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Review Article

Exosomes as mediators of tumor immune escape and immunotherapy in hepatocellular carcinoma☆

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ABSTRACT

Hepatocellular carcinoma (HCC), a typical inflammatory-related cancer, mainly occurs in patients with chronic liver diseases. Moreover, the liver is an immunologically privileged apparatus with multiple immunosuppressive cell groups. The long process of inflammation-mediated carcinogenesis turns the HCC tumor microenvironment (TME) into one with strong immunosuppression, facilitating the immune escape of HCC cells. Accumulated data have manifested that tumor-associated cell-derived exosomes carry diverse molecular cargoes (*e.g.*, proteins and nucleic acids) for mediating cell-to-cell communication and are implicated in TME remodeling to promote tumor-infiltrating immune cell reprogramming, ultimately creating a tumor-friendly microenvironment. Characterized by several intrinsic attributes, such as good stability (bilayer-like structure) and high biocompatibility (cell secretion), exosomes can be modified or engineered as nanocarriers to deliver tumor-specific antigens or antitumor drugs to targeted cells or organs, thus effectively triggering the HCC cell elimination by the immune system. This review aimed to highlight the pivotal role of exosomes in regulating immune escape mechanisms in HCC and recent advances in exosome-mediated immunotherapy for HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is a typical inflammatory-related cancer that most commonly occurs with chronic hepatitis.[1,2] During the inflammation-mediated carcinogenesis process, various inflammatory cells interact with each other, creating an immunosuppressive tumor microenvironment (TME), which is beneficial for HCC cells to initiate an immune escape by eluding the surveillance and attack of the immune system.[3] Different from other organs, the liver is an immunologically privileged apparatus with special immunosuppressive cell groups that can protect the liver from autoimmune damage and chronic inflammation under physiological conditions.[4] Hence, HCC yields a special microenvironment with more potent immunosuppression as compared with other tumors; a wide range of cell groups and varied regulatory mechanisms are conducive to driving an immune escape of HCC. However, the mechanism of activating an immune escape in HCC remains unclear.

Following an in-depth investigation in recent years, accumulated data have demonstrated that exosomes derived from tumor-associated cells can be involved in TME remodeling to promote the reprogramming of tumor-infiltrating immune cells, thus creating a tumor-friendly microenvironment.[5–7] Exosomes, a group of small extracellular vesicles ranging from 30 to 150 nm in size, are manufactured and released by the vast majority of cell types (*e.g.*, epithelial cells, various immune cells, and cancer cells) and ubiquitous in human body fluids.[8,9] Multiple cargoes are capsulized in

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exosomes, including deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), proteins, lipids, and metabolites, which enable to estimate the cell's characteristics and status.[10] Physiologically and pathologically, exosomes facilitate cell-to-cell communication, characterized by intrinsic attributes, such as good stability (bilayer-like structure) and high biocompatibility (cell secretion).[11] Given these unique peculiarities, exosomes can be modified or engineered as nanocarriers to deliver tumor-specific antigens or antitumor drugs to the targeted cells or organs, the deemed promising therapy to effectively trigger the antitumor immune responses.[12,13]

Therefore, this review aimed to accentuate the pivotal role of exosomes in regulating the immune escape mechanisms in HCC and assess how patients with HCC can benefit from using exosomes to trigger the elimination of HCC cells by the immune cells (Fig. 1).

2. The exosome biogenesis

The exosome is typically synthesized by a multistep procedure involving (i) inward budding of the cytoplasmic membrane, (ii) the endosome manufacture, (iii) the shaping of intracellular multivesicular bodies (MVBs), and (iv) the initiation of exocytosis (Fig. 2).[14] First, early endosomes are produced following the cell membrane endocytosis;[15] apart from a preexisting early endosome, an endoplasmic reticulum (ER) or a trans-Golgi network is also involved in the early endosome formation and content.[16–19] These endosomes mature into late endosomes, forming MVBs containing intraluminal vesicles (ILVs). Ultimately, the MVBs can be degraded inside the cell during the identification and ingested by lysosomes or autophagosomes; or following the MVB fusion with the plasma membrane, ILVs will be secreted into the intercellular space as exosomes.[10,17,20]

