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The Implications of Extracellular Vesicles in HBV-induced Hepatocellular Carcinoma: From Pathogenesis to Diagnostic and Therapeutic

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1. Abstract

Hepatocellular carcinoma (HCC) induced by hepatitis B virus (HBV) infection represents a significant global health challenge. A comprehensive understanding of the molecular mechanisms driving HBV-induced hepatocarcinogenesis and the identification of effective diagnostic and therapeutic strategies are essential for enhancing patient outcomes. Extracellular vesicles (EVs) have recently emerged as pivotal mediators of intercellular communication, playing diverse roles in cancer progression. HBV infection initiates a cascade of molecular events, and the EV-mediated transfer of viral proteins, nucleic acids, and immune-regulatory molecules has been implicated in the dysregulation of critical cellular processes, thereby promoting tumor cell proliferation, angiogenesis, immune evasion, and drug resistance. Moreover, EVs hold considerable promise as non-invasive biomarkers for the early detection and monitoring of HBV-induced HCC. Additionally, EVs exhibit significant potential as targeted drug delivery vehicles and immunomodulatory agents in the treatment of HBV-induced HCC. In summary, elucidating the intricate interplay between EVs and HBV-infected hepatocytes will offer valuable insights into novel diagnostic and therapeutic approaches, ultimately improving patient outcomes in HBV-related HCC. This review synthesizes the pathogenic role of EVs in HBV-related HCC, their contribution to tumor growth, their role in immune regulation of liver cancer, their application in HCC diagnosis, and their potential in HCC treatment.

2. Introduction

Hepatitis B virus (HBV) is a highly infectious pathogen that primarily targets hepatocytes. Unlike direct cytotoxicity, HBV induces liver damage through the immune response triggered by the interaction between infected cells and the immune system. Globally, HBV constitutes a significant public health concern, with an estimated 31.695 billion people affected worldwide as of 2019, accounting for 48.8% of all hepatitis-related fatalities [1]. Transmission of HBV occurs through contact with contaminated blood or bodily fluids, including semen, vaginal fluid, and saliva, primarily via unsafe injections, sexual contact, or mother-to-child transmission during childbirth [2]. The hepatitis B virus exhibits a highly restricted species tropism, infecting limited species, including humans and chimpanzees [3]. Although recent research has expanded the range of HBV hosts [4], humans remain one of the primary hosts. HBV infection can manifest as either acute or chronic hepatitis. In certain cases, the virus persists within the body, leading to chronic infection that can result in long-term complications, such as liver cirrhosis and HCC, a form of liver cancer. Liver cancer is a major contributor to the global cancer burden, with its incidence rate remaining high in recent years. HBV-induced HCC is one of the primary causes of cancer-related deaths worldwide. Prospective cohort studies have demonstrated that chronic HBV infection increases the risk of developing hepatocellular carcinoma by 5 to 100 times [5]. Another study suggests that HBV carriers have a lifetime risk of developing hepatocellular carcinoma ranging from 10% to 25% [6].Extracellular vesicles (EVs) are small membranous structures released by cells into the extracellular space. Based on their biogenesis and diameter, EVs can be categorized into three main types: ectosomes, microvesicles, and apoptotic bodies. EVs are lipid bilayer vesicles with diameters ranging from 40 to 200 nanometers, which can be produced by most types of cells and

