AFP and a poor prognosis in HCC.[121,122]

5.13. Lysine

Proteins must undergo the essential post-translational modification (PTM) known as lysine acetylation, which is crucial for cancer growth. In healthy human liver tissues, several nonhistone proteins showed acetylation alterations; nevertheless, it is yet unclear how acetylated proteins affect the development of HCC.[123,124]

5.14. Protein tyrosine phosphatase (PTP)

Several pathological and physiological processes depend on the PTP family, which is involved in several cellular functions. For the treatment of various chronic diseases, including cancer, PTP is a potential therapeutic target.[71] Numerous PTP inhibitors, which target certain PTP molecules, have been created in the past 20 years as medicines.[125] Recent research has shown that PTP exhibits both tumor-promoting and tumor-suppressive roles in HCC.[126]

6. Role of gut microbiome in HCC

Research published over the last ten years has shown that the gut microbiota has a significant influence on human health.[127] Usually, the host's gut microbiota is of great help, especially in terms of immunity and metabolism. However, it is becoming clear that disease processes such as chronic inflammation in inflammatory bowel disease (IBD) are also associated with gut microbiota.[128] Previous research on the microbiome-gut-liver axis has enhanced our understanding of how the gut microbiota influences the onset and progression of liver disease, with transmission between the liver and gut bacteria.[129] The gut microbiota, along with their metabolites and byproducts, are known to play significant roles in the development of HCC. In addition, the gut microbiota is thought to have a crucial role in several aspects of the evolution of liver disease, which in turn contributes to the hepatic environment that promotes the formation and progression of HCC.[130]

There are two mechanisms by which the gut microbiota promotes the development of liver disease and HCC. One is dysbiosis, which results in altered bacterial metabolites including the cancer-promoting secondary bile acid (deoxycholic acid). The other is intestinal barrier dysfunction, which fosters chronic hepatic inflammation through Toll-like receptor-mediated signals.[131] Also, the gut microbiome utilizes bile acids as a messenger to modulate the expression of the CXC chemokine ligand 16 (CXCL16) on liver sinusoidal endothelial cells (LSEC), thereby limiting the development of natural killer T cells with the $CXCR6^{+}$ receptor on the liver.[132]

Emerging research has highlighted several gut-derived metabolites that play a critical role in liver cancer development. For example, microbial metabolites such as secondary bile acids (*e.g.,* deoxycholic acid) are involved in hepatocarcinogenesis by their ability to induce oxidative stress and inflammation in liver tissue. Similarly, lipopolysaccharides (LPS) derived from gut bacteria can promote liver inflammation by activating Toll-like receptor pathways, contributing to fibrosis and HCC.[133,134] These metabolites create a protumorigenic environment, facilitating the transition from liver injury to cancer. The gut microbiome also affects systemic metabolic profiles. For instance, microbial dysbiosis can alter levels of short-chain fatty acids (SCFAs) and tryptophan metabolites, which are associated with modulating immune responses and tumor progression.[135,136] These metabolites are promising biomarkers for the early detection of HCC and potential therapeutic targets. Recent studies, including therapeutic interventions like fecal microbiota transplantation and probiotics, have shown potential in modifying gut microbiota composition to reduce liver cancer risk.[137] Such strategies underscore the importance of gut-derived metabolites in both the pathogenesis and management of HCC.[138] This review holds significant promise for advancing biomedical applications in the diagnosis and treatment of liver cancer.[139] Understanding the role of metabolites in HCC progression provides important insights into the metabolic reprogramming that promotes the development and progression of liver cancer.