Generally, the predominant mechanism of exosome production is through the endosomal sorting complex required for transport (ESCRT)-dependent and ESCRT-independent pathways.[15] An essential part of exosome biogenesis is the interaction between ESCRT complexes and certain accessory protein molecules (*e.g.*, apoptosis-linked gene 2-interacting protein X (ALIX), syntenins, syndecans).[21–23] Moreover, their formation and secretion can occur independent of the ESCRT but involve neural sphingomyelinase 2 (nSMase 2),[24] tetraspanin family (*e.g.*, CD9, CD63, or CD81),[25,26] and RAS-related protein (*e.g.*, RAB31),[27] among others.[28]

3. Exosome remodeling the immune microenvironment in HCC

A tumor's growth is commonly fostered by the frequent communication between abundant cancer cells and diverse immune cells in the TME. Increasing evidence has demonstrated that molecular signals delivered by exosomes in TME are capable of triggering tumor immune escape mechanisms and immune microenvironment reprogramming, thereby resulting in uncontrolled tumor progression (Fig. 3).[29,30]

3.1. Exosome-mediated delivery of cargoes between macrophages/monocytes and HCC cells

Macrophages, a group of phagocytic immune cells, exert a significant role in TME; tumor-associated macrophages (TAMs) are generally defined to infiltrate the neoplasm and play a “double-edged sword” role in HCC occurrence and development, polarizing the M1 type with immunologic surveillance effects at the early cancer stages and the M2 type with tumor promotion effects at later stages.[31,32] Reportedly, exosomal microRNA (miRNA)-326 released by M1 macrophages inhibited the proliferation, migration, and invasion and promoted HCC apoptosis by reducing the expression of nuclear factor-kappa B (NF-κB).[33] Conversely, M2 macrophage-derived exosomes loading miRNA-660-5p contributed to HCC progression by downregulating the Kruppel-like factor 3.[34] Furthermore, exosomal miRNA-27a-3p from M2 macrophages fostered cancer stemness in HCC by downregulating thioredoxin-interacting protein.[35] Previous studies reported that HCC may be initiated and partly progressed by androgen receptor (AR); Liu *et al.*[36] revealed that TAM-derived exosomal miRNA-92a-2-5p targeting the 3′-untranslated region of AR messenger RNA could restrain the AR translation, thus triggering the regulation of PH domain leucine-rich repeat protein phosphatase/AKT/beta (β)-catenin pathway to allow a more readily HCC invasion. In macrophages, their activation and plasticity can be regulated by a transcriptional regulator known as recombination signal binding protein-Jkappa. TAMs overexpressing this transcriptional regulator were found to suppress HCC development through exosomal hsa_circ_0004658 by targeting miRNA-499b-5p/junctional adhesion molecule 3.[37]

Under normal physiological conditions, immune checkpoints, such as the programmed death-1 (PD-1) and its ligand (PD-L1), keep the immune response by controlling the prevention of autoimmunity and upregulation of immune checkpoints; however, tumor immune escape facilitates the TME.[38] A recent study has reported that the tumor-derived exosomal PD-L1 transport into TAMs regulated by Golgi membrane protein 1 yielded suppressive effects on $CD8^+$ T cells in HCC.[39] With the absence of underlying mechanisms on ER stress leading to tumor cells' escape in immune surveillance, another research team discovered that the PD-L1 expression in macrophages was upregulated by exosomal miRNA-23a-3p originating from HCC cells exposed to the ER stress, which subsequently inhibited the antitumoral T-cell functions via