released into various bodily fluids, including blood, cerebrospinal fluid, urine, saliva, and serous cavity effusions. The membrane provides a protective barrier that shields the internal cargo from degradation by extracellular enzymes or harsh environmental conditions. Furthermore, EVs serve as vehicles for transporting bioactive molecules from the parent cells to recipient cells. They can transfer various types of cargo, including proteins, lipids, RNAs, and DNA fragments. This cargo exchange between cells can modulate the physiological and pathological states of recipient cells, influencing processes such as tissue repair, immune regulation, and disease progression [7]. Once released, EVs exhibit diverse activities, including remodeling the extracellular matrix and transmitting signals and molecules to other cells. Numerous reports indicate that EVs are crucial for intercellular communication and play key roles in various physiological and pathological processes, including cancer. EVs have increasingly been recognized as important players in the development and progression of HCC induced by HBV infection. Carcinogenesis results from the simultaneous destruction or reintegration of the HBV gene sequence and the host cell's gene sequence, leading to the activation of oncogenes and inactivation of tumor suppressor genes. EVs derived from HBV-infected cells can exert profound effects on HCC pathogenesis and contribute to disease progression. As carriers of cellular communication from various sources in the tumor microenvironment, EVs participate in a variety of tumor microenvironment processes, including the induction of angiogenesis, cell migration and proliferation, inflammatory responses, immune suppression, escape from immune surveillance, and metastasis [8, 9]. Due to their widespread presence and stability in body fluids, EVs hold potential as biomarkers for the diagnosis and prognosis of HCC [10, 11].The primary objective of this review is to provide a comprehensive overview of the multifaceted roles of extracellular vesicles (EVs) in HCC induced by Hepatitis B Virus (HBV) infection. By elucidating the pathogenic mechanisms through which EVs contribute to HBV-related HCC, this review aims to highlight their significance in tumor growth, immune regulation, diagnostic applications, and therapeutic potential. By systematically addressing these aspects, this review aims to provide valuable insights into the complex interplay between EVs and HBV-infected hepatocytes, may guide future research and clinical applications to improve patient outcomes in HBV-related HCC.

3. The Pathogenic Role of Extracellular Vesicles in HBV Induced HCC

Extracellular vesicles (EVs) play a significant role in the pathogenesis of Hepatocellular Carcinoma induced by Hepatitis B Virus (HBV) infection. EVs have the ability to facilitate HBV transmission, contributing to the replication and progression of HBV-related diseases. This occurs as HBV-infected cells discharge EVs, which comprise the viral genome and protein components. Many enveloped viruses, including HBV, form enveloped viral particles with EV-associated proteins that aid in HBV transmission by transferring substances from infected cells to uninfected ones. Firstly, the spread of EVs can facilitate HBV replication and the progression of HBV-related diseases. HBV-infected cells release EVs that contain the viral genome and protein components. These EVs, through their lipid bilayer, protect the viral cargo from degradation and facilitate its transfer to uninfected cells. This process enhances the spread of HBV within the liver, contributing to the chronic infection that underlies HCC development. Additionally, EVs have shown inhibitory effects on the proliferation of hepatocellular carcinoma cells. While EVs generally promote tumor growth, certain subpopulations of EVs derived from HBV-infected cells exhibit anti-proliferative properties. These EVs can deliver molecules that inhibit cell cycle progression or induce apoptosis in HCC cells, thereby counteracting the oncogenic effects of HBV[12]. This dual role of EVs in both promoting and inhibiting tumor growth underscores their complex and context-dependent functions in HBV-induced HCC.In summary, EVs play a crucial role in the pathogenesis of HBV-induced HCC by facilitating HBV transmission, enhancing viral replication, and exhibiting both pro-tumorigenic and anti-tumorigenic effects. Understanding these mechanisms is essential for developing targeted therapeutic strategies and improving patient outcomes.

3.1. EVs Modulate Tumor Cell Proliferation in HBV-Induced HCC

EVs derived from HBV-infected hepatocytes undergo significant alterations in gene expression profiles, reflecting the molecular changes induced by the viral infection. These altered genes are encapsulated within EVs and subsequently released into the extracellular environment. The exosomes, acting as intercellular messengers, can transfer these altered genetic materials to neighboring or distant recipient cells, thereby influencing their metabolic processes. The gene expression changes in HBV-infected hepatocytes often involve the upregulation or downregulation of key regulatory genes, including oncogenes, tumor suppressor genes, and genes involved in immune response and cell signaling pathways. miRNAs, due to their small size, stability, efficient packaging mechanisms, regulatory role, intercellular communication capabilities, and cell-specific signaling, are easily encapsulated in exosomes. This encapsulation is crucial for their transport and functional delivery to recipient cells, enabling them to exert their regulatory effects across cell boundaries. Moreover, EVs derived from HBV-infected cells can carry miRNAs that modulate the expression of target genes in recipient cells. Exosomes derived from HepG2.2.15 cells, which express hepatitis B virus (HBV)-related proteins, trigger the activation of LX2 liver stellate cells and promote liver fibrosis and cell proliferation. DESeq2 analysis identified a total of 27 miRNAs that were differentially expressed

