H-TEX-mediated signaling between hepatocellular carcinoma cells and macrophages and exosome-targeted therapy for hepatocellular carcinoma

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There is increasing evidence for the key role of the immune microenvironment in the occurrence and development of hepatocellular carcinoma. As an important component of the immune microenvironment, the polarization state and function of macrophages determine the maintenance of the immunosuppressive tumor microenvironment. Hepatocellular carcinoma tumor-derived exosomes, as information carriers, regulate the physiological state of cells in the microenvironment and control cancer progression. In this review, we focus on the role of the exosome content in disease outcomes at different stages in the progression of hepatitis B virus/hepatitis C virus-induced hepatocellular carcinoma. We also explore the mechanism by which macrophages contribute to the formation of hepatocellular carcinoma and summarize the regulation of macrophage functions by the heterogeneity of exosome loading in liver cancer. Finally, with the rise of exosome modification in immunotherapy research on hepatocellular carcinoma, we summarize the application prospects of exosome-based targeted drug delivery.

KEYWORDS
exosomes, hepatocellular carcinoma, liver cancer, hypoxia, TAM, macrophage, therapy, drug resistance
Introduction

Liver cancer has a 5-year relative survival rate of only 20% (1). Liver cancer caused by chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) accounts for approximately 50%–80% of cases (2). Other risk factors include aflatoxin exposure, tobacco and alcohol use, non-alcoholic fatty liver disease, obesity, and diabetes. The distribution of these risk factors varies according to the population, time, and region (1, 3). Therefore, as liver cancer is a chronic inflammation-related cancer, it is crucial to study the role of exosomes during disease progression and in the immune microenvironment.

Macrophages are abundant in the liver and are essential cells in the tumor microenvironment (TME) in liver cancer. During the initial stages of liver cancer at the time of niche formation, hepatic macrophages display an inflammatory phenotype, namely, the M1 type; these cells damage neighboring cells by continuous secretion of reactive oxygen species. In solid tumors that successfully escape immune surveillance, macrophages disproportionately differentiate into the M2 phenotype with anti-inflammatory activity, that is, tumor-associated macrophages (TAMs), which have proangiogenic, matrix remodeling, distal metastasis, and immunosuppressive effects (4). The recruitment of hepatic macrophages in human liver cancer is correlated with disease progression and a poor prognosis (5). Hepatoma cells also play crucial regulatory roles in macrophage proliferation and differentiation during tumor progression (6).

Hypoxia has become one of the most intensively studied features of the TME (7). In this context, in addition to directly secreting cytokines (8), exosome-mediated communication between tumor cells and the stroma is considered an important step in remodeling the TME (9). Multiple studies have demonstrated the adaptive tuning of extracellular vesicle (EV) secretion and contents of liver cancer cells during progression, providing a basis for subsequent remodeling of the surrounding niche (10, 11) and, specifically, for altering TAMs.

In this review, we summarize the roles of exosomes and macrophages during the progression of viral hepatitis to liver cancer, including but not limited to the effects of exosomal contents on macrophages and exosome-based therapeutic prospects for liver cancer.

Involvement of exosomes in hepatocarcinogenesis

Analyses of liver cancer progression associated with chronic inflammation are still needed. Hepatocyte exosomes function as messengers in the formation and evolution of the liver cancer niche (12). Exosomes are released into the intercellular space or enter the hepatic microvasculature to participate in intercellular signal communication or material transport, thereby regulating the pathophysiological state of the liver (13).

In the physiological state, liver parenchymal cells, which make up approximately 80% of the liver volume, secrete exosomes loaded with neutral ceramidase and sphingosine kinase 2 (SK2), which are recognized by recipient hepatocytes and upregulate sphingosine-1-phosphate (SIP) production by target cells, thereby promoting hepatocyte repair and regeneration (14).

In some pathological conditions, liver parenchymal cells, hepatic stellate cells (HSCs), and Kupffer cells (KCs) are the main donor and recipient cells of exosomes and are associated with hepatitis, cirrhosis, and liver cancer (15). The hepatocyte exosomal cargo plays diverse roles in the microenvironment during liver cancer progression (Figure 1).

