The emerging roles of microvesicles in liver diseases

Sara Lemoinne, Dominique Thabut, Chantal Housset, Richard Moreau, Dominique Valla, Chantal M. Boulanger and Pierre-Emmanuel Rautou

Abstract | Microvesicles (MVs) are extracellular vesicles released by virtually all cells, under both physiological and pathological conditions. They contain lipids, proteins, RNAs and microRNAs and act as vectors of information that regulate the function of target cells. This Review provides an overview of the studies assessing circulating MV levels in patients with liver diseases, together with an insight into the mechanisms that could account for these changes. We also present a detailed analysis of the implication of MVs in key processes of liver diseases. MVs have a dual role in fibrosis as certain types of MVs promote fibrolysis by increasing expression of matrix metalloproteinases, whereas others promote fibrosis by stimulating processes such as angiogenesis. MVs probably enhance portal hypertension by contributing to intrahepatic vasoconstriction, splanchnic vasodilation and angiogenesis. As MVs can modulate vascular permeability, vascular tone and angiogenesis, they might contribute to several complications of cirrhosis including hepatic encephalopathy, hepatopulmonary syndrome and hepatorenal syndrome. Several results also suggest that MVs have a role in hepatocellular carcinoma. Although MVs represent promising biomarkers in patients with liver disease, methods of isolation and subsequent analysis must be standardized.

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Introduction

Microvesicles (MVs) are part of a group of extracellular vesicles that also includes exosomes and apoptotic bodies. Schematically, these three different types of vesicles have been defined by their size, mechanisms of production, and protein and lipid content. The main features of these extracellular vesicles are shown in Figure 1 and Box 1, although caution is required, as the precise definition of each type of extracellular vesicle is still a matter of debate.[1,2] Moreover, owing to a lack of standardization of the isolation procedures and of the detection methods, a strict separation of these vesicles (in particular MVs and exosomes) has not been achieved in several studies. As limited specific data on apoptotic bodies and exosomes in liver diseases exist, this Review is focused on MVs, taking into account the above mentioned limitations.

Although MVs were previously considered to be cellular debris, they are now recognized as vectors for intercellular information exchange. MVs—also referred to as microparticles or ectosomes—usually refer to vesicles 0.1–1 μm in diameter produced by budding from the plasma membrane. Another classic, but still debated, feature of MVs is the exposure of phosphatidylserine at their surface, which can be detected by labelling of annexin V or lactadherin using flow cytometry analysis.[3] Moreover, as MVs harbour most of the membrane-associated proteins of the cells they stem from, their cellular origin can be determined using flow cytometry using antibodies against specific cell-surface markers (Box 1). MVs also contain lipids, cytosolic proteins, RNAs and/or microRNAs, and regulate the function of the target cell. MVs can interact with their target cells in a variety of ways: release of the soluble mediators they contain; ligand–receptor interactions; fusion of MV membranes with those of the target cell and transfer of MV contents (especially RNAs and microRNAs); or phagocytosis/endocytosis by macrophages or endothelial cells.[4,5] The lipid, protein and nucleic acid fractions contained in MVs vary greatly depending upon the cell of origin and the stimulus initiating MV release.[6] Their diverse content translates into different downstream effects; for example, MVs isolated from human atherosclerotic plaques stimulate endothelial cell proliferation, whereas circulating MVs from the same patients do not.[7] As a result, although the information obtained using MVs generated *in vitro* provides important information regarding the mechanisms of these vectors, application of these results *in vivo* must still be elucidated. Thus, this Review pays particular attention to studies evaluating the effects of MVs isolated from patients or from animal models.

MVs are now implicated in several major fields of hepatology. This Review describes studies assessing circulating MV levels in patients with liver diseases, together with insights into the mechanisms that could

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Key points

- Microvesicles (MVs) are 0.1–1.0 μm vesicles containing lipids, proteins, RNAs and microRNAs; they are formed by budding from the cellular plasma membrane
- Circulating levels of several subpopulations of MVs are increased in patients with liver diseases, probably due to enhanced MV production and decreased MV clearance, related to the individual liver disorder
- MVs are now implicated at many stages of liver disease progression, including liver fibrogenesis, portal hypertension and activation of coagulation
- Several results suggest that MVs have a role in hepatocellular carcinoma by conveying information between tumour cells and between tumour and neighbouring cells
- High levels of circulating procoagulant MVs have been found in patients with acute liver failure and might contribute to normal or hypercoagulable global haemostasis in this setting
- MVs have promise as diagnostic and prognostic biomarkers in patients with liver diseases