within these exosomes. Gene Ontology (GO) analysis indicated that the target genes of these DE-miRNAs were associated with cell differentiation, intracellular signal transduction, negative regulation of apoptosis, extracellular exosomes, and RNA binding [13]. For instance, a cohort study demonstrated that Hepatitis B core antigen (HBc) expression significantly enhances miR-135a-5p levels in HCC cells and their derived EVs. The encapsulated miR-135a-5p, upon entering distant recipient cells, downregulates VAMP2 transcription, ultimately enhancing anti-apoptotic mechanisms, cell proliferation, and drug resistance in HCC [14]. Another study showed that in HBV-infected hepatocytes, miR-222 is upregulated. The upregulated miR-222 can be transmitted into LX-2 cells via exosomes, thereby activating LX-2 cells and promoting liver fibrosis by inhibiting TFRC-induced ferroptosis [15]. miRNA microarray screening revealed that miR-483-5p was significantly upregulated in recurrent hepatocellular carcinoma tissues. This upregulation significantly promoted the in vitro migration and invasion of HCC cells and increased intrahepatic metastasis in nude mice in vivo. The activated leukocyte cell adhesion molecule (ALCAM) was identified as a direct target of miR-483-5p, which significantly suppressed the migration and invasion of HCC cells. Reintroduction of ALCAM expression could antagonize the promoting effects of miR-483-5p on the migration and invasion capacity of HCC cells [16]. Exosomal miR-142-3p is highly expressed in HCC patients infected with HBV. miR-142-3p promotes the proliferation, migration, and invasion of HCC cells by inhibiting SLC3A2 [17]. circHDAC1_004 is upregulated in HCC, and its overexpression enhances HCC proliferation and metastasis via the miR-361-3p/NACC1 axis. Additionally, circHDAC1_004 upregulates its expression in human umbilical vein endothelial cells (HUVECs) through exosomes, promoting angiogenesis in HCC. This upregulation is significantly associated with poor overall survival [18]. CircCCAR1 levels increased in tumor tissues and plasma exosomes, as well as in culture supernatants and HCC cells of HCC patients. CircCCAR1 acted as a sponge for miR-127-5p, upregulating its target Wilms tumor 1-associated protein (WTAP), and accelerated the growth and metastasis of HCC both in vitro and in vivo [16]. Several ncRNAs we have listed exhibit significant expression changes in HCC and are involved in key processes such as cell proliferation, apoptosis, invasion, and metastasis. Although we have not listed all ncRNAs, these representative ncRNAs reflect the general role of ncRNAs in HCC. Through functional enrichment analysis and clinical correlation studies, we found that the target genes of these ncRNAs primarily involve HCC-related metabolic pathways. Future research should further validate the roles of other miRNAs, explore the regulatory networks of miRNAs, and develop diagnostic and therapeutic strategies based on miRNAs. Through this summary, we not only demonstrate the representativeness of the listed miRNAs and their relationship with HCC progression but also provide directions and suggestions for future research.

