REVIEW

The Role of Extracellular Vesicles in Liver Pathogenesis

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Extracellular vesicles (EVs) are generated by cells in the form of exosomes, microvesicles, and apoptotic bodies. They can be taken by neighboring cells, and their contents can have functional impact on cells that engulf them. As the mediators of intercellular communication, EVs can play important roles in both physiological and pathologic contexts. In addition, early detection of EVs in different body fluids may offer a sensitive diagnostic tool for certain diseases, such as cancer. Furthermore, targeting specific EVs may also become a promising therapeutic approach. This review summarizes the latest findings of EVs in the field of liver research, with a focus on the different contents of the EVs and their impact on liver function and on the development of inflammation, fibrosis, and tumor in the liver. The goal is to provide a succinct account of the various molecules that can mediate the function of EVs so the readers may apply this knowledge to their own research. (Am J Pathol 2022, 10; https://doi.org/10.1016/j.ajpath.2022.06.007)

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https://doi.org/10.1016/j.ajpath.2022.06.007
(CYP) family 2 subfamily E member 1 and asialoglyco-
protein receptor 1.20 Cholangiocyte-derived exosomes are
usually with specific marker of cytokeratin 19.19 Sphingo-
sine kinase 1 is found in exosomes isolated from liver si-
nusoidal endothelial cell line, SK-HEP-1,21 whereas
connective tissue growth factor (CTGF/CCN2) is found in
exosomes from highly activated mouse HSCs.22 Liver cells
can be both the donor and the recipient of EVs, resulting in
a multiway communication network among the various cells
in the liver.23,24 Intercellular communications can be
mediated by proteins, and perhaps more important, by
mRNAs and miRNAs, with functional consequence in
target cells.25 In this review, recent findings on the role of
EVs in liver pathology are summarized, and their potential
clinical applications are discussed.

**EVs in Liver Physiology**

Although the most studies investigate the role of EVs in the
disease status, EVs do seem to participate in the physiolo-
gical function of the liver with some of the well-
characterized molecules found in the EVs (Figure 2 and
Table 1).26–32

**Drug Metabolism**

In the late 1980s, Johnstone et al.26,33 reported the shedding
of plasma membrane in the process of maturation of reti-
culocytes to erythrocytes. In addition, they showed that
some superfluous proteins were eliminated through released
membrane vesicles. These vesicles were found with
acetylcholinesterase activities that are associated with the
metabolism of certain drugs.26,33 That is the first report that
implicated a role of EVs in drug metabolism–related function. The CYP superfam-
ily is mainly responsible for drug metabolism in the liver, kidney, and small intestine.
Functional CYP isoforms can be sorted into EVs that are
found in plasma of healthy persons.27 In particular, CYP
family 2 subfamily E member 1 is expressed at a much
higher level than other CYPs, such as 1B1, 2A6, 2E1, and
3A4, in EVs isolated from healthy subjects.27 and thus be-
comes a well-recognized marker for hepatocyte-derived
EVs. In addition, exosome-derived CYP3A and uridine 5’-
diphospho-glucuronosyltransferase proteins have also been

![Figure 1](image)

*Figure 1* Generation of exosomes from multivesicular bodies (MVBs). MVBs are important for the intracellular trafficking, connecting early endosomes to lysosomes. MVBs are characterized by the presence of multiple intraluminal vesicles (ILVs). The formation of ILVs requires the endosomal sorting complexes required for transport (ESCRT) machinery, which is made up of cytosolic protein complexes, known as ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. They are recruited to the precursors of MVBs sequentially as indicated and promote membrane invagination and vesicle formation. A variety of protein and RNA molecules can be incorporated into the ILVs. Exosomes are a type of extracellular vesicles (EVs) with the size around 100 nm in average. They are derived from the ILVs of MVBs when the later fuses with the cytoplasmic membranes. Thus, EVs derived from MVBs can contain cargos contained in the ILVs, which may carry unique signatures reflecting the functional or structural features of the cells. These cargo molecules can have functional impact on cells that engulf the exosomes.