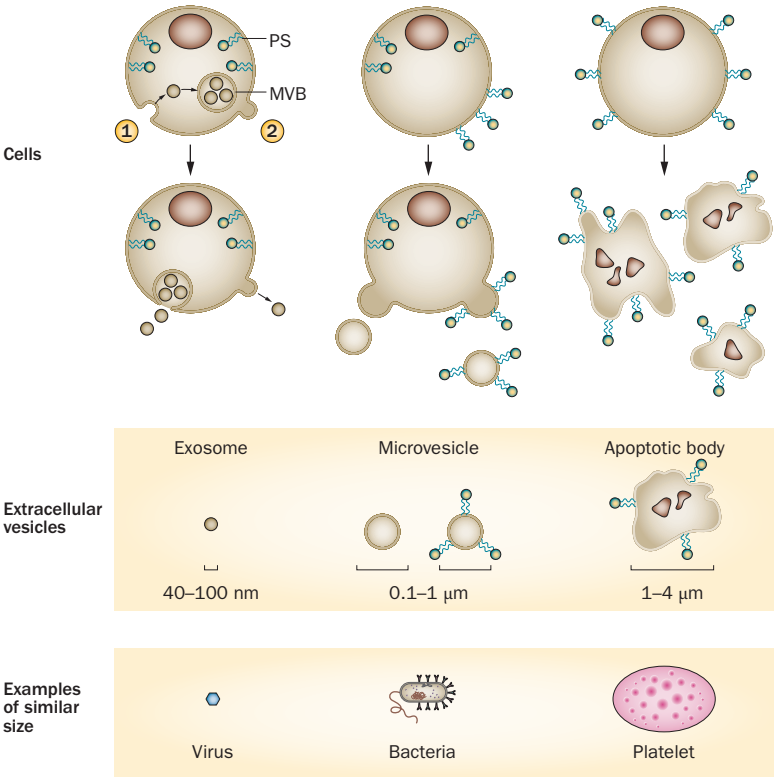

Figure 1 | Definitions of microvesicles. Left: Exosomes. Cells release exosomes via two mechanisms. In the classic pathway (1), intracellular vesicles appear from inward membrane budding and form MVBs before fusing with the PM and being released into the extracellular space. The direct pathway (2) involves the release of vesicles, indistinguishable from exosomes, directly from the PM without involvement of MVBs. Exosomes expose little or no PS at their surface, but harbour relatively specific markers. Middle: Microvesicles. Under normal conditions, PS is primarily located on the PM inner leaflet. After exposure to a stimulus, PS is exposed at the cell surface and cytoskeleton reorganization occurs leading to outward blebbing of the PM and release of MVs into extracellular space. However, formation of MVs might happen without externalization of PS as some MVs might be PS negative. Right: Apoptotic bodies. The early stages of apoptosis are characterized by changes in mitochondrial membrane potential and PM asymmetry leading to exposure of PS at the cell surface, but without increased cell permeability. Later stages of apoptosis are characterized by DNA fragmentation and increased PM permeability. Apoptotic cells shrink and can break up into smaller apoptotic bodies. Like MVs, apoptotic bodies harbour PS and contain various materials from mother cells (PM markers, proteins, RNA and microRNA). Unlike MVs, they contain nuclear fragments. Abbreviations: MV, microvesicle; MVB, multivesicular body; PM, plasma membrane; PS, phosphatidylserine.

Box 1 | Proposed markers of extracellular vesicles*

Exosomes

Alix, CD9, CD63, CD81, heat shock proteins, TSG101

Microvesicles

Phosphatidylserine (detected using annexin V or lactadherin)

Cell type marker

- Leukocyte: CD45, CD11a, CD11b
- Granulocyte: CD66b
- Monocyte: CD14
- Lymphocyte: $CD4^+$ and $CD8^+$ T cells, CD20 (B cell)
- Platelet: CD41a, CD42a, CD42b, $CD31^+/CD42^+$, CD61, CD62b, CD62p
- Erythrocyte: CD235a
- Endothelial cell: $CD31^+/CD41^-$, CD62e, CD51, CD105, CD144, CD146

Apoptotic bodies

Phosphatidylserine and/or DNA (detected using annexin V or propidium iodide), histones, DNA

*These markers are largely used and the best identified so far, although none of them can be considered as 100% specific.
Abbreviation: TSG, tumour susceptibility gene.

account for these changes. The potential roles of MVs in key processes of liver diseases, such as fibrosis, portal hypertension, complications of cirrhosis, thrombosis and hepatocellular carcinoma, are also presented. If specific studies in the context of liver diseases are lacking, we postulate on the potential effects of MVs on the basis of available data from other organs. Finally, we interpret available results and propose clinical situations in which MVs could be useful as biomarkers.