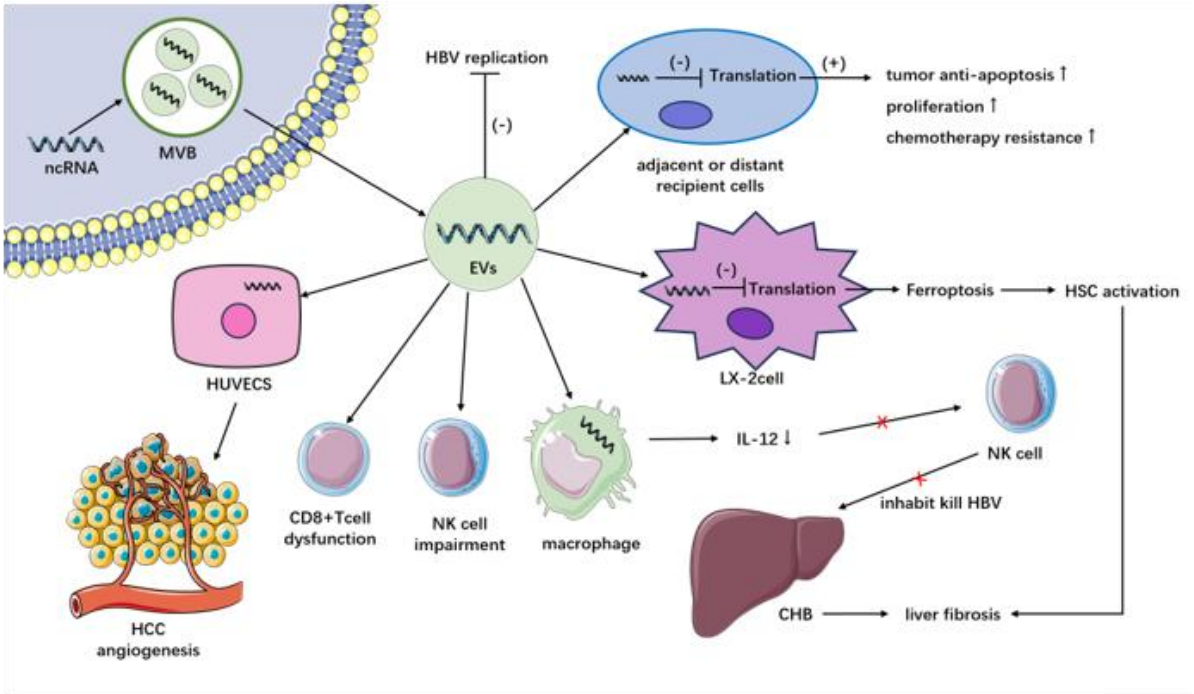

Figure 1: The combination of EVs and other related tests can significantly increase the rate and accuracy of early HCC diagnosis.