![Figure 2](image)

*Figure 2* Extracellular vesicles (EVs) can affect both physiological and pathologic processes of liver diseases. EVs can carry molecules that can be related to normal liver physiology. The cargos are responsible for its regulatory functions (*Table 1*). EVs are also important to injury, inflammation, fibrosis, and tumorigenesis in the development of various liver diseases. EVs may be produced and secreted by hepatocytes and nonhepatocytes, and they can be taken by hepatocytes and nonhepatocytes. EVs function by delivering the cargos from the producing cells (*Tables 2–4*) to the target cells and thus modify the function of the latter with a consequence in the pathogenesis in alcoholic liver disease, nonalcoholic fatty liver disease, biliary diseases, liver cancer, and viral hepatitis.
found metabolically active under ex vivo conditions and exhibit kinetics comparable to subcellular fractions prepared from human liver tissue. CYP and uridine 5'-diphospho-glucuronosyltransferase families of drug-metabolizing enzymes account for the metabolic clearance of >90% of drugs. EVs may play a critical role in drug metabolism. It is possible that the liver releases CYP-containing EVs to promote drug metabolism in other cells that internalize these vesicles. However, the mechanism of CYP/uridine 5'-diphospho-glucuronosyltransferase sorting into EVs and target cell selection are largely unknown.

**Regeneration**

Liver is the main organ for detoxification of noxious endobiotics and xenobiotics, and it is susceptible to various harmful conditions that can lead to injuries. The capability of regeneration is important to the liver. Interestingly, exosomes isolated from human liver stem cells could induce proliferation and prevent apoptosis of hepatocytes. The proliferation was abolished when the isolated EVs were treated with anti-ζ4-integrin-blocking antibody and RNase, indicating that ζ4-integrin and RNAs played a critical role in the internalization of EVs and regulation of regeneration, respectively. In addition, Nojima et al reported that hepatocyte-derived EVs increased hepatocyte proliferation and liver regeneration. This effect was abolished when EVs were isolated from sphingosine kinase 2-deficient mice or when the function of sphingosine kinase 2 was blocked, indicating that sphingosine kinase 2, an enzyme that catalyzes the formation of sphingosine-1-phosphate, was important in the process. Sphingosine kinase 2/sphingosine-1-phosphate may play a positive role in cell growth.

Other than stimulating hepatocyte growth, EVs isolated from bile could have influences on cholangiocyte proliferation. Bile can contain exosomes that decrease phosphorylated level of extracellular-regulated protein kinase 1/2, and subsequently increased the level of miR-15A, causing decreased proliferation of cholangiocytes.

**EVs in Liver Pathology**

EVs can be released by various liver cells under pathologic conditions, which, in turn, contribute to the occurrence and development of inflammation, fibrosis, and cancer. This important role of EVs has now been well recognized. Table 1 summarizes some of the molecules found in EVs that are involved in liver pathology.

**Liver Inflammation**

The key cellular events of liver inflammatory responses are the activation of liver-resident macrophages, also known as Kupffer cells, and the recruitment and activation of inflammatory monocytes originated from the extraliver space. Excessive alcohol drinking, one of the most common causes of liver disease, can cause multiple pathologic effects. The number of exosomes is significantly increased in mice after binge or chronic alcohol consumption, and in the serum of healthy individuals after alcohol binge drinking. Early on, it was found that alcohol treatment in vitro induced EV release from primary hepatocytes and HepG2 cells. The released EVs could stimulate cytokine production from THP-1 macrophage cells. EV release required caspase activation, and EVs were enriched with the molecule CD40 ligand. Mice receiving a pan-caspase or a Rho kinase blocking antibody and 4-integrin and RNAs30