Increased MV levels in liver diseases

MVs have been detected in numerous human body fluids, including saliva,[8] urine,[9] bile,[10] synovial fluid,[11] vitreous fluid[12] and semen,[13] as well as in muscles,[14] atherosclerotic plaques[7] and liver tissue[15] (Figure 2a). Nevertheless, most studies have focused on plasma MVs, because they are easily accessible. Circulating levels of MVs are increased in patients with cardiovascular disorders, thrombosis and cancer.[16–18] Over the past ~10 years, several groups have measured the levels of circulating MVs in patients with liver diseases (Table 1).[19–30] The increased levels of several subpopulations of circulating MVs reported in these studies are a result of increased formation and/or decreased clearance of MVs (Figure 2b; Box 2).

Enhanced MV formation

Several causes of liver disease trigger MV production, in particular alcohol consumption,[31] viral infection[32] and features of the metabolic syndrome including diabetes,[33] obesity,[34] dyslipidaemia[35] and physical inactivity.[36] Liver disease itself might also induce MV release, as the main processes of MV formation (namely, apoptosis and cell activation) are common in this context.[37,38] Furthermore, many stimuli that promote MV release, including oxidative stress,[39] shear stress,[40,41] systemic inflammation and bacterial translocation, are present in liver diseases (Table 2).[10,39,42–49]

Decrease in MV clearance

The mechanisms of MV clearance have been reviewed elsewhere.[5] Under healthy conditions, spleen and liver macrophages are the primary contributors to MV clearance from the circulation.[5,50,51] Interestingly, several lines of evidence show that cirrhosis is associated with a defect in macrophage function. Indeed, the function of the macrophage Fc gamma receptor is impaired in patients with alcoholic cirrhosis and is correlated with the degree of liver insufficiency.[52] Similarly, macrophage dysfunction has been observed in animal models of cirrhosis[53] and the clearance of certain molecules (radiolabelled colloid or microaggregated human serum albumin) has been shown to be decreased in patients with cirrhosis.[54,55] As such, we speculate that clearance of MVs might also be decreased in these patients. During pathological conditions that are associated with elevated levels of circulating MVs, such as endotoxaemia, another pathway for MV clearance is 'turned on' in the liver and lung endothelium.[56] As cirrhosis is associated with detectable endotoxaemia,[42] it would be interesting to determine whether this pathway is activated or defective in cirrhosis. In healthy individuals, platelets are the major source of MVs; the reason why platelet-derived MVs are not systematically increased in patients with cirrhosis is unclear.[21,24]

MVs and liver fibrosis

MVs and fibrolysis

MVs have been suggested to promote the regression of fibrosis in the liver.[22] Indeed, circulating levels of T-cell MVs are elevated in patients with active hepatitis C compared with patients with mild hepatitis C and healthy controls. MVs released *in vitro* from T cells fused with hepatic stellate cell (HSC) membranes, leading to an upregulation of matrix metalloproteinases *MMP1*, *MMP3*, *MMP9* and *MMP13* gene expression; downregulation of the gene encoding procollagen-α1(I); and blunting of profibrogenic activities of transforming growth factor β1 (Figure 3). Although zymography was not performed to confirm these results, the induced fibrolytic activity seemed evident with MVs from activated $CD4^+$ T cells and even higher with MVs from activated and apoptotic $CD8^+$ T cells. Basigin (also known as CD147 and EMMPRIN) exposed on T-cell MVs contributed to the induction of HSC fibrolytic activity.[22] These results are in-line with previous reports showing that MVs generated *in vitro* from T cells and monocytes strongly induce synthesis of matrix metalloproteinases in fibroblasts.[57] MVs also carry proteases themselves, as reported for MVs generated *in vitro* from endothelial cells and neutrophils under static conditions.[58,59] It should be highlighted that all these results have been obtained using MVs generated *in vitro* and tested on cultured cells (Box 3).

Although not yet investigated in the context of liver diseases, indirect evidence indicates that MVs could also promote fibrolysis via the microRNAs they contain.[60] MicroRNAs are short noncoding RNAs that bind to messenger RNAs to regulate gene expression.[61]