3.2. EVs Play a Role in Immune Regulation of Liver Cancer

Extracellular vesicles (EVs) have emerged as critical mediators of intercellular communication, playing a pivotal role in the immune regulation of liver cancer. These nanoscale vesicles, derived from various cellular sources, encapsulate a diverse array of bioactive molecules, including proteins, lipids, and nucleic acids, which they deliver to recipient cells. In the context of liver cancer, EVs serve as potent modulators of the tumor microenvironment, influencing both innate and adaptive immune responses. HBV-miR-3 expression increased in a dose- and time-dependent manner in HBV-infected HepG2-NTCP cells. By downregulating SOCS5 in hepatocytes, HBV-miR-3 activated the JAK/STAT signaling pathway, thereby enhancing the IFN-induced anti-HBV effect. Additionally, HBV-miR-3 in EVs promoted M1 macrophage polarization. EVs containing HBV-miR-3 enhanced IL-6 secretion by inhibiting SOCS5-mediated EGFR ubiquitination [19]. HBV infection induces the upregulation of exosomal miR-142-3p, which regulates the ferroptosis of M1 macrophages during HBV infection by targeting SLC3A2 [17]. Using StarBase, the expression of proteasome subunit alpha 5 (PSMA5) in liver hepatocellular carcinoma (LIHC) tissues was predicted to be associated with compromised survival of LIHC patients. PSMA5 knockdown inhibited the migration and invasion of HCC cells and reduced PSMA5 levels in exosomes. Exosomes successfully internalized into macrophages, promoting M2 polarization and JAK2/STAT3 pathway activation. PSMA5 knockdown in exosomes secreted by HCC cells inhibited the exosome-induced effects on macrophages and attenuated the promotion of HCC cell migration/invasion and tumorigenesis by macrophages [20]. M1-type macrophages can inhibit tumor growth and enhance immunity, whereas M2-type macrophages can promote tumor growth and development. EVs can influence the progression of hepatocellular carcinoma (HCC) by modulating macrophage polarization through the delivery of key molecules into macrophages. The study of these mechanisms provides valuable insights for HCC treatment. CircUHRF1 expression is elevated in human HCC tissues relative to matched adjacent nontumor tissues. Furthermore, circUHRF1 levels are inversely correlated with the clinical prognosis of HCC patients. Elevated plasma exosomal circUHRF1 is associated with a reduced proportion of NK cells and diminished NK cell tumor infiltration. CircUHRF1 inhibits NK cell secretion of IFN-γ and TNF-α by upregulating TIM-3 expression and degrading miR-449c-5p [21]. The synergistic anti-tumor effects of IL-15 and IL-21-stimulated NK-exos (NK-exosIL-15/21) on Hep3B cells were investigated. The study revealed that NK-exosIL-15/21 enhanced Hep3B cell cytotoxicity and apoptotic activity by activating pro-apoptotic proteins (Bax, cleaved caspase 3, cleaved PARP, perforin, and granzyme B) and inhibiting the anti-apoptotic protein (Bcl-2) [22]. Compared to adjacent tissues, the level of miR-17-5p in exosomes from HCC tissues increased, and miR-17-5p specifically targeted RUNX1, downregulating the expression of RUNX1 and NKG2D, subsequently reducing the in vitro and in vivo cytotoxic capabilities of NK cells against HCC cells [23]. Studies have demonstrated that EVs released by tumor cells can regulate tumor progression by modulating the immune response. CircCCAR1 in tumor tissues and plasma EVs of HCC patients was taken up by CD8+ T cells, leading to CD8+ T cell dysfunction by stabilizing the PD-1 protein. CircCCAR1 promoted resistance to anti-PD1 immunotherapy [16]. DC-derived exosomes loaded with ubiquitinated HBV core antigen (Dexs-Ub-HBcAg) induced T lymphocytes to release more IFN-γ and IL-2, effectively stimulating T cell proliferation and inducing the activation of antigen-specific cytotoxic T lymphocytes (CTLs) [24].EVs derived from various cell types, including immune and cancer cells, modulate immune responses through multiple mechanisms. For example, EVs from immune cells enhance anti-tumor immunity by promoting the activation and function of immune effector cells, whereas EVs from cancer cells suppress immune responses by inducing immune checkpoint expression and inhibiting immune cell function. Additionally, EVs facilitate the recruitment and activation of immune cells to the tumor site, thereby influencing the tumor microenvironment. Overall, EVs are emerging as crucial factors in shaping the immune landscape of liver cancer, presenting both therapeutic opportunities and challenges in the development of immunotherapies.

Another study utilized microRNA sequencing and mass spectrometry to characterize the exosomal microRNAs and proteins released from HBV-related HCC cell lines SNU-423 and SNU-182, as well as immortalized normal hepatocyte cell lines (THLE2 and THLE3). The findings revealed that, compared to normal liver cells, more than 40 microRNAs (such as mir-483, mir-133a, mir-34a, mir-155, mir-183, mir-182) and 200 proteins (such as POSTN, STAM, EXOC8, SNX9, COL1A2, IDH1, FN1) were significantly dysregulated in the exosomes released from HCC cells. The differentially expressed miRNAs, their predicted targets, and the exosomal differentially expressed proteins were primarily associated with pathways related to HBV, virus activity and invasion, exosome formation and adhesion, and exogenous protein binding [25].