Viral Hepatitis

Hepatitis caused by HBV/HCV infection is one of the main causes of hepatocellular carcinoma (HCC) (2). Exosomes secreted from hepatocytes infected with HCV carry virus-derived Ago2, heat shock protein 90 (Hsp90), and miR-122, which mediate the stable transmission of HCV in the liver (16–18). Exosome-mediated viral transport helps the virus evade immune system surveillance. MicroRNAs (miRNAs) released from virus-infected hepatocytes inhibit natural killer (NK) cell proliferation and survival and facilitate the evasion of host innate immunity (19). Exosomes containing HCV RNA reduce Toll-like receptor 3 (TLR3) activation and interfere with antiviral interferon-stimulated gene activation (20). T-cell immunoglobulin and mucin domain-containing molecule 3 (TIM-3)/galectin 9 (Gal-9) in exosomes increase HCV-infected hepatocytes, affect monocyte differentiation, and inhibit the immune response (21).

Cirrhosis

During liver cirrhosis, HSCs along with other cells of the liver parenchyma (liver sinusoidal endothelial cells and KCs) play important roles in the development and progression of liver fibrosis (22). Exosomes released by injured hepatocytes are internalized by stellate cells, leading to phenotypic switching of quiescent stellate cells. HSC activation is a major driver of the initiation, progression, and resolution of liver fibrosis (23).

Exosomes released from injured hepatic stellate structures contain abundant fibrotic components that promote fibrosis via multiple pathways, such as by stimulating fibroblasts and myofibroblasts to produce collagen from the bone marrow and portal fibrocytes. Connective tissue growth factor (CTGF), a multifunctional heparin-binding glycoprotein, contributes to the promotion of multiple fibrotic processes (24). CTGF, which is widely expressed in activated HSC-derived exosomes, regulates the activation and migration of HSCs and immune responses, whereas exosomes produced by quiescent HSCs are enriched in
miR-214 and twist, attenuating the profibrotic function of activated HSCs (25, 26). Exosomes derived from liver sinusoidal endothelial cells regulate the migratory capacity of HSCs via adhesion.

**Hepatocellular Carcinoma**

HCC is a common malignancy with poor overall survival. The main risk factors for HCC include viral hepatitis, excessive alcohol consumption, and smoking. However, the pathogenesis of HCC is complicated and difficult to determine. Extensive evidence suggests that exosomes derived from cells carry tumor-specific markers, which mediate intercellular communication between cancer cell populations and promote the migration and invasion of recipient cells (27). For non-immune cells, HCC exosomes regulate tumor niche formation by promoting tumor-associated fibroblast transformation and angiogenesis by altering the endothelial vascular phenotype (28, 29). Liver cancer exosomes mainly mediate tumor cell immune escape by inhibiting their maintenance and proliferation, promoting phenotypic transformation, and blocking functional activation (30).

These effects promoting HCC progression depend on proteins and non-coding RNAs (ncRNAs) in exosomes. They are transferred by exosomes and participate in the communication between HCC cells and targeted cells in the TME, thereby affecting tumor angiogenesis, metastasis, and drug and radiotherapy resistance. Therefore, we summarized the current research status of proteins and ncRNAs in HCC exosomes to further emphasize the potential value of these abnormally expressed exosome molecules in HCC as biomarkers for the diagnosis, prognosis, and treatment of HCC (Table 1).