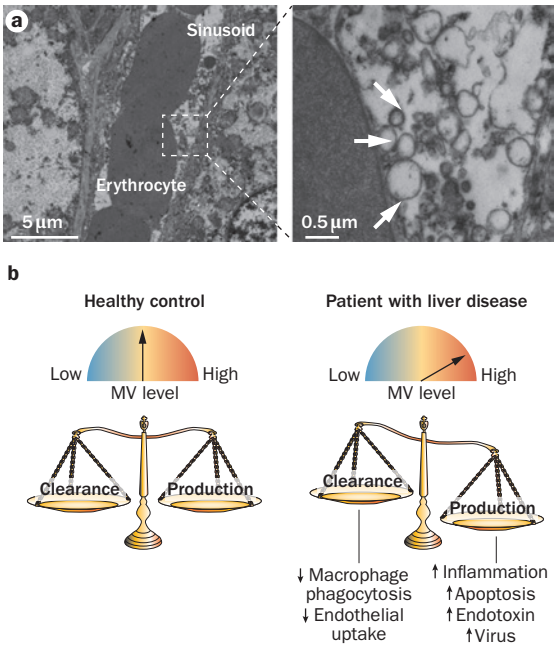

Figure 2 | Microvesicles in liver diseases. **a** | Transmission electron microscopy image of a liver biopsy performed in a patient with chronic HCV infection. Within sinusoids, erythrocytes can be seen. Insert: presence of vesicles (white arrows) of MV size. **b** | In a healthy individual, the formation and clearance of MVs are balanced whereas in a patient with a liver disease, MV formation is likely enhanced and MV clearance decreased. This imbalance leads to an increase in the levels of several MV subpopulations in patients with liver diseases. Abbreviation: MV, microvesicle.

Circulating microRNAs are transported by MVs, as well as by exosomes, lipoproteins and ribonucleoprotein complexes (such as Argonaute 2), which protect them from rapid degradation by plasma RNAses. A quantitative analysis published in 2012 of circulating microRNAs in patients with coronary artery disease showed that the majority of plasma microRNAs are associated with MVs.[62] The profiles of microRNAs in MVs were found to be markedly different from those in their maternal cells, indicating an active mechanism of selective MV 'packaging'.[62] Interestingly, MVs released from cultured endothelial progenitor cells *in vitro* prevented tubulo–interstitial fibrosis and preserved renal function 180 days after injection into a rat model of ischaemia–reperfusion injury.[63] Several convincing experiments have revealed that the protective effect of these MVs is based on the microRNAs (specifically miR-126 and miR-296) they contain.[63] Other microRNAs also contribute to the regression of fibrosis in the liver, as reviewed elsewhere.[60] It has not yet been determined if these microRNAs are also harboured by MVs.

MVs and fibrogenesis

Although data show that MVs mitigate liver fibrosis, the process seems to be complex (Figure 3). Angiogenesis is proposed to promote fibrogenesis in the liver and hepatic fibrosis usually decreases when angiogenesis is pharmacologically inhibited.[64] Angiogenesis is increased by

Table 1 | Circulating MVs in patients with liver diseases

Liver disease (number of patients)	Method	Analysed subpopulations of MVs	Potential interest of MVs as a biomarker	Reference
Hepatitis B (n=11) Hepatitis C (n=25)	FCM	Platelets	Marker of liver fibrosis in chronic hepatitis C	Fusegawa *et al.* (2002)[19]
Hepatitis C (n=16) HCC (n=8)	FCM	Annexin V-positive, endothelial, hepatocyte	Marker of HCC and post-transplantation outcome	Brodsky *et al.* (2008)[20]
Hepatitis B and C related cirrhosis (n=60)	FCM	Platelets	ND	Sayed *et al.* (2010)[21]
Hepatitis C (n=21)	FCM	Annexin V-positive, lymphocyte, macrophages, neutrophils, platelets	Marker of liver inflammation and fibrosis	Kornek *et al.* (2011)[22]
Alcoholic liver disease (n=20) Hepatitis C related cirrhosis (n=9)	FCM	Platelets	ND	Ogasawara *et al.* (2005)[23]
Hepatitis C (n=20) Alcoholic cirrhosis (n=71)	FCM	Annexin V-positive, leukoendothelial, lymphocyte, platelets, hepatocyte, erythrocyte	Marker of cirrhosis severity	Rautou *et al.* (2012)[24]
NAFLD (n=67) Hepatitis C (n=42)	FCM	Annexin V-positive, lymphocytes, natural killer T cells or macrophage, neutrophils, platelets	Marker of liver inflammation	Kornek *et al.* (2012)[25]
Liver transplantation (n=19)	FCM	Platelets	Marker of ischaemia–reperfusion injury	Esch *et al.* (2010)[26]
Acute liver failure (n=20)	MV PS activity assay	PS-positive	ND	Agarwal *et al.* (2012)[27]
Acute liver failure with (n=20) and without (n=20) kidney injury	MV PS activity assay	PS-positive	ND	Agarwal *et al.* (2013)[28]
Acute liver failure (n=50)	FCM; ISADE™; MV tissue factor activity	Annexin V-positive, endothelial, hepatocyte, monocytes, platelet, tissue-factor-positive	Prognostic marker	Stravitz *et al.* (2013)[29]
Acute liver failure (n=5) Acute-on-chronic liver failure (n=5)	FCM	Hematopoietic stem cells	Marker of acute liver insults	Schmelze *et al.* (2013)[30]

Abbreviations: FCM, flow cytometry; HCC, hepatocellular carcinoma; ISADE™, Invitrox sizing, antigen detection and enumeration (Invitrox, NC, USA); MV, microvesicle; ND, not determined; PS, phosphatidylserine.