3.3. Application of EVs in the Diagnosis of HCC

The application of exosomes (EVs) in the diagnosis of hepatocellular carcinoma (HCC) represents a promising frontier in cancer biomarker research. EVs, particularly those derived from cancer cells, carry a unique cargo of proteins, lipids, and nucleic acids that reflect the molecular characteristics of the tumor microenvironment. These vesicles can be detected in various biological fluids, including blood, urine, and ascites, making them attractive candidates for non-invasive diagnostic tools. The distinct molecular signatures of EVs, such as specific miRNAs, proteins, and surface markers, offer potential for early detection, differential diagnosis, and monitoring of HCC progression and treatment

response.The current diagnosis of HCC is mainly divided into two categories: imaging tests and diagnostic marker detection including alpha-fetoprotein (AFP). However, AFP exhibits low sensitivity and specificity for HCC [26]. While Imaging methods are highly specific, but its sensitivity is relatively low and unable to detect very small tumors [27]. Therefore, the potential of the EV biomarkers level as diagnostic markers for HCC has garnered significant interest from biologists and clinicians in recent years.

Studies examining circulating exosomes in HCC patients showed that the expression of miRNA-18a, miRNA-221, miRNA-222, and miRNA-224 in exosomes was significantly elevated in HCC patients compared to those with chronic hepatitis B. Conversely, the expression levels of miRNA-101 and miRNA-122 in exosomes were significantly reduced in HCC patients relative to those with chronic hepatitis B [28]. Significant upregulation of miR-143 and miR-215 expression was observed in the serum of patients with chronic hepatitis and hepatocellular carcinoma [29]. Circulating exosomal miRNA-21 and lncRNA-ATB were associated with hepatocellular carcinoma TNM stage and other prognostic factors, including T stage and portal vein thrombosis. Elevated miRNA-21 and lncRNA-ATB levels were independent predictors of mortality and disease progression, along with larger tumor size and higher C-reactive protein. Patients with higher circulating exosomal miRNA-21 and lncRNA-ATB levels exhibited significantly reduced overall survival and progression-free survival [30]. Another study utilized microRNA sequencing and mass spectrometry to characterize the exosomal microRNAs and proteins released from HBV-related HCC cell lines SNU-423 and SNU-182, as well as immortalized normal hepatocyte cell lines (THLE2 and THLE3). The findings revealed that, compared to normal liver cells, more than 40 microRNAs (such as mir-483, mir-133a, mir-34a, mir-155, mir-183, mir-182) and 200 proteins (such as POSTN, STAM, EXOC8, SNX9, COL1A2, IDH1, FN1) were significantly dysregulated in the exosomes released from HCC cells. The differentially expressed miRNAs, their predicted targets, and the exosomal differentially expressed proteins were primarily associated with pathways related to HBV, virus activity and invasion, exosome formation and adhesion, and exogenous protein binding [25]. Analysis of plasma EV circRNA expression levels in 256 liver cancer patients and 125 healthy controls revealed that circ_0000690, circ_0001359, and circ_0000396 were elevated in the HCC group compared to the healthy control group. The combined detection model utilizing these three circRNAs (circ_0000690, circ_0001359, and circ_0000396) exhibited good diagnostic efficacy for HCC [31].Clinical trials have shown that relying solely on a single biomarker for HCC diagnosis does not yield optimal accuracy. For instance, miRNA-21 is highly expressed in EVs secreted by hepatoma cells but is not specific to liver cancer. Combining miRNA-21 with AFP for early liver cancer diagnosis can significantly enhance diagnostic accuracy [32]. The development trends of HCC markers encompass the integration of multiple biomarkers, early detection strategies, and advancements in liquid biopsy technologies. Nonetheless, challenges such as low specificity and sensitivity persist. Future development should prioritize the integration of multiple biomarkers, extensive validation studies, personalized medicine, and the implementation of continuous monitoring systems to significantly enhance the accuracy and specificity of HCC detection, ultimately improving patient outcomes.