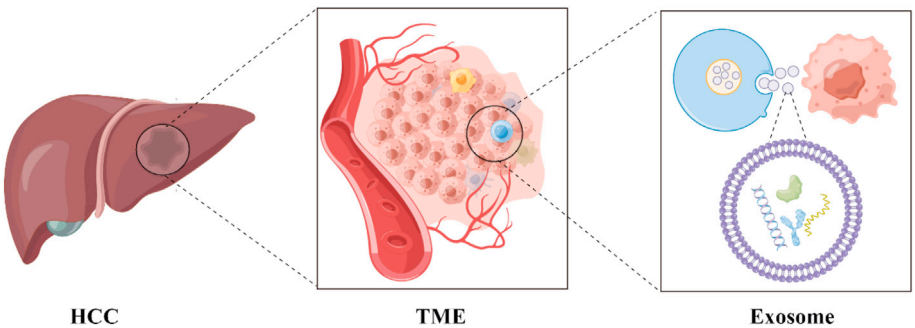

Fig. 1. Immunosuppressive tumor microenvironment (TME) in hepatocellular carcinoma (HCC). HCC, a typical inflammatory-related malignancy, most commonly occurs with chronic hepatitis. During the inflammation-mediated carcinogenesis process, exosomes derived from tumors or immune cells mediate the cell-to-cell communication and are implicated in TME remodeling to promote the reprogramming of tumor-infiltrating immune cells, thus creating a tumor-friendly microenvironment.

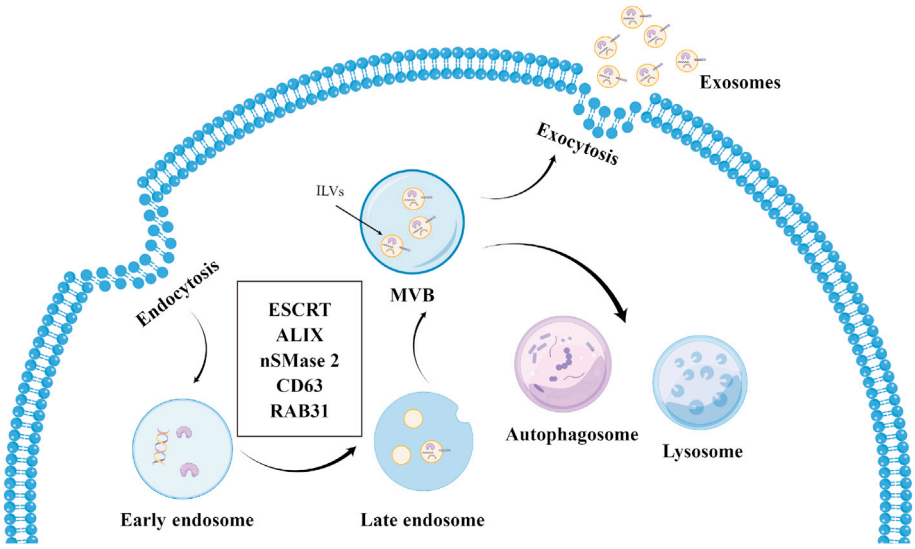

Fig. 2. The biogenesis of exosome. First, early endosomes are produced with the endocytosis of the cytoplasmic membrane; these endosomes then mature into late ones, followed by the MVB formation containing ILVs. Ultimately, the MVBs can be degraded inside the cell during the identification and ingested by lysosomes or autophagosomes, or following the MVB fusion with the cell membrane, ILVs are secreted into the intercellular space as exosomes. Generally, several molecules (*e.g.*, ESCRT complexes, ALIX, syntenins, syndecans, nSMase 2, tetraspanin CD63, and RAB 31) are involved in exosome biogenesis. Abbreviations: ALIX, apoptosis-linked gene 2-interacting protein X; ESCRT, endosomal sorting complex required for transport; ILVs, intraluminal vesicles; MVB, multivesicular body; nSMase 2, neural sphingomyelinase 2; RAB31, RAS-related protein 31.