These insights can be leveraged in several ways. First, the identification of specific metabolites as biomarkers can significantly improve early detection, addressing the asymptomatic nature of HCC in its initial stages. Metabolite-based diagnostic tools could enable noninvasive screening, facilitating timely intervention, and improving patient outcomes. Second, metabolic profiling might improve patient classification by showing stage-specific metabolite changes in HCC, thereby laying the framework for personalized treatment options suited to the metabolic features of distinct patient subgroups.[140] Such profiling allows clinicians to tailor therapies to target specific dysregulated pathways, improving the efficacy of treatments. Furthermore, key metabolites and their associated pathways represent promising therapeutic targets. Developing interventions that modulate these pathways could inhibit tumor growth and metastasis, particularly in advanced HCC cases. Additionally, the role of the gut microbiome in influencing liver metabolism offers potential for therapeutic innovation. Modulating the gut microbiota through dietary, probiotic, or prebiotic strategies could influence metabolite production, reducing HCC risk or complementing existing treatments. Lastly, insights into metabolite roles can support patient stratification for targeted therapies, ensuring treatments are given to those most likely to benefit, thereby reducing costs and enhancing outcomes. Overall, these applications highlight the significant biomedical advantages of understanding metabolite involvement in HCC, providing a foundation for improved diagnostics, therapies, and preventive measures.[141]

6.1. Role of metabolites in the progression of HCC

Research in the field typically utilizes a combination of *in vitro* and *in vivo* models to simulate liver cancer development and progression.[142–144]

6.1.1. In vitro models

Cell lines such as HepG2, Huh7, and SMMC-7721 are widely used to study metabolic alterations in liver cancer. These models enable a controlled examination of how specific metabolites influence cell proliferation, migration, and invasion. Experimental conditions often include culturing cells in media supplemented with specific nutrients or inhibitors to mimic metabolic stress or investigate the effects of therapeutic agents targeting metabolic pathways.[145]

6.1.2. In vivo models

Animal models, including xenograft models using immunocompromised mice and chemically induced liver cancer models (*e.g.,* diethylnitrosamine-induced HCC), are essential for understanding the systemic and tumor-specific metabolic changes within a physiological context. These models provide insights into how alterations in metabolites and pathways contribute to tumor growth and metastasis.[98]

6.1.3. Biomarkers and analytical techniques

Studies consistently identify key metabolites such as glucose, lactate, glutamine, and bile acids as critical to liver cancer progression.[146] Techniques such as liquid chromatography-mass spectrometry, nuclear magnetic resonance spectroscopy, and gas chromatography-mass spectrometry are extensively employed to quantify these key metabolites and map altered metabolic pathways. The integration of metabolomics with genomic and proteomic data further enhances understanding and reinforces the relevance of this approach.[147,148]

6.1.4. Relevance of the method

Collectively, the consistent data reporting across diverse experimental settings demonstrates the resilience of the analytical approaches used to elucidate the pathophysiology of HCC.

The ability to trace metabolic shifts from *in vitro* studies to *in vivo* applications strengthens the translational potential of these findings. This approach represents a novel alternative for identifying therapeutic targets and diagnostic biomarkers, offering a more personalized and precise way of managing liver cancer.[149]

6.2. Metabolic targeting as a solid alternative to existing therapies

The exploration of metabolite involvement in HCC provides a promising alternative to conventional therapies. Unlike standard treatments such as chemotherapy and radiation, which often come with significant systemic toxicity and limited specificity, metabolic targeting offers unique advantages by addressing the altered metabolic pathways, such as glycolysis, glutaminolysis, lipid metabolism, and the tricarboxylic acid cycle (TCA cycle), that are fundamental to HCC progression.[150]

Metabolic therapies focus on disrupting the tumor-specific metabolic dependencies that sustain rapid proliferation and survival. For instance, targeting pathways, such as glycolysis, glutaminolysis, and bile acid metabolism, allows for selective inhibition of cancer cell growth while sparing normal cells. This specificity minimizes off-target effects and reduces the toxicity often observed with conventional treatments.[151] Moreover, metabolic approaches have demonstrated the potential to overcome resistance associated with traditional therapies. For example, hexokinase-2 inhibitors that target glycolysis make resistant HCC cells more sensitive to sorafenib, whilst inhibiting glutaminolysis increases the effectiveness of chemotherapy.[152]

Tumor heterogeneity and the adaptive nature of cancer cells often limit the efficacy of existing treatments. These approaches can potentially disrupt the compensatory mechanisms of HCC, thereby enhancing therapeutic outcomes.[153]

A comparative analysis reveals that metabolic therapies are also more amenable to integration with existing treatment modalities. For example, combining metabolic inhibitors with immunotherapy has shown promise in enhancing the immune response against tumors. This approach not only complements current therapeutic strategies but also paves the way for innovative, personalized treatments in liver cancer management.[154]

6.3. Metabolite changes across different stages of HCC and their role as biomarkers

These changes are critical for understanding the metabolites' potential as indicators of disease progression and treatment response.