3.4. The Role of EVs in the Treatment of HCC

EVs hold significant promise in cancer treatment, offering potential applications in drug delivery, immunomodulation, stem cell therapy, combination therapy, personalized medicine, and biomarker discovery. Engineered to carry therapeutic agents such as nucleic acids and small molecules directly to cancer cells, EVs enhance efficacy while minimizing side effects. They also boost anti-tumor immune responses by carrying immunomodulatory molecules or delivering checkpoint inhibitors to counteract immune evasion. EVs derived from stem cells promote liver tissue repair and regeneration. Combining exosomes with conventional therapies improves treatment outcomes, and personalized strategies based on individual tumor molecular profiles enhance specificity and efficacy. Additionally, EVs provide abundant information about the tumor microenvironment, serving as a valuable source for identifying novel biomarkers. Angiogenesis has been an extremely important aspect of tumor development. The EVs miR-3174 derived from Hep-3B and HCC-LM3 cell lines has been reported to promote HCC progression and metastasis by targeting and inhibiting the HIPK3/p53 and HIPK3/Fas signaling pathways to induce angiogenesis and enhance vascular permeability. Up-regulation of HIPK3 or GW4869 reverses this process, which may provide a new potential target for targeted therapy [33]. In another study, miR-23a-5p carried by EVs in HepG2 and SKHep-1 cells was shown to negatively target PRDX2 to inhibit HCC cell proliferation and angiogenesis [34]. Therefore, inhibition of miR-23a-5p or upregulation of PRDX2 may provide new therapeutic ideas. Recent studies have found that hepatitis B core antigen (HBc) can modulate the release of EVs [35]. Studies found that in the HBV transfected HepG2.2.15 cell line, HBc can upregulate the content of miR-135a-5p within EVs, miR-135a-5p can block DOX induced apoptosis of receptor cells by downregulating VAMP2, which enhances the chemotherapy resistance of HCC. From this, it can be proposed that miR-135a-5p may serve as a potential therapeutic target for delaying the development of HCC and improving its chemotherapy resistance [14]. Exosomes enriched with circUPF2 enhance the formation of the circUPF2-IGF2BP2-SLC7A11 ternary complex by acting as a scaffold for circUPF2, which helps stabilize SLC7A11 mRNA, promotes SLC7A11 expression, and enhances the function of system Xc- in hepatocellular carcinoma cells, thereby reducing sensitivity to

ferroptosis and resistance to sorafenib [36]. This provides new ideas for the problem of Sorafenib resistance. Research has found that circTMEM181 is highly expressed in HCC patients who respond poorly to PD1 therapy and in those with poor prognosis after surgery, and it is also highly expressed in exosomes. Exosomal circTMEM181 sponges miR-488-3p and upregulates CD39 expression in macrophages, affecting the eATP-adenosine pathway, favoring an immunosuppressive microenvironment, and conferring resistance to PD1 in HCC [37]. This indicates that targeting macrophage CD39 is a potential therapeutic strategy to reverse anti-PD1 resistance in HCC. Currently, several clinical trials targeting CD39 are ongoing (ClinicalTrials.gov identifiers: NCT03884556, NCT04261075, NCT04336098). Research has found that the long non-coding RNA Lnc-CCNH-8 is highly expressed in HCC and is associated with poor prognosis. Mechanistically, up-regulated Lnc-CCNH-8 can sponge miR-217 to regulate PD-L1 expression and also stabilize PD-L1 through the miR-3173/PKP3 axis. This discovery reveals a novel mechanism of PD-L1 regulation in HCC, and exosomal Lnc-CCNH-8 can serve as a predictive marker for immunotherapy response in HCC [38].It was found that CircDCAF8 is upregulated in HCC tissues and cell lines and is associated with poor prognosis in HCC patients. CircDCAF8 is also upregulated in regorafenib-resistant HCC cells, and exosome-mediated circDCAF8 transfer promotes angiogenesis in HUVECs by sponging miR-217 and upregulating NAP1L1 expression. Additionally, exosomes may transfer circDCAF8 from regorafenib-resistant HCC cells to sensitive cells, conferring a resistant phenotype [39]. Circ_0032704 is overexpressed in sorafenib-resistant hepatocellular carcinoma (HCC) tissues and cells, and positively regulates PD-L1 expression by targeting miR-514a-3p, thereby promoting tumor growth. Circ_0032704 can be transported by exosomes, and exosomal circ_0032704 serves as a biomarker for HCC-resistant diagnosis. Knockdown of circ_0032704 in Huh-7/SR and SK-HEP-1/SR cells decreases PD-L1 expression, subsequently reducing sorafenib resistance, inhibiting cell malignant phenotypes, and promoting CD8+ T-cell-induced cytotoxicity [40]. The resistance of hepatocellular carcinoma is a multifaceted and multilevel complex process involving various aspects such as genes, signaling pathways, drug metabolism, cell cycle and apoptosis regulation, and the microenvironment. EVs, as crucial mediators of intercellular communication, play a significant role in HCC drug resistance by transferring drug-resistant genes and proteins, mediating immune suppression and angiogenesis, and regulating intercellular communication, thereby promoting tumor drug resistance. Therefore, investigating the role of EVs in HCC drug resistance and developing therapeutic strategies targeting EVs may provide new insights and approaches to overcoming HCC drug resistance.