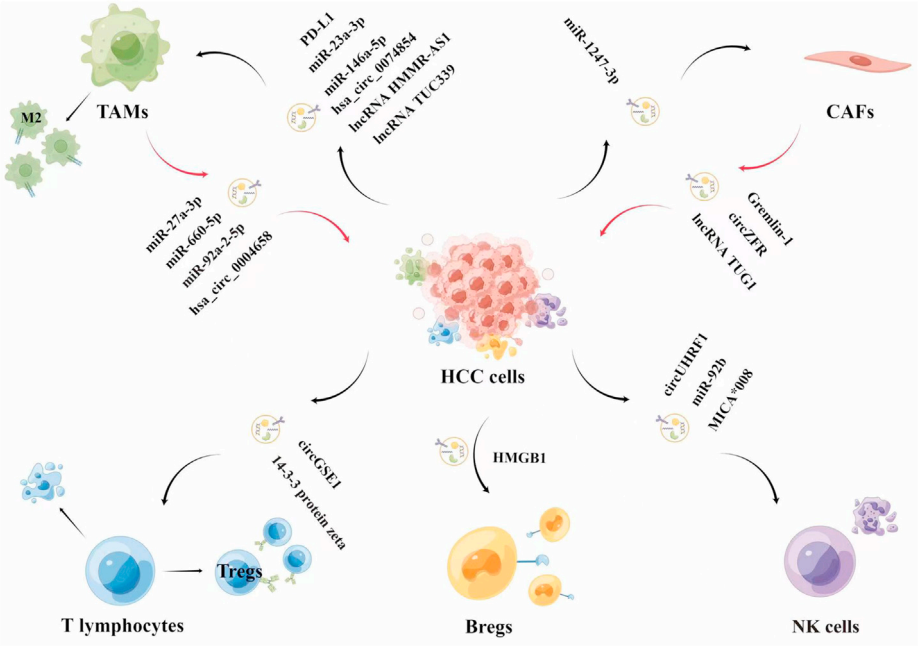

Fig. 3. Exosome-mediated delivery of cargoes between cells remodeling the immune microenvironment in HCC. A tumor's growth is fostered by the frequent communication between cancer cells and various immune cells in the TME. Molecular signals delivered by exosomes in TME are capable of triggering tumor immune escape mechanisms and immune microenvironment reprogramming, thereby resulting in uncontrolled HCC progression. Black arrows indicate the delivery of cargoes from HCC cells to immune-related cells via exosomes, inducing the immunocyte apoptosis or inactivation or promoting the cell conversion or expansion with immunosuppressive function; conversely, red arrows represent the delivery of cargoes from immune-related cells to HCC cells via exosomes, facilitating tumor progression. Abbreviations: Bregs, regulatory B cells; CAFs, cancer-associated fibroblasts; HCC, hepatocellular carcinoma; HMGB1, high mobility group box 1; MICA, major histocompatibility complex class I chain-related protein A; NK, natural killer; PD-L1, programmed death-ligand 1; TAMs, tumor-associated macrophages; Tregs, regulatory T cells; TUG1, taurine-upregulated gene 1.

the phosphatase and tensin homolog (PTEN)/AKT1 pathway.[40] Notably, macrophages can be remodeled into M2-polarized TAMs by HCC via exosomes loaded with cargoes (*e.g.*, miRNA-146a-5p); furthermore, T-cell exhaustion was driven by exosomal miRNA-146a-5p from HCC with high Sal-like protein-4 expression.[41] Similarly, exosomes can promote the macrophage M2 polarization by converting hsa_circ_0074854 or long non-coding RNA (lncRNA) HMMR-AS1 from HCC cells to macrophages.[42,43] M1 and M2 polarization are dynamically reversible. Moreover, a previous study reported the crucial role of HCC-derived exosomal lncRNA TUC339 based on macrophage M1/M2 polarization.[44] As TAM precursors, monocytes usually initiate apoptosis within 2

days; however, they are capable of surviving in the inflammatory TME for prolonged periods and producing sufficient TAMs. Reportedly, the delivery of receptor tyrosine kinases through tumor-derived exosomes prompted monocyte survival wherein mitogen-activated protein kinase (MAPK) signaling was regulated.[45] Briefly, the immune system can be reactivated in HCC by targeting inhibitory molecules or signaling in the M2 type or reversing M1/M2 polarization.