**Table 2 EV-Derived Molecules Involved in Liver Inflammation and Fibrosis**

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation (alcoholic liver disease)</td>
<td>miR-12237</td>
</tr>
<tr>
<td></td>
<td>CD40L38</td>
</tr>
<tr>
<td></td>
<td>HSPP9029</td>
</tr>
<tr>
<td></td>
<td>miR-19239</td>
</tr>
<tr>
<td></td>
<td>miR-27a40</td>
</tr>
<tr>
<td></td>
<td>miR-192-5p20</td>
</tr>
<tr>
<td>Inflammation (nonalcoholic fatty liver disease)</td>
<td>ζ4-Integrin and RNAs30</td>
</tr>
<tr>
<td></td>
<td>SK231</td>
</tr>
<tr>
<td></td>
<td>CXCL1041</td>
</tr>
<tr>
<td></td>
<td>S1P72</td>
</tr>
<tr>
<td></td>
<td>miR-130a43</td>
</tr>
<tr>
<td></td>
<td>Ceramides44</td>
</tr>
<tr>
<td></td>
<td>ITGB147</td>
</tr>
<tr>
<td>Liver fibrosis</td>
<td>CCN2 protein or mRNA32</td>
</tr>
<tr>
<td></td>
<td>miR-128-3p65</td>
</tr>
<tr>
<td></td>
<td>TLR3 ligands46</td>
</tr>
<tr>
<td></td>
<td>SK147</td>
</tr>
<tr>
<td></td>
<td>KIT36</td>
</tr>
</tbody>
</table>

Listed molecules are increased in EVs under the disease status. CCN2, connective tissue growth factor; CD40L, CD40 ligand; EV, extracellular vesicle; HSPP90, heat shock protein 90; ITGB1, integrin β1; S1P, sphingosine-1-phosphate; SK, sphingosine kinase; TLR3, toll-like receptor 3.
inhibitor and mice with genetic deletion of CD40 or the caspase-activating TRAIL receptor were protected from alcohol-induced injury and associated macrophage infiltration, suggesting that these EVs contributed to the pathogenesis. Saha et al \(^ {30} \) also found that the total number of circulating EVs was increased in mice with chronic ethanol feeding, compared with pair-fed controls, and the mRNA and protein level of C-C motif chemokine ligand 2 were increased in hepatocytes isolated from mice treated with these EVs. Furthermore, the transcriptional levels of tumor necrosis factor-α and IL-1β were increased in macrophages treated with these EVs derived from mice with chronic ethanol exposure or alcoholic liver disease condition. The EVs thus play a proinflammatory role by affecting both inflammatory cells and hepatocytes.

This proinflammatory effect of alcoholic liver disease–derived EVs is determined by their cargos, particularly miRNAs. Studies have found the significance of several different cargos in alcoholic liver disease, such as heat shock protein 90, miR-192, \(^ {39} \) and miR-122. \(^ {37} \) Exosome-derived miR-122 could inhibit heme oxygenase-1 pathway in the target THP-1 monocytes so that the cells become sensitized to stimulation of lipopolysaccharide, leading to increased production of proinflammatory cytokines. Similarly, miR-27a, a cargo of exosomes derived from alcohol-treated monocytes, could activate naive monocytes and promote inflammatory response by IL-10 and transforming growth factor-β. \(^ {40} \)

Nonalcoholic fatty liver disease (NAFLD) is a common disorder with accumulation of excess fat in the liver of people who drink little or no alcohol. Recent studies have found that EVs can play a considerable role in NAFLD. Lipid accumulation in the liver under NAFLD can lead to toxicity in hepatocytes, which can stimulate the release of EVs. EVs from hypoxic fat-laden hepatic cells can induce proinflammatory responses in Kupffer cells, \(^ {40} \) which may be mediated by CXCL10 contained in the EVs. \(^ {41} \) Another study shows that hepatocyte-derived EVs have a higher level of proinflammatory lipids, such as sphingosine-1-phosphate, which can also induce macrophage chemotaxis. \(^ {42} \) Adipocytes are also a major source of EVs in NAFLD. Co-culturing adipocyte-derived EVs, isolated from high-fat–fed obese mice, with primary macrophages activated the latter and promoted the production of macrophage colony-stimulating factor, IL-6, and tumor necrosis factor-α. \(^ {40} \)