**Hypoxia promotes the production and release of exosomes**

HCC is a hypermetabolic tumor of the digestive system. Based on the high rate of cell proliferation, the altered blood supply system participates in the exchange of substances within the tumor (66). Therefore, hypoxic signals contribute to liver cancer formation, proliferation, and metastasis (67). In addition to the adaptive changes in cellular components within the TME in response to hypoxia, hepatoma cells transmit post-hypoxic regulatory signals to other cells by secreting EVs (68). Cancer cells with different
| Contents | Mechanism | Function | References |
|----------|-----------|----------|------------|
| **Proteins** | | | |
| LOXL4 | Activation of FAK/SRC pathway alters cell matrix adhesion and migration ability | Promotes migration and angiogenesis | (31) |
| GOLM1 | Activated glycogen synthase kinase-3 β / MMPs (GSK-3 β/ MMPs) of the recipient cells signaling axis | Accelerates cell proliferation and migration | (32) |
| S100A4 | Activation of OPN transcription by STAT3 phosphorylation | Promotes tumor metastasis | (33) |
| HMGB1 | Activation of the TLR-MAPK pathway | Promotes TIM-1(+) B-cell proliferation and inhibits CD8(+) T-cell activity | (34) |
| SMAD3 | Enhanced TGF-β-Smad3-ROS signaling | Promotes proliferation and adhesion | (35) |
| ENO1 | Upregulation of integrin α6β4 expression | Activates the FAK/Sc-p38MAPK pathway to promote the growth and metastasis of HCC cells | (36) |
| CLEC3B | Promotes the phosphorylation of AMPK, thereby decreasing the expression of VEGF | Attenuates migration and invasion of recipient cells and relieves angiogenesis | (37) |
| CHD3L1 | Activation of MAPK and Akt signaling pathways | Promotes tumor metastasis | (38) |
| EIF3C | Activation of S100A11 expression | Promotes angiogenesis and tumor development | (39) |
| **miRNAs** | | | |
| miR150 | Promotes vascular endothelial growth factor (VEGF) secretion in TAMs | Promotes tumorigenesis | (40) |
| miR-23a-3p | Upregulation of PD-L1 expression in macrophages via STAT3 signaling pathway | Attenuates the anti-HCC immune response | (41) |
| miR-32-5p | Inhibits PTEN and activates the PI3K/Akt pathway | Induction of multidrug resistance by angiogenesis and EMT | (42) |
| miR-1247-3p | Downregulation of B4GALT3 and activation β1-integrin/NF-κB axis | Promotes tumor status, EMT, chemoresistance, tumorigenicity, and metastasis | (43) |
| miR-638 | By downregulating the expression of VE-cadherin and ZO-1 in endothelial cells | Promotes vascular permeability | (44) |
| miR-27a-3p | By regulating thioedoxin-interacting protein (TXNIP) | Promotes the stemness of liver cancer | (45) |
| miR-125b | Disrupted TGF-β1-induced epithelial-mesenchymal transition and TGF-β1/SMAD signaling pathway | Antimetastatic effect | (46) |
| miR-15a-5p | Inhibits PDI expression in CD8+ T cells | Inhibits the development of HCC | (47) |
| miR-210 | Entry into endothelial cells inhibits SMAD4 and STAT6 | Promotes tumor angioinvasion | (48) |
| miR-93 | Inhibits CDKN1A, TP53INP1, and TIMP2 | Promotes proliferation and invasion | (49) |
| miR-374a-5p | Possibly by regulating GADD45A | Promotes proliferation, migration, and invasion of HCC cells | (50) |
| miR-92a-3p | By inhibiting PTEN and activating the Akt/Snail signaling pathway | Promotes EMT | (51) |
| miR-320a | Inhibits PRX3/ERK1/2/CDK2 axis | Inhibits proliferation and metastatic ability | (52) |
| miR-21 | Inhibits PTEN, upregulate PDK1/AKT pathway | Transforms normal hematopoietic stem cells into cancer-associated fibroblasts | (53) |
| miR-451a | Targeting LPIN1 regulates tumor cell apoptosis and angiogenesis | Inhibits hepatocellular tumorigenesis | (54) |
| **lncRNAs** | | | |
| TUC339 | May be involved in cytokine receptor signaling pathway and CXCR chemokine receptor-binding pathway | Promotes macrophage polarization to M2 (IL-4) phenotype | (55) |
| IncRNA H19 | By upregulating the miR-520a-3p/LIMK1 axis | Promotes the proliferation, migration, and invasion of HCC cells after propofol treatment and inhibits the apoptosis of HCC cells | (56) |
| SENP3-EIF4A1 | Regulation of ZFP36 expression by competitive binding to miR-9-5p | Able to inhibit tumor growth in vivo | (57) |
| FAL1 | Upregulation of ZEB1 and AFP by inhibiting miR-1236 | Promotes proliferation and migration | (58) |
| ASMTL-AS1 | by activating the YAP signaling pathway | Accelerates tumor progression | (59) |
| **circRNAs** | | | |