3.2. Exosome-mediated delivery of cargoes between cancer-associated fibroblasts (CAFs) and HCC cells

A recent study reported that CAFs can induce antitumor immunity suppression and immune cell activity modulation, in addition to functioning as crucial components of the tumor extracellular matrix.[46] A study demonstrated that CAF-derived exosomes loaded with gremlin-1 could promote HCC progression by triggering epithelial–mesenchymal transition of tumor cells. Moreover, by regulating Wnt/β-catenin and bone morphogenetic protein signaling, the sensitivity of HCC cells to sorafenib has been abated.[47] Furthermore, CAF-derived exosomes enabled the delivery of circular RNA (circRNA) ZFR to HCC by inhibiting the signal transducers and activators of transcription (STAT) 3/NF-κB signaling, thus promoting the HCC growth and chemoresistance to cisplatin.[48] HCC progression might be facilitated by regulating the miRNA transport from CAFs to tumor cells via exosomes. Due to its oncogenesis role, CAF-derived exosomal lncRNA taurine-upregulated gene 1 (TUG1) that targeted the miRNA-524-5p/Sine oculis homeobox homolog 1 axis was reported to be implicated in stimulating HCC progression and glycolysis.[49] Conversely, the downregulation of antitumoral miRNA-150-3p or miRNA-320a in CAF-derived exosomes facilitated the HCC proliferation, migration, and metastasis.[50,51]

Tumor-derived exosomes can induce CAF activation in HCC, with direct or indirect effects on the immune microenvironment. HCC cells with high metastatic potential were well documented to be capable of driving the conversion of normal fibroblasts into CAFs by releasing miRNA-1247-3p in the form of exosomes, which targeted beta-1,4-galactosyltransferase III directly and activated the beta1-integrin/NF-κB signaling in fibroblasts. In turn, activated CAFs could accelerate the HCC development by releasing pro-inflammatory cytokines.[52] Moreover, the exosomal miRNA-21 originating from HCC triggered the transformation of hepatic stellate cells into CAFs by regulating the PTEN/3-phosphoinositide-dependent protein kinase-1/AKT axis; thus, cancer progression further accelerated the release of angiogenic cytokines from activated CAFs.[53]

3.3. Exosome-mediated delivery of cargoes between T/B lymphocytes and HCC cells

Regulatory B cells (Bregs), a B-cell subgroup, are reportedly essential for regulating immune responses. A research team from China determined the function of exosomal high mobility group box 1 (HMGB1) secreted by HCC on stimulating the expansion of T-cell immunoglobulin mucin (TIM)-1^+ Bregs by regulating the HMGB1/Toll-like receptor/MAPK axis, and TIM-1^+ Bregs enabled stimulating the release of interleukin-10 and even reducing the antitumoral function of T cells, thus creating an immunosuppressive TME.[54] The tumor immune escape can be accomplished with the help of regulatory T cells (Tregs). HCC-derived exosomal circGSE1 prompted tumor progression by promoting Treg expansion through the miRNA-324-5p/TGFBR1/Smad3 axis.[55] Increasing evidence reported significant inhibition of tumor-infiltrating T lymphocytes (TILs) in TME. A study demonstrated that HCC 14-3-3 protein zeta inhibited TILs with an antitumorigenic capacity via exosomes.[56] These novel findings may provide new insights into obstructing the inhibitory immune cell expansion by targeting these signaling pathways.

3.4. Exosome-mediated delivery of cargoes between natural killer (NK) cells and HCC cells

NK cells are deemed robust antitumoral weapons by their cell-killing activity and cytokine secretion. However, the immunosuppressive TME maintains NK cells predominantly in a dysfunctional state, ultimately allowing the tumors to escape.[57] A study demonstrated the role of HCC-derived exosomal circUHRF1 by inducing NK cell exhaustion; circUHRF1 was reported to suppress the antitumoral function of NK cells by upregulating TIM-3 and downregulating miRNA-449c-5p expression, and the relationship between the circRNA and anti-PD1 therapy was observed.[58] Another study revealed exosomal miRNA-92b transferring from HCC cells to NK cells, eventually resulting in low CD69 expression and NK cell cytotoxicity.[59] A binding of major histocompatibility complex (MHC) class I chain-related protein (MIC) A/B to NKG2D, one of the activating receptors expressed on NK cells, triggers an immune system activation. Exosomal MICA*008 derived from HCC cells facilitated downregulation of NKG2D expression on NK cells, ultimately suppressing the cytotoxic function and promoting HCC immune escape.[60] It follows that targeting the downregulation of these exosomal cargoes may activate NK cells to perform the antitumor effects to some degree. Moreover, Table 1 summarizes the functions of exosomal cargoes transported between immune-related cells and HCC cells.