Nonalcoholic steatohepatitis is one form of NAFLD in which the liver develops inflammation and cell damage in addition to fat accumulation. As in alcoholic liver disease, miRNAs play a role in the inflammation of NAFLD and could be a major force driving the development of nonalcoholic steatohepatitis. Hepatocyte-derived exosomes isolated from high-fat diet mice have an increased level of miR-130a, \(^ {43} \) which promotes proinflammatory phenotype and suppresses anti-inflammatory phenotype of THP-1 cells via directly inhibiting peroxisome proliferator-activated receptor-γ (PPAR-γ). \(^ {31} \) In rats fed with a high-fat, high-cholesterol diet, exosomes derived from hepatocyte are enriched with miR-192–5p that plays a critical role in the activation of proinflammatory macrophages and disease progression by targeting the RPTOR-independent companion of MTOR complex 2/serine/threonine kinase kinase endoribonuclease-1 signaling pathway. \(^ {20} \) Interestingly, miR-192–5p level in serum collected from patients with NAFLD positively correlates with hepatic inflammation activity score and disease progression. \(^ {20} \)

Other factors associated with EVs can be also important for nonalcoholic steatohepatitis development. Dasgupta et al \(^ {44} \) reported that activation of inositol-requiring transmembrane kinase endoribonuclease-1α stimulated X-box–binding protein 1 to increase transcription of serine palmitoyltransferase gene, resulting in ceramide biosynthesis and production of hepatocyte-derived EVs in a mouse model of nonalcoholic steatohepatitis. Consistently, mice given i.v. injections of inositol-requiring transmembrane kinase endoribonuclease-1α–stimulated, hepatocyte-derived, ceramide-enriched EVs recruited monocytes into the liver, resulting in inflammation and injury. Another mechanism related to EVs involves integrin β1 (ITGβ1). Guo et al \(^ {45} \) reported that lysophosphatidylcholine treatment in hepatocytes activates ITGβ1 and mediates its endocytic trafficking and sorting into EVs. The ITGβ1-enriched EVs can then mediate monocyte adhesion to liver sinusoidal cells through an ITGβ1-dependent manner. The resulting inflammation could be thus attenuated by anti-ITGβ1 antibody.

Liver Fibrosis

Liver fibrosis results from excessive accumulation of extracellular matrix proteins, such as collagens. Fibrosis can diminish blood flow throughout the liver. It can progress to cirrhosis, leading to liver failure and even liver cancer. \(^ {52} \) HSCs are one of the major types of cells driving fibrosis, and their functions can be regulated by EVs as they internalize these vesicles coming from different types of cells. Hepatocytes are the major source of EVs that can activate HSCs. \(^ {45,53} \) Increased fat deposit in hepatocytes is an early event of NAFLD. It was found that EVs derived from fat-laden hepatocytes contain various miRNAs, some of which are known repressors of PPAR-γ, and HSC activators, such as miR-128–3p. \(^ {45} \) Thus, EVs may promote progression of liver pathology from simple steatosis to fibrosis. An in vitro study also found that cobalt chloride, a chemical hypoxia inducer, caused the release of EVs from fat-laden HepG2 cells. \(^ {53} \) These EVs, in turn, enhanced the expression of profibrotic markers in LX-2 cells, an immortalized HSC cell line. This in vitro finding was confirmed by an in vivo model in which C57BL/6 mice given CDA diet and intermittent hypoxia exposure led to an increased production of exosomes to promote fibrosis. \(^ {53} \) In yet another example of carbon tetrachloride–induced liver injury,
exosomes isolated from damaged hepatocytes contained toll-like receptor 3 ligands, which induced activation of toll-like receptor 3 in HSCs to exacerbate liver fibrosis.  

Endothelial cells are another source of EVs that could affect HSCs in livers. It was found that exosomes derived from SK1-overexpressing endothelial cells could promote the migration of HSCs via AKT signaling, which were blocked by disruption of fibronectin-integrin interaction or by inhibition of dynamin. The findings suggested that the effect of endothelial cell-derived SK1-containing EVs on HSC migration relied on fibronectin-integrin-dependent EV adherence and dynamin-dependent EV internalization.  

Another study found that small EVs (exosomes) isolated from the serum of patients with systemic mastocytosis, accounting for approximately 90% of cases, and it is the most common form of liver cancer, accounting for approximately 90% of cases, and it is the second leading cause of cancer-related death globally. Exosomes can act as a regulatory means to transmit signaling cargos between neighboring cells and heterogeneous populations of tumor cells to affect the nature and microenvironment of tumor cells (Table 3[^57-57]).