4. Discussion

As one of the five most common cancers in the world, liver cancer has a high incidence rate and mortality, increasing the global burden [41]. Hepatocellular carcinoma is the primary form of liver cancer, comprising approximately 75% of all cases [42]. Chronic infection with hepatitis B virus is a high-risk factor for hepatocellular carcinoma, and HBV related liver cancer accounts for approximately 80% of cases [43]. Therefore, it is necessary to pay attention to this. With the continuous recognition of EVs, more and more evidences suggest that small molecule substances such as RNAs, proteins, lipids and viruses paticles can be transported and spread between cells through tumor derived EVs, and can even affect some parts of the immune systems [44].In the process of hepatitis B virus induced hepatocellular carcinoma, extracellular vesicles have a promoting effect on tumor growth. At the same time, in order to evade the host's immune response, the virus loads its viral components and certain gene encoded proteins into the extracellular vesicles to promote the replication of HBV and the progress of HBV-related diseases. In addition, EVs can promote tumor progression by promoting tumor angiogenesis.The expression of specific molecules such as miRNA and IncRNA in EVs derived from HCC cells induced by HBV infection is different from that of non-hepatocellular carcinoma cells. Recent discoveries of circular RNA may also offer fresh perspectives on the early detection of HCC. Additionally, proteins like G3BP may distinguish between diagnosing HCC and similar disorders and can predict the onset of HCC early. Therefore, EVs have bright prospects to be applied to the auxiliary diagnosis of HCC. At present, the metastasis, recurrence, and drug resistance of liver cancer remain a major challenge in the treatment of patients with advanced tumors. As of now, the US Food and Drug Administration (FDA) has approved three different molecules of ICIs for tumor treatment, but due to individual differences and other reasons, many patients have developed resistance to this. With the continuous discovery of mechanisms related to EVs in tumor progression, it may provide new therapeutic targets. For example, substances carried by EVs secreted by tumor tissue can affect tumor growth and immune escape by upregulating or downregulating the content of related targeted proteins. At the same time, they can also affect related immune cells by directly or indirectly acting on the surrounding immune environment, such as upregulating miR-142-3P, increasing M1 macrophage cell death, or targeting TIM-3. These findings can provide a theoretical basis for future intervention and treatment. Specific antigens can be labeled on the outer membrane of EVs to induce specific immune responses and achieve anti-tumor effects. Furthermore, due to the stable nature of EVs, they can be artificially synthesized in vitro for drug delivery.However, extraction and purification methods are complex and costly, making them difficult to apply on a large scale

in clinical and practical operations. Many studies on the application of extracellular vesicles are still in the preclinical experimental stage. Therefore, there is still a lot of room for research on extracts and cancer.

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