4. Exosome-mediated immunotherapy in HCC

Immunotherapy, a highly anticipated treatment enhancing antitumor immunity, has emerged as a frontline therapeutic approach in multiple types of cancer including HCC.[10,61,62] Exosomes, a natural nanocarrier, can effectively deliver drugs or cargoes to augment tumor immunogenicity and trigger antitumor immune responses.[13,63]

Dendritic cell (DC)-derived exosomes (DEXs), loading signature molecules from their parental cells (*e.g.*, costimulatory molecules and/or MHC), can be used as novel DC vaccines for cancer immunotherapy.[64] Alpha-fetoprotein-enriched DEXs were demonstrated to potently enable stimulating antitumor immune responses; in addition, these DEXs could reshape the TME where interferon-gamma (IFN-γ)-expressed $CD8^+$ T cells, interleukin-2, and IFN-γ increased, but $CD25^+Foxp3^+$ Tregs, transforming growth factor-beta, and interleukin-10 decreased.[65] Another study manifested the robust anti-HCC effects of DEXs with antigens, targeting ligands, and peptide immunoadjuvants, which could enhance the immune responses by purposefully recruiting and activating DCs in HCC tumors in mice.[66] Furthermore, DEXs combined with other therapies, such as microwave ablation, can elevate the anti-HCC efficacy.[67]

Growing evidence suggests that tumor cell-derived exosomes (TEXs) carry multiple antigens, revealing the activation of immune response and the improvement of TME mediated by TEX-pulsed DCs in murine HCC models, according to a study.[68] As a hormone with certain cytotoxicity, melatonin can modulate immune function and inhibit tumor growth. Another study investigated the effects of exosomes secreted by HCC cells with melatonin by co-culturing these TEXs with macrophages, a decrease in PD-L1 expression on macrophages and inflammatory cytokine secretion by macrophages.[69]

Table 1
The delivery of cargoes between immune-related cells and HCC cells via exosomes.

Exosomal cargoes	Secreted cells	Recipient cells	Functions	References
miRNA-660-5p	TAMs	HCC cells	Contributing to HCC development through downregulating the Kruppel-like factor 3	34
miRNA-27a-3p	TAMs	HCC cells	Fostering cancer stemness in HCC by downregulating thioredoxin-interacting protein	35
miRNA-92a-2-5p	TAMs	HCC cells	Regulating the androgen receptor/PH domain leucine-rich repeat protein phosphatase/AKT/β-catenin signaling pathway to enhance HCC invasion	36
hsa_circ_0004658	TAMs	HCC cells	Suppressing HCC progression by miRNA-499b-5p/junctional adhesion molecule 3	37
PD-L1	HCC cells	TAMs	Provoking $CD8^+$ T cell suppression in HCC	39
miRNA-23a-3p	HCC cells	TAMs	Upregulating PD-L1 expression in macrophages	40
miRNA-146a-5p	HCC cells	TAMs	Remodeling macrophages into M2-polarized TAMs and driving the T-cell exhaustion	41
hsa_circ_0074854	HCC cells	TAMs	Promoting macrophage M2 polarization	42
lncRNA HMMR-AS1	HCC cells	TAMs	Promoting macrophage M2 polarization	43
lncRNA TUC339	HCC cells	TAMs	Playing a critical role in macrophage M1/M2 polarization	44
Receptor tyrosine kinases	HCC cells	Monocytes	Suppressing apoptosis	45
Gremlin-1	CAFs	HCC cells	Triggering epithelial-mesenchymal transition in HCC cells and reducing the sensitivity of HCC cells to sorafenib by regulating Wnt/β-catenin and bone morphogenetic protein signaling	47
circZFR	CAFs	HCC cells	Inhibiting the signal transducers and activators of transcription 3/nuclear factor-kappa B pathway	48
lncRNA TUG1	CAFs	HCC cells	Targeting the miRNA-524-5p/Sine oculis homeobox homolog 1 axis and stimulating HCC migration, invasion, and glycolysis	49
miRNA-1247-3p	HCC cells	Fibroblasts	Targeting β-1,4-galactosyltransferase III directly and activating the β1-integrin/nuclear factor-kappa B signaling pathway in fibroblasts	52
miRNA-21	HCC cells	Hepatic stellate cells	Activating the conversion of hepatic stellate cells into CAFs via phosphatase and tensin homolog (PTEN)/3-phosphoinositide-dependent protein kinase-1/AKT axis	53
HMGB1	HCC cells	Bregs	Stimulating TIM-1^+ Breg expansion and facilitating HCC immune evasion	54
circGSE1	HCC cells	Tregs	Promoting Treg expansion through the miRNA-324-5p/TGFBR1/Smad3 pathway	55
14-3-3 protein zeta	HCC cells	TILs	Inhibiting antitumoral TILs	56
circUHRF1	HCC cells	NK cells	Inducing NK cell exhaustion	58
miRNA-92b	HCC cells	NK cells	Resulting in low CD69 expression and NK cell cytotoxicity	59
MICA*008	HCC cells	NK cells	Facilitating downregulation of NKG2D expression on NK cells and suppressing the cytotoxic function	60