### Table 3 EV-Derived Molecules Involved in Liver Tumorigenesis

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Molecules</th>
</tr>
</thead>
</table>
| **EMT**        | - ZEB5[^57]  
- N-cadherin[^57]  
- β-SMA[^57]  
- Vimentin[^57]  
- OVOL1 (negative effect)[^57]  
- HGF[^58]  
- miR-21[^59]  
- ANGPT2[^59]  
- Vaso[^60]  
- miR-155[^61]  
- miR-210[^62]  
- Circ-CCAC1[^63]  
- LncRNA H19[^64]  
- CircRNA-100338[^65]  
- CircRNA-100338[^66]  
- miR-181[^67]  
- ncRNA-TUC39[^68]  
- ITGα[^69] and CD147[^70]  
- lncRNA-FAL1 and miR-1236[^71]  
- miR-21 and miR-10b[^72]  
| **Angiogenesis** | - linc-ROR[^73]  
- miR-21[^74]  
| **Proliferation, invasion, and metastasis** | - NF-kB[^75]  
- Angiopoietin 2[^76]  
- Circ-CCAC1, circular RNA 1[^77]  
- CircRNA, circular RNA; EMT, epithelial-mesenchymal transition; EV, extracellular vesicle; HGF, hepatocyte growth factor; ITGα[^78]  
- Interferon-γ[^79]  
- miR-181[^80]  
- miR-21[^81]  
- linc-ROR[^82]  
| **Liver Cancer** |

**Effect of Exosomes in EMT**

Epithelial-mesenchymal transition (EMT) is a pathophysiological process in which epithelial cells lose polarity and cell-cell adhesion, and acquire the motile and invasive characteristics to become mesenchymal stem cells. EMT is the critical step for the invasion and metastasis of cancers. The emerging evidence has shown that EVs can promote EMT. Most of these studies treated in *vitro* one type of cells with exosomes derived from another type of cells. It was found that the expression of EMT markers, such as E-cadherin and vimentin, could be increased in the recipient cells. Notably, exosome-treated cells could become more proliferative through increased mitogen-activated protein kinase/extracellular-regulated protein kinase signaling. Similarly, HCC-derived exosomes have the regulatory role on hepatocyte growth factor/hepatocyte growth factor receptor/AKT signaling.

**Effect of Exosomes in Angiogenesis**

Angiogenesis is critical for cancer growth through increased supply of oxygen, nutrients, and growth factors. Expanded vascular network, along with altered permeability, could also promote metastasis. Liver cancer—derived EVs could...
be involved in the promotion of angiogenesis when they are taken up by vascular endothelial cells. A variety of molecules contained in the EVs have been linked to this process. These molecules may include protein molecules and small RNA molecules. For example, liver cancer cells can produce angiopoietin 2–containing exosomes, which can be internalized by vascular endothelial cells. In another study, HCC cells were found to produce vasorin-containing exosomes that can stimulate human umbilical vein endothelial cells for angiogenesis.

miRNAs were often found to transmit the angiogenesis effect after delivery to the target cells via EVs. miR-155–containing exosomes, produced by HCC cells under hypoxic condition, elevated the level of vascular endothelial growth factor and hypoxia-inducible factor-1α in target human umbilical vein endothelial cells to promote angiogenesis. On the other hand, miR-21–containing exosomes, secreted by HCC cells, induce angiogenesis via SMAD family member 4 and STAT6 signaling pathway. Cholangiocarcinoma can also release EVs that are taken up by endothelial cells. Circular RNA 1 contained within the EVs could promote angiogenesis. The effect of miRNA in angiogenesis could be also indirect. Quiescent HSCs can take up miR-21–containing exosomes derived from HCC cell lines to become cancer-associated fibroblasts, which can then stimulate angiogenesis in addition to other effects.