Lactate levels are often elevated in the early stages of liver cancer due to increased anaerobic glycolysis (Warburg effect), and they further increase as the disease progresses to advanced HCC.[155,156] Similarly, alterations in amino acid metabolism, particularly BCAAs, are observed as the disease advances, with their levels decreasing significantly in the later stages of HCC due to liver dysfunction. Before treatment, certain metabolites such as α-ketoglutarate and glutamine can indicate tumor aggressiveness and metabolic alterations associated with malignancy.[157] After treatment, including chemotherapy or targeted therapies, these metabolites may show a significant reduction or normalization, reflecting the therapeutic response. Conversely, persistent metabolic dysregulation could signal resistance to treatment or disease recurrence.[158] Liquid biopsy techniques or metabolic profiling could help clinicians track disease progression and evaluate the effectiveness of treatments, enabling more personalized therapeutic strategies.[159]

6.4. Limitations and challenges associated with using metabolites as biomarkers for HCC

While metabolites show considerable potential for monitoring disease progression and therapeutic responses, several factors hinder their clinical application. One key limitation is the lack of specificity and sensitivity; many metabolites, such as glutamine and lactate, are altered in not only HCC but also other liver diseases, such as cirrhosis and hepatitis, as well as in systemic conditions, reducing their diagnostic accuracy.[160] Additionally, confounding factors like comorbidities, medication use, diet, and gut microbiota composition can influence metabolite levels, making it difficult to interpret the results consistently. Standardized methodologies for measuring metabolites, including techniques like mass spectrometry and nuclear magnetic resonance spectroscopy, are also lacking, affecting the reproducibility and reliability of the data. Furthermore, the dynamic nature of metabolite changes throughout the disease process complicates the establishment of definitive "cut-off" values, particularly when patients undergo treatment, as these levels can fluctuate due to therapeutic interventions.[161] Lastly, while promising results have been seen in preclinical models, the translation of these findings into clinical practice requires validation through large-scale, multicenter clinical trials to confirm the diagnostic and prognostic value of metabolites in diverse patient populations. These limitations emphasize the need for further research to overcome these challenges and enhance the clinical utility of metabolites as biomarkers for HCC.[160]

7. Conclusions

This review looked into the significance of important metabolites in the development of HCC, including L-carnitine, PC, serine, glutamine, glutamate, BCAAs, methionine, uric acid, GCA, AA, FA, proline, lysine, and PTP. It is considered that an important factor contributing to the development of HCC is the hepatic manifestation of the metabolic imbalance. While this review remains centered on metabolite-based mechanisms and interventions, nanomaterials are briefly noted as a supportive future tool that may enhance the delivery and metabolite-targeted therapies; however, their clinical application is still evolving. Moreover, the part of the microbiome in HCC is also explored in the review, which helps to completely understand the mechanism of microbiota. Although the gut microbiota is a relatively new area of study, it may be a potential target for HCC prevention and therapy. Changes in metabolites appear to be linked to the development of HCC as well, but many concerns remain unanswered.

Authors' contributions

Anurag Kumar Gautam: Writing – review & editing, Writing – original draft, Conceptualization. **Vipin Kumar:** Writing – review & editing. **Archana Bharti Sonkar:** Writing – review & editing. **Amita Singh:** Writing – review & editing. **Deepankar Yadav:** Writing – review & editing. **Nitin Rajan:** Writing – review & editing. **Pranesh Kumar:** Writing – review & editing, Supervision, Conceptualization. **Sanjay Singh:** Writing – review & editing, Conceptualization. **Sudipta Saha:** Funding acquisition, Conceptualization. **Vijayakumar Mahalingakam Rajamanickam:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

All authors declare that there are no competing interests.

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