Abbreviations: Bregs, regulatory B cells; CAFs, cancer-associated fibroblasts; HCC, hepatocellular carcinoma; HMGB1, high mobility group box 1; MICA, major histocompatibility complex class I chain-related protein A; NK, natural killer; PD-L1, programmed death-ligand 1; TAMs, tumor-associated macrophages; TILs, tumor-infiltrating T lymphocytes; TIM, T-cell immunoglobulin mucin; Tregs, regulatory T cells; TUG1, taurine-upregulated gene 1.

Given the STAT6 function in inducing TAMs toward the M2 phenotype, Kamerkar *et al.*[70] designed an exosome loaded with an antisense oligonucleotide targeting STAT6, which was capable of selectively silencing the expression of STAT6 in TAMs; besides inhibiting the HCC growth, this exosome could remodel the TME by augmenting the $CD8^+$ T- and M1-cell generation.[70] Another study reported that exosomes were engineered with pegylated iron oxide nanoparticles loaded with chlorin e6 to augment anti-HCC immunological activity by fostering M1-polarized macrophages.[71]

5. Conclusions

Currently, exosome-mediated immunoregulation in HCC represents a promising field of study for deciphering novel mechanisms underlying cancer progression. Despite great advances in exploring the immune regulation mechanisms mediated by exosomes, challenges still exist. First, efficient identification of exosomes is one of the major technical difficulties. Owing to the low exosomal levels in body fluids, effective separation and detection devices must be optimized, and a quantitative evaluation system must be established. Second, how they were selectively loaded into exosomes and their biological functions remain unidentified, even though multiple cargoes including proteins, DNA, and RNA have been recognized in exosomes from the HCC or HCC-associated cells. An intensive investigation of these exosomal cargoes could facilitate the identification of new HCC-specific biomarkers for the diagnosis and/or therapy. Third, exosomes investigated in most research reports are extracted from either body fluids or cultured HCC cell lines. Remarkably, exosomes isolated from body fluids are a conglomeration mixed with ones from other organs or cells, and the characteristics of HCC cell lines can be altered after long-term culture, directly affecting the exosomal function. By contrast, liver tissue-derived exosomes are capable of providing sufficient information about TME, which truly reflects the exosomal function of the body. Ultimately, assessing the potential peril in administering exosomal vaccines will be of significance before its safe utilization in clinical practice.

In conclusion, as a mediator of substance transmission and signaling transduction between the tumor and immune cells, exosomes demonstrate crucial roles in the dynamic immune regulation of HCC, influencing cancer growth and immune escape; in turn, they can also initiate TME alteration or decrease immune suppression after modification or engineering.

Authors' contributions

M.-C. Guan, M.-D. Wang, and W.-Y. Wang contributed equally to this work. M.-C. Guan and C. Li searched literature. M.-C. Guan, M.-D. Wang, and W.-Y. Wang drafted the initial manuscript. M.-C. Guan, M.-D. Wang, W.-Y. Wang, and L.-Q. Yao drew the figures by Figdraw. H. Zhu and T. Yang conceived the idea and revised the manuscript critically.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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