(Continued)
phenotypes communicate via exosomes to complete the phenotypic transformation and promote the progression of liver cancer (69). For example, exosomes from highly metastatic MHCC97H cells can communicate with less metastatic HCC cells, increasing their migration, chemotaxis, and invasion (70). Similarly, the EVs of cisplatin-resistant non-small-cell lung cancer cell lines secreted pyruvate kinase M2 (PKM2) under hypoxic conditions. The phagocytosis of these EVs by cisplatin-sensitive cells, increasing their migration, chemotaxis, and invasion (70).

The adaptive response of tumor cells to hypoxia is mostly regulated by hypoxia-inducible factor 1 (HIF1). HIF1α/2α is also highly expressed in liver cancer (72). Under normoxia, the two proline residues of the HIF-1α/2α subunit are hydroxylated by prolyl hydroxylase domain (PHD) enzymes, promoting binding to von Hippel–Lindau (VHL), which mediates the degradation of the hydroxylated HIF-1α/2α subunit via the ubiquitin-proteasome pathway. However, under hypoxic conditions, the generation and release of EVs are regulated by HIF. During EV biogenesis, RAS superfamily proteins (RABs) are involved in the formation and fusion of membrane buds, and HIF can directly affect the RAS. That is, under hypoxic conditions, HIF is activated to promote the transcription of RABs and finally promote the generation and secretion of exosomes (73, 74). HIF can promote the expression and activation of a series of cell surface receptors, such as epidermal growth factor receptor, glucose transporter receptor, and transferrin receptor, and promote cell internalization and endocytosis (75).

The mechanism by which EV contents (nucleic acids, proteins, etc.) are specifically sorted under hypoxia remains unclear. This process is related to endosomal sorting complex required for transport (ESCRT) complexes and ceramides and may be related to posttranslational processes (76). This modified protein complex is closely related to ubiquitin-like 3 (UBL3)/membrane-anchored Ub-fold protein (MUB). In models of lung injury, proteins and peptides in vesicles were more ubiquitinated than non-hypoxic conditions (77). This indicates that ubiquitination regulates the loading process of exosome contents under hypoxia.

In addition, HIF1-independent regulation of adaptive responses to hypoxia has been reported, such as phosphoinositide 3-kinase (PI3K), serine-threonine kinase (AKT), mammalian target of rapamycin (mTOR), Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) Rab-GTPase, Wnt/b-catenin, mitogen-activated protein kinases, and oxidative stress (78).

Hepatocellular Carcinoma (HCC)-associated macrophages

Origin and function of macrophages

Under physiological conditions, the liver has a rich blood supply and abundant innate immune cells (such as KCs, NK cells, and T cells). Resident macrophages in the liver are mainly composed of KCs and monocyte-derived macrophages (79). In healthy liver, KCs are the main resident hepatic macrophages. KCs are generally believed to have originated from yolk sac-derived colony-stimulating factor 1 receptor (CSF1R) + erythroid/myeloid progenitors (EMPs), which are present in the fetal liver during embryogenesis. They can maintain liver homeostasis by removing metabolic waste and cell debris, regulating cholesterol homeostasis, maintaining iron homeostasis and iron cycling, mediating immune responses, and promoting immune tolerance (80). Some
circulation-derived monocyte-macrophage populations recognize liver-invading bacteria and recruit neutrophils. In the human liver, hepatic macrophages consist of CD68+macrophage receptor with collagenous structure (MARCO)+KCs, CD68+MARCO-macrophages, and CD14+monocytes. CD68+MARCO+KCs usually overexpress immune tolerance-related genes and have anti-inflammatory effects, and CD68+MARCO-macrophages and CD14+monocytes have pro-inflammatory effects (5).