MVs generated *in vitro* from platelets,[65] lymphocytes,[66] or endothelial progenitor cells,[67] and by those obtained from mouse ischaemic hind-limb muscles.[14] The pro-angiogenic effect of MVs suggests that they promote fibrogenesis, a hypothesis supported by data from Feldstein and colleagues who observed that hepatocytes exposed to saturated, but not monounsaturated, free fatty acids released MVs *in vitro* that induced marked angiogenesis both *in vitro* and *in vivo*, in a manner dependent on the ectoenzyme pantetheinase (also known as vanin-1).[49] Vanin-1 is found primarily in the liver, kidney epithelia and intestine, and has been linked with promotion of cell adherence and migration.[49] In a common mouse model of dietary-induced steatohepatitis, this group detected high levels of hepatocyte-derived MVs associated with marked angiogenesis and early liver fibrosis. Genetic inhibition of caspase-3 limited the increase in circulating hepatocyte-derived MVs and protected mice from angiogenesis and fibrosis, independent of lipid accumulation and inflammation.[49] Although these results are not direct, they suggest that hepatocyte-derived MVs contribute to angiogenesis and liver fibrosis in steatohepatitis.

HSCs are the main precursor of liver myofibroblasts and have a key role in fibrosis.[68] A pioneer study by Diehl and colleagues showed that cultured HSCs release MVs that expose Hedgehog ligands on their surface. These ligands induce the expression of inducible nitric oxide synthase (iNOS) by primary sinusoidal endothelial cells *in vitro*;[10] MVs isolated from the plasma or bile of bile-duct-ligated rats also have a similar effect.[10] iNOS expression in the liver increases in cirrhosis,[69] and iNOS-deficient mice have less fibrosis after chronic carbon tetrachloride administration than wild-type mice,[70] supporting a profibrogenic role for iNOS. Therefore, HSC MVs might enhance fibrogenesis by inducing iNOS expression.[10] Finally, as discussed in a later section, MVs have procoagulant properties.[71,72] Results from various studies suggest that coagulation activation promotes liver fibrosis[73] and, as such, MVs might also promote fibrogenesis.

In conclusion, the overall effect of MVs on liver fibrosis is still unclear and dedicated studies in animal models are required to fully elucidate their role. However, such studies are hampered by the short half-life of MVs in the circulation precluding assessment of their long-term effects,[5] and by the lack of genetically modified mice that are deficient in MV formation without other marked defects. An acceptable option would be to use daily injections of diannexin, an annexin V homodimer that cloaks phosphatidylserine residues on the surfaces of MVs and acts as an inhibitor of MVs.[74]

Box 2 | Challenges in assessing microvesicles in the portal vein

Analysis of the levels and cell origin of microvesicles (MVs) present in the portal and hepatic veins would provide some insight into the role of the liver in MV clearance and production. However, obtaining such blood samples suitable for MV analysis is complex in patients, as well as in animal models.

In patients, portal blood can be obtained during transhepatic intravenous portosystemic shunt (TIPS) placement or revision, or during abdominal surgery. TIPS placement is associated with liver injury that immediately activates blood coagulation and thus induces a strong release of MVs. Extrapolating results obtained from portal vein blood at the time of TIPS revision to what happens in patients without TIPS would also be hazardous because of the systemic changes associated with TIPS. Besides ethical considerations, data obtained using blood drawn from the portal vein at the time of a surgery should be interpreted cautiously, as the reason for such a surgery is usually a digestive tract disease, which can also induce MV release.

Concerns have also been raised about the use of mouse models. In particular, we observed that circulating levels of annexin V-containing MVs are much lower in rats with carbon-tetrachloride-induced cirrhosis than in controls injected with the vehicle only (Rautou, P.-E. and Boulanger, C. M., unpublished data), which is not what has been reported in patients with cirrhosis (Table 1). These low MV levels probably result from the high phospholipase A2 activity induced by carbon tetrachloride. Indeed, phospholipase A2 is able to hydrolyze phospholipids, such as phosphatidylserine or phosphatidylcholine, exposed at the surface of MVs. Bile duct ligation seems to be a good chronic liver disease model to study MVs (Rautou, P.-E. and Mackman, N., unpublished data). Yet, drawing blood from the portal vein of bile-duct-ligated animals without activating coagulation, that is, by using a sufficiently large needle, is tricky.