Other small RNAs can also serve as the signaling messenger. One study has shown that exosomes isolated from the medium of cultured cancer stem cell–like CD90+ liver cells contained long noncoding RNA (lncRNA) H19, which increased the transcripts of vascular endothelial growth factor in human umbilical vein endothelial cells to induce the formation of tube-like structures. Another study found that high-metastatic HCC cells produced more exosomes containing circular RNA-100338, which promoted the proliferation, angiogenesis, and permeability of human umbilical vein endothelial cells.

Effect of Exosomes in Proliferation, Invasion, and Metastasis

Most of the studies focused on the exosomes derived from tumor cells, although EVs from nontumor cells, such as immature myeloid cells, macrophages, mast cells, and T cells, were examined as well. Although the target cells are mainly studied for hepatic tumor cells, vascular endothelial cells and other nonparenchymal cells have also been reported. The overall effects in cancer cell proliferation, invasion, and metastasis can be direct or indirect through distinct mechanisms mediated by the content in the exosomes, which may include protein molecules, miRNA, IncRNAs, and circular RNA. The following are a few examples illustrating the heterogeneity in the way of action.

One study showed that exosomes derived from HCC cells can facilitate tumorigenesis in recipient normal hepatocytes by activating phosphoinositide 3-kinase/AKT/mitogen-activated protein kinase pathway and promote production of metalloprotease matrix metallopeptidases 2 and 9. Another study reported that exosomes isolated from the culture medium of HCC cells contained lncRNA TUC339, which was transferred to perhaps other cells within the same microenvironment, and consequently promoted HCC proliferation and invasion. Exosomes may communicate between tumor cells to enhance viability and survival of target cells through a series of regulations involving multiple small RNAs, including long intergenic non–protein-coding RNA, regulator of reprogramming, which eventually lead to the activation of hypoxia-inducible factor-1α and/or hypoxia-inducible factor-2α pathway. The miRNAs may be regulated by other factors under tumor microenvironment. For example, it was reported that acidic microenvironment triggered the activation of hypoxia-inducible factor-1α/2α, which enhanced the level of miR-21 and miR-10b in exosomes sufficiently, thus promoting Hep3B cell proliferation, migration, and invasion in vivo and in vitro. Unfortunately, how deep inside the tumor EVs can reach is unclear.

Interestingly, many of the studies found that tumor-derived exosomes target to a variety of nontumor cells, which then, in turn, promote proliferation, invasion, and metastasis. For example, HCC cells, such as HepG2, MHC97H, or QGY-7703 cells, can release exosomes that are internalized by mesenchymal stem cells. miR-181d-5p released from these exosomes targets suppressor of cytokine signaling 3 to activate focal adhesion kinase/SRC proto-oncogene, nonreceptor tyrosine kinase signaling pathway, which promotes the differentiation of mesenchymal stem cells into fibroblasts and accelerates EMT, invasion, and migration of tumor cells. Another study showed that such effects can cause the differentiation of mesenchymal stem cells to fibroblasts, which regulate local microenvironment to enhance tumor cell growth. In addition, adipocytes had been shown to have the capacity to internalize exosomes isolated from HepG2 cell culture medium. When co-injected with HepG2 cells to mice, these exosome-treated adipocytes could release inflammatory cytokine through the activation of NF-kB pathway, which recruits macrophages to the tumor region, leading to enhanced cell proliferation and tumor growth. Finally, another cell population that could be modified by tumor-derived exosomes is endothelial cells, which led to increased vascular permeability via the down-regulation of tight junction protein 1, cadherin 5, and p120-catenin by miR-103, thus facilitating tumor metastasis.

Potential Values of EVs in Clinical Applications

Considering the many effects of EVs in cell-cell communications, it is tempting to consider the possibility of...
administering EVs with proper cargos in some clinical scenarios to produce desirable outcomes. There are some advantages of using EVs for this purpose, including that i) EVs do not replicate after administration; ii) EVs may have a lower immunogenicity; iii) EVs possess a high ability to cross tissue and cellular barrier; and iv) EVs can escape from the degradation of proteases in circulation, and they are stable with freeze/thaw cycles during long-term storage. However, it is not likely that EVs will be used in a form directly isolated from an in vivo situation because of limitations in achieving uniform purification and variations in the nature of cargos and tissue source. More plausible is that engineered EVs will be used with only intended cargos and with an enhanced delivery ability. Thus, it is the knowledge of the specific cargos and the membrane components of the EVs that may be translated to a better engineered form of artificial EVs for clinical applications.