From the progression of chronic hepatitis to fully developed tumors, there is a high degree of heterogeneity in the intratumoral microenvironment, with a highly invasive anterior and middle hypoxic and necrotic areas and tumor cells with high and low proliferation (81). There are also different TAM phenotypes in liver cancer, and research on the classification of TAMs and their heterogeneity is still in its infancy (82). In short, TAMs are collections of macrophages, including infiltrating and resident macrophages, originating from various cellular sources. TAM polarization shows plasticity, and cells can exhibit either phenotype. Several studies have provided evidence that the acquisition of an M2-like polarized macrophage phenotype by TAMs promotes tumor progression by promoting angiogenesis, immunosuppression, and growth factor secretion, ultimately leading to metastasis (83).

TAMs secrete excessive proangiogenic factors [e.g., vascular endothelial growth factor (VEGF)], platelet-derived growth factor, and transforming growth factor beta (TGFβ) and cell proliferation-stimulating factors (e.g., Interleukin(IL)-1β, IL-6, chemokine (C-C motif) ligand 2 (CCL2), tumor necrosis factor, and VEGF), which strongly promote tumor growth and development (84, 85).

Mechanisms underlying macrophage uptake of exosomes

When exosomal vesicles come in contact with the surface of macrophages, they trigger a functional response (e.g., proliferation and differentiation) via membrane surface ligand–receptor recognition signals and/or transport of their contents into the cell, antigen presentation, etc. (86).

Macrophages are initially recognized by protein receptors and adhesion molecules (e.g., tetraspanins, integrins, proteoglycans, and lectins) on the exosome surface. Exosomes are then taken up by activating cell membrane-expressed receptors, fusion with the macrophage plasma membrane, or endocytosis (87). The final contents are delivered to macrophages to exert biological functions. An increasing number of studies have evaluated the mechanism underlying the uptake. For example, exosomes derived from pancreatic cancer preferentially bind to F4/80+ and CD11b+ KCs in the liver, which is promoted by intercellular adhesion molecules and CD11b ligands (88). A study of the liver metastasis of pancreatic cancer cells suggested that endoplasmic reticulum aminopeptidase 1 (ERAP1)-secreting exosomes enhance the phagocytic capacity and NO synthesis activity of macrophages (89). However, some circulating exosomes can protect against phagocytosis by macrophage CD47 enrichment.

The effect of exosomes from non-metastatic K7 and Dunn osteosarcoma cells and the metastatic sublines K7M3 and DLM8 on macrophage phagocytosis was evaluated in a study of osteosarcoma lung metastasis (85). Exosomes secreted by the highly metastatic K7M3 and DLM8 cell lines were incubated with MHS mouse alveolar macrophages, which induced the mRNA expression of IL-10, TGFB2, and CCL22 (markers of M2 macrophages). Reduced macrophage phagocytosis, exocytosis, and macrophage-mediated tumor cell killing were also observed. By contrast, exosomes from non-metastatic K7 or Dunn cells failed to inhibit macrophage phagocytosis, exocytosis, and cytotoxicity and did not induce increases in the mRNA expression of IL10, TGFB2, or CCL22.

The uptake of exosomes by macrophages is inseparable from clathrin-dependent endocytosis in which caveolin-1 is essential for the formation of pits (membrane depressions) and accumulates in membrane depressions (90). Clathrin protein heavy chain 1 (Cltc) is encoded by the cltc gene and is highly expressed in macrophages (91). When Cltc1 is knocked out, phagocytosis by monocyte-macrophages is inhibited.

HCC Tumor-Derived exosomes are involved in the regulation of the polarization and function of macrophages

Liver macrophages can be activated to M1 and IL-13 via the classical activation pathway (bacterial lipopolysaccharide and interferon-gamma secreted by Th1 cells) and alternative activation pathways (cytokines IL-4, IL-10, and IL-13 secreted by Th2 cells). There are two subtypes of M2 macrophages, and these can be further subdivided into M2a, M2b, M2c, and M2d. M1-type macrophages mainly secrete pro-inflammatory factors, such as IL-12, IL-6, IL-18, IL-23, and tumor necrosis factor, and increase the expression of nitric oxide synthase, which is responsible for the defense against pathogen infection. The M2 type expresses high levels of IL-10, IL-1a/b inhibitor, mannose receptor (MRCl), arginase 1 (Arg1), and other anti-inflammatory factors. These two polarization modes are classic models for studies of macrophages (92, 93).