MVs and portal hypertension

MVs and intrahepatic vascular resistance

Portal hypertension is a frequent and severe complication of cirrhosis, a leading cause of death and an indication for liver transplantation. The pathogenesis of portal hypertension is mainly related to a combination of structural and dynamic components that cause an increase in hepatic vascular resistance to portal blood flow.[75] The structural components include fibrosis, regenerative nodule formation and vascular remodelling. The potential role of MVs in liver fibrosis has already been discussed. MVs might also contribute to vascular remodelling in cirrhosis through the release of MVs expressing Hedgehog ligands from HSCs. These MVs induce changes in sinusoidal endothelial cell gene expression *in vitro*, presumably contributing to their activation and to sinusoidal remodelling.[10] Portal myofibroblasts also release MVs that stimulate angiogenesis both *in vitro* and in mice, in a VEGF-dependent manner, thus enhancing vascular remodelling.[76]

The dynamic components of portal hypertension include increased hepatic vascular tone, related to elevated activity of several endogenous vasoconstrictors, and reduced bioavailability of intrahepatic vasodilators. Of note, cirrhotic livers exhibit reduced nitric oxide (NO) availability, owing to decreased endothelial nitric oxide synthase (eNOS) activity and increased NO scavenging during elevated oxidative stress.[75] By contrast, liver iNOS expression is greater in the liver of cirrhotic rats than in sham controls.[69] This contrast is reminiscent of the local inhibition of eNOS by iNOS, possibly via NO autoinhibition, described in the kidneys.[77] As bile-duct-ligated rats produce MVs that increase iNOS expression in sinusoidal endothelial cells (Figure 4),[10] MVs might promote intrahepatic vasoconstriction via an increase in hepatic iNOS expression leading to eNOS inhibition.

MVs and splanchnic arterial vasodilatation

Progressive splanchnic arterial vasodilatation also enhances portal blood flow, aggravating and perpetuating portal hypertension. Vasodilation is associated with an increased release of vasodilators and a vascular hyporesponsiveness to vasoconstrictors. MVs seem to contribute to this so-called vascular hypocontractility in patients with advanced cirrhosis; indeed, we have shown that patients with Child–Pugh B or C cirrhosis have increased circulating levels of MVs of leukoendothelial, pan-leucocyte, lymphocyte, erythrocyte and hepatocyte origin.[24] These MVs impair the response of aortic rings to vasoconstrictive agents *ex vivo*, and decrease arterial blood pressure in mice via the transfer of phospholipids from MVs to endothelial cells. Phospholipid metabolism leads to the formation of prostacyclin, which diffuses abluminally and causes vascular smooth muscle relaxation (Figure 4). Although the origin of the MVs causing this effect was not identified, it is interesting to note that this hyporeactive effect is specific to circulating MVs in patients with advanced cirrhosis and was not

Table 2 | Stimuli of MV release relevant for liver diseases

Stimulus	Source of MVs	Content of MVs	Effect of MVs	Study
Oxidative stress	Rat primary hepatocye	ND	ND	Miyoshi *et al.* (1996)[39]
High shear stress	Platelets	ND	Procoagulant	Nomura *et al.* (2000)[43]
PDGF BB	Rat HSC cell line Mouse cholangiocyte cell line	Hedgehog ligands	Activation of sinusoidal endothelial cells	Witek *et al.* (2009)[10]
Lipopolysaccharide	HCC cell line H22	miRNA let7b	Decrease in inflammation	Li *et al.* (2012)[48]
IL-1-α	Endothelial cells	Caspase-3	ND	Abid Hussein *et al.* (2005)[44]
TNF	Endothelial cells	Tissue factor	Procoagulant	Szotowski *et al.* (2007)[46]
Fas ligand	Smooth muscle cells	ND	Endothelial dysfunction	Essayagh *et al.* (2005)[45]
Cholesterol	Monocytes	Tissue factor	Procoagulant	Liu *et al.* (2007)[47]
Fatty acid	Primary rat hepatocyte Human HCC cell line	Vanin-1	Proangiogenic	Povero *et al.* (2013)[49]

Abbreviations: HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; MV, microvesicle; ND, not determined; PDGF BB, platelet-derived growth factor BB.