On the other hand, EVs may be more readily analyzed for diagnostic purposes. The content of EVs could serve as biomarkers for a given pathologic condition. There are several advantages of EVs being diagnostic biomarkers, including that EVs can be secreted by almost all types of cells and found in all major bodily fluids, which will make the detection easy for most diseases for early diagnosis and follow-up; and that the cargos vary from cell types or situations and specific cargo may be linked to specific scenarios. In the subject of liver-related condition, the focus has been the liver cancer, in which several EV cargo molecules have been examined for their differential expression in the normal versus the cancer conditions (Table 4).

Early diagnosis of HCC has always been a critical issue in the prompt management. There are no specific clinical symptoms for the early-stage HCC, and later-stage HCC is difficult to treat. Detection of serum α-fetoprotein and ultrasonography have been the two widely used methods, but both have limitations in the early detection of HCC. An increased number of studies have discovered that tumors can secrete unique exosomes, with the number and content distinctly different from healthy population. For example, it is reported that the levels of galectin-3–binding protein and polymeric immune receptor are significantly higher in exosomes isolated from serum of HCC patients, compared with those in healthy subjects. SMAD family member 3 and cyclase-associated actin cytoskeleton regulatory protein 1 have also been found to be rich in exosomes isolated from the peripheral blood of HCC patients and to be closely associated with HCC stage and metastasis, respectively.

However, the small RNAs are perhaps the most promising biomarkers for HCC. Recent studies have shown that the levels of miR-21, miR-210, miR-224, and miR-93 in exosomes isolated from HCC patients’ serum are significantly higher than those in EVs from healthy donors. A recent study reported that HCC patients, particularly those with low α-fetoprotein levels, had much higher levels of miR-21-5p, miR-10b-5p, miR-221-3p, and miR-223-3p in exosomes, compared with those in chronic hepatitis/non-HCC patients. Similarly, exosomes from HCC patients are enriched with more miR-18a, miR-221, miR-224, and miR-223, compared with the patients with liver cirrhosis or chronic hepatitis B. In contrast, miR-101, miR-106b, miR-122, miR-125b, and miR-195 are found to be significantly lower in exosomes from HCC patients.

Other kinds of noncoding RNAs, including lncRNAs and long intergenic noncoding RNAs, are also reported to be associated with HCC, even though lncRNAs account for only 3% of total RNA in EVs. TUC339, lncRNA H19, lnc-ROR, lnc-VLDLR, and Linc00161 are significantly up-regulated in HCC cells. Nevertheless, there is still a lot to do to develop a clinically proven diagnostic test detecting EVs and/or the specific content for a particular disease.

**Conclusion**

The research of exosomes has made great progress in recent years. Novel mechanisms regarding cell-to-cell communications have been discovered. The physiological and
pathologic roles of exosomes have been explored in various liver conditions. EVs contain various cargos that can be important to the pathogenesis of liver diseases, including metabolic disturbance, inflammation, fibrosis, and tumorigenesis. However, many questions remain to be addressed, particularly about the in vivo role and dynamics of EVs, considering that most of the works were done in vitro using cell lines. It is far from clear how EVs interact with the extracellular matrix and other microenvironment elements to gain access to cells that uptake them. Toward that end, an efficient and practical way to label these EVs may be necessary, trackable by methods for in vivo imaging.

Inhibition of the biogenesis or release of EVs, or blockage of EV uptake, may evolve into potential therapies to arrest or even prevent the development of the diseases. In addition, the structural features of EVs, which help them from lysosomal degradation, can help to design a new way to deliver RNAs or drugs for therapeutic purpose. However, it is still far away to have this approach ready for clinical application with the current knowledge of EVs. More likely, diagnostic approaches based on specific EV cargos may become available, which should provide the necessary sensitivity and specificity for early detection, thus facilitating the clinical management of the disease.

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