Immunity and metabolism are highly integrated and coordinated. In the initial stage of tissue hypoxia, the anaerobic glycolysis and pentose phosphate pathways of M1 macrophages are activated, whereas M2 macrophages mainly use oxidative phosphorylation and aerobic glycolysis to meet the energy requirements for tissue repair and remodeling. M1 macrophages are considered the most likely precursors of tumor-infiltrating macrophages, and TAMs are frequently M2 macrophages (94).

The long non-coding RNA (lncRNA) TUC339 is highly expressed in HCC-derived exosomes, which can be transferred
across HCC cells to promote tumor growth and metastasis (55, 95). Furthermore, the exosomal long non-coding RNA (lncRNA) TUC339 can be transferred to neighboring macrophages to modulate M1/M2 polarization and suppress antitumor immune responses in vitro. Microarray studies have demonstrated that exosomal TUC339 downregulated TLR signaling and Fcγ receptor (FcγR)-mediated phagocytosis pathways in macrophages, and TUC339 knockdown increased the phagocytic activity of macrophages. TUC339 is also involved in cytokine and chemokine receptor signaling, although the exact mechanism is unclear. Tumor cell-derived exosomes also carry miRNAs that regulate the expression of immune response-related genes. miR150 is highly expressed in the plasma of patients with HCC and in HCC-derived exosomes and promotes the growth of vascular endothelial cells by secreting the TAM-derived cytokine factor VEGF (40). VEGF levels are reduced in the plasma and tumor tissues of tumor-bearing mice treated with miR150 inhibitors. HCC exosomal miR-23a-3p upregulates the programmed cell death ligand 1 (PD-L1) expression in macrophages via Signal transducer and activator of transcription 3 (STAT3) signaling, which significantly attenuates melatonin-treated HCC cell-derived exosomes (41). PD-L1 expression in phagocytes has been demonstrated in vivo. HCC-derived exosomes significantly increased CD11b+F4/80+CD206+ macrophages, accompanied by upregulation of M2-specific markers, including C-C chemokine ligand 17 (ccl17), C-C chemokine ligand 22 (ccl22), and arg-1. M2 polarization in vitro and in HCC-bearing mouse models is driven by miR146a, which is directly regulated by the zinc finger transcription factor Sal-like protein-4 (SALL4) in HCC cells (96, 97). The exosomal lncRNA HMMR-AS1 mediates macrophage polarization via the miR-147a/ARID3A axis under hypoxia and affects the progression of HCC (98) (Figure 2).

**Therapeutic prospects related to exosomes in liver cancer cells**

**Role of H-TEXs in liver cancer drug resistance**

In the TME, exosomes act as key regulators of the effects of chemotherapeutics by modulating drug efflux, epithelial–mesenchymal transition (EMT), autophagy phenotype, and immunosuppression (Table 2).

**Exosome-related drug delivery system based on liver cancer therapy**

Additionally, exosomes can assist in the early diagnosis of tumors, monitoring, and prognostic analyses. Because exosomes are endogenous vesicles, they benefit from low immunogenicity,
TABLE 2 The role of H-TEXs in liver cancer drug resistance.

| Donor cells | Contents | Recipient cells | Functions | Mechanism | References |
|-------------|----------|-----------------|-----------|-----------|------------|
| MHCC-97L    | HGF      | SMCC-7721       | Induce sorafenib resistance in vitro and in vivo | HGF/cMET/Akt signaling | (99)       |
| HepG2       | linc-ROR | HepG2           | Induce resistance to doxorubicin and camptothecin | Modulate TGF-β/Caspase3/CD133 signaling | (100)      |
| HepG2       | linc-VLDLR | HepG2 and KBMC | Induce resistance to sorafenib and doxorubicin | Enhance ABCG2 expression | (101)      |
| hepav-6     | Tumor-associated antigen | DCs | Increase sorafenib efficacy with PD-1 antibody | Regulate Treg accumulation via PD-1/PD-L1 pathway | (102)      |
| HBV-infected HepG2 | HRX | HepG2 | Facilitate OXA resistance | Activate CMA pathway | (103)      |
| AMSCs       | miR-199a | HCC cells       | Improve HCC chemosensitivity | nTOR pathway | (104)      |
| HCC cells   | circ-UHRF1 | HCC cells | Anti-PD1 therapy resistance | NK cell dysfunction by upregulating TIM-3 | (62)       |
| HepG2       | circ-SORE | HepG2           | Induce resistance to sorafenib | Stabilize YBX1 | (105)      |