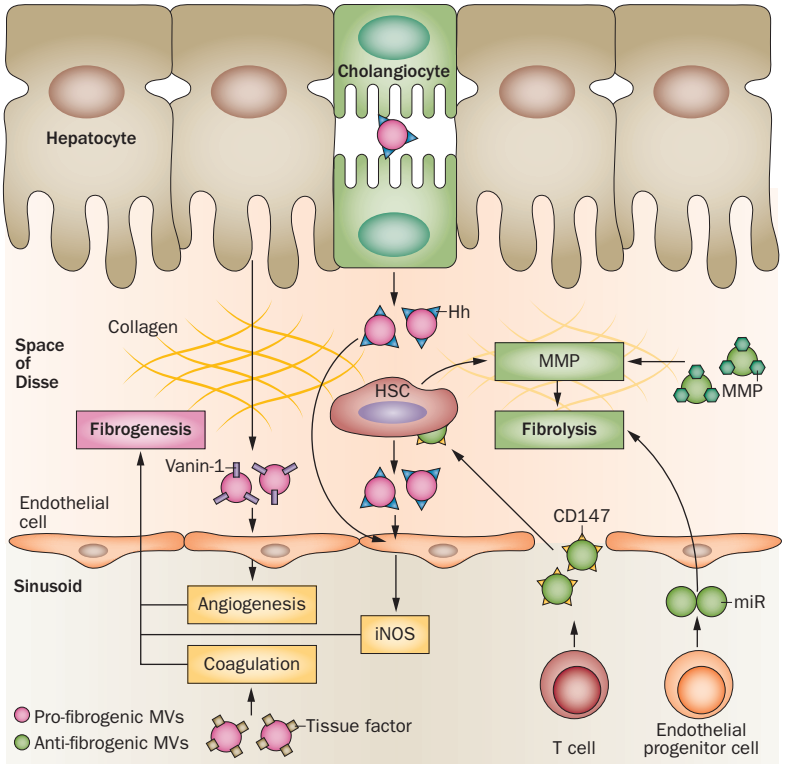

Figure 3 | Microvesicles in liver fibrosis. Some MVs (pink) promote fibrogenesis such as those produced by hepatocytes containing vanin-1 (which induce angiogenesis) or those that expose phosphatidylserine and/or tissue factor on their surface (activating coagulation). Cholangiocytes and HSCs release MVs containing Hh, which might also promote fibrogenesis by increasing iNOS expression. Other subpopulations of MVs decrease fibrosis (green). CD147-containing MVs released by T cells can be taken up into HSCs and upregulate MMP secretion. MVs containing MMP as well as miR-containing MVs released by endothelial progenitor cells might also promote fibrolysis. Abbreviations: Hh, Hedgehog ligand; HSC, hepatic stellate cell; iNOS, inducible nitric oxide synthase; miR, microRNA; MMP, matrix metalloproteinase; MV, microvesicle.

Box 3 | Liver sinusoidal endothelial cells and MV traffic

A key feature of sinusoidal endothelial cells should be kept in mind when interpreting the effects of microvesicles (MVs) present in the sinusoids on hepatic stellate cells or on hepatocytes: fenestrae (open pores that act as a dynamic filter). As the diameter of fenestrae ranges from 0.05–0.3 μm in humans, only some MVs can cross them in the healthy physiological state. Given the reported differential effect of MVs according to their size, this feature might confound the interpretation of consequences of MVs in the liver. The changes in fenestrae size in liver diseases further complicate this matter. For instance, fenestrae diameter increases after exposure to endotoxin. Finally, although MV size is defined as ranging from 0.1–1.0 μm whatever the species, fenestrae size varies between mice and humans, so observations made in one might not apply to the other.

observed with MVs from patients with Child–Pugh A cirrhosis or in patients with end-stage renal disease.[24] By contrast, circulating MVs in patients with septic shock increase thromboxane A2 production, which enhanced the sensitivity of mouse aorta to vasoconstrictors. According to the authors of this study, these findings support a role for MVs in protecting against vascular hyporeactivity and hypotension in patients with septic shock.[78] Specific studies to determine the vascular consequences of MVs in patients with cirrhosis and septic shock are needed. As the outcome in these patients is poorer than in those with septic shock without cirrhosis, it is tempting to speculate that the effect of MVs related to cirrhosis predominate, enhancing vascular hyporeactivity and contributing to the severity of septic shock in cirrhosis.

Outside the liver, angiogenesis has been shown to contribute to the formation of portosystemic collaterals and increase in splanchnic blood flow.[75] Further studies are needed to address the role of MVs in this setting.

MVs and the complications of cirrhosis

To date, no direct evidence has shown that MVs have a role in the complications of cirrhosis, with the exception of portal hypertension, as stated earlier. However, several properties of MVs demonstrated in various non-digestive diseases suggest that MVs contribute to the development of ascites, hepatic encephalopathy, hepatopulmonary syndrome, portopulmonary hypertension and hepatorenal syndrome.

Ascites

Enhanced endothelial permeability in the mesenteric vascular bed contributes to the formation of ascites in cirrhosis,[79] and endothelial MVs might directly increase endothelial permeability.[80] This effect was initially reported in the pulmonary capillaries of C57BL/6 mice injected with endothelial MVs.[80] By contrast, another study found no increase in permeability with MVs generated following exposure of endothelial cells to exogenous activated protein C.[81] However, this stimulus is not relevant to cirrhosis as protein C is synthesized by the liver, and the plasma levels of this protein are decreased in cirrhosis.[82] MVs have been detected in the ascites of patients with ovarian[83,84] and colorectal cancer,[85] but have not been measured in the ascites of patients with cirrhosis. In the past, the presence of ascites was considered to have little or no influence on the homeostasis of the peritoneal vasculature. However, this view has been challenged by several studies demonstrating that this liquid might possess vasodilatory, proinflammatory and proangiogenic properties.[86–88] Further studies are needed to show if MVs contribute to these properties.

Hepatic encephalopathy

Hepatic encephalopathy is a result of astrocyte swelling and is aggravated by systemic inflammation related to increased levels of proinflammatory cytokines and lipopolysaccharide (LPS).[89] These systemic factors could interact with the brain parenchyma via the endothelial cells of the blood–brain barrier (BBB), in barrier-deficient areas, or by stimulation of peripheral nerves.[90] Some data suggest that MVs contribute to hepatic encephalopathy. First, patients with cirrhosis and encephalopathy have 3.5-fold more leukoendothelial

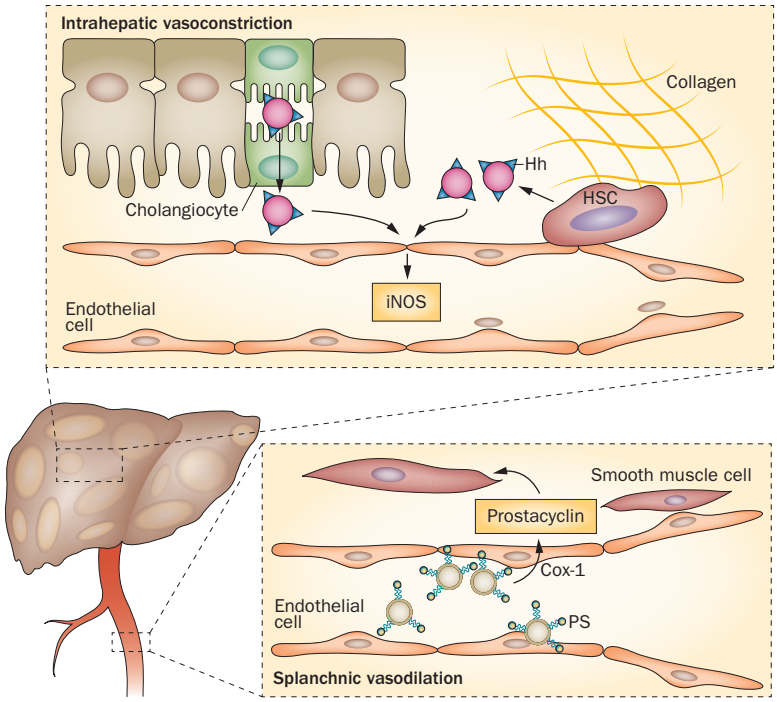

Figure 4 | Microvesicles in portal hypertension. Upper insert: In the cirrhotic liver, cholangiocytes and HSCs produce MVs containing Hh. These Hh-containing MVs induce endothelial expression of iNOS, probably contributing paradoxically to the intrahepatic vasoconstriction associated with cirrhosis. Vascular resistance is also increased by collagen deposition and contraction of HSCs, wrapped around endothelial cells. Lower insert: In cirrhosis, the splanchnic vascular bed is dilated. Levels of leukoendothelial, lymphocyte, erythrocyte and hepatocyte MVs are increased in the systemic circulation. These MVs expose PS at their surface that can be transferred (with other membrane phospholipids) to endothelial cells. Phospholipids are then used as substrates for the arachidonic acid pathway, including Cox-1 (which is an enzyme involved in the formation of vasodilator agents, such as prostacyclin), leading to smooth muscle cell relaxation. Abbreviations: Cox-1, cyclo-oxygenase-1; Hh, Hedgehog ligand; HSC, hepatic stellate cell; iNOS, inducible nitric oxide synthase; MV, microvesicle; PS, phosphatidylserine.

MVs than those without encephalopathy.[24] Second, endothelial MVs might increase BBB endothelial cell permeability as they were shown to increase pulmonary capillary permeability in mice.[80] Third, brain endothelial cells might release MVs that induce astrocyte swelling. Indeed, the conditioned