Belhadj et al. (110) described an “eat/don’t-eat” decision switch for macrophages to evade phagocytosis by modifying CD47 outside of EVs. The effectiveness of this switch was verified by Du et al. (111). They engineered an exosome armed with three moieties, surface functionalization with CD47, membrane loading with ferroptosis inducer erastin, and core with photosensitizer RB. The exosomes displayed high delivery efficiency to tumors. Upon irradiation with a 532-nm laser in the tumor region, Erastin (Er) and Rose Bengal (RB) synergistically induced cell death.

Exosome-based immunotherapy in HCC

Cancer immunotherapy reverses the immunosuppressive TME (112). Exosome-targeted immunotherapy of HCC is often associated with dendritic cell (DC)-derived exosomes (DEXs), which have great potential for immunotherapy applications (113, 114). Lu et al. (115) infected a DC cell line (DC2.4), which was established by transfecting Granulocyte-macrophage colony-stimulating factor (GM-CSF) (Csf2), Myc, and Raf genes into C57BL/6 mice, with a lentivirus-expressing murine α-fetoprotein (AFP). They found that DC-AFP-derived exosomes (DEX-AFP) elicited strong antigen-specific immune responses, resulting in significantly delayed tumor growth and prolonged survival in various HCC mouse models (115). Zuo et al. (116) also used DEX as a carrier for a liver cancer vaccine to initiate a specific immune response against HCC. They decorated DEX with an HCC-targeting peptide (P47-P), an AFP epitope (AFP212-A2), and a functional domain of high-mobility group nucleosome-binding protein 1 (N1ND-N) and demonstrated its potential for the individualized treatment of HCC via universal DEX vaccines.
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its immunostimulatory proper ties are easily manipulated

strategy. DEX acts as a stable vesicle with a long shelf life, and

challenges, DEX remains a pr omising immunotherapeutic

expanded the use of DEX-based cancer treatments in clinical

(119). Research advancements have

TEX and a programmed cell death protein 1 (PD-1) antibody

that liver cancer-derived exosomes elicited
durable large-scale antitumor immunity in mouse liver
tumors. Shi et al. (102) showed that the combination of DC-

HCC (118) demonstrated that alarmin -coated exosomes elicited

discussed. Due to this, the treatments of HCC are often aimed at

antibody (Ab) enhances the efficacy of sorafenib.

However, immunotherapy clinical trials have shown that

substantial work is still needed before these findings can be

applied to the treatment of cancer in clinical settings. Despite the

challenges, DEX remains a promising immunotherapeutic

strategy. DEX acts as a stable vesicle with a long shelf life, and

its immunostimulatory properties are easily manipulated

(through donor DCs). Research advancements have

expanded the use of DEX-based cancer treatments in clinical

settings (119).


discussion

In this review, we evaluated the important role of exosomes in HCC progression and immunotherapy. Given that TAMs are a key component of the microenvironment, we summarize the regulatory mechanism by which liver cancer-derived exosomes regulate macrophage polarization, demonstrating that exosomes are a promising tool to target macrophages for HCC immunotherapy.

It should be emphasized that the interaction between the tumor and immunity is dynamic, heterogeneous, and bidirectional, including the immune response to drugs or external stimuli. Furthermore, the tumor cell state and even the genome are altered (120). Even so, the regulatory function of exosomes as messengers cannot be ignored, especially in the treatment of HCC. Chemotherapy resistance has become a major obstacle in improving the prognosis of patients. Targeting exosomes could be a promising strategy for reversing drug tolerance. In addition, the improvement of the efficacy of chemotherapy in patients with HCC by exosome anticancer drug delivery provides a new perspective for clinical treatment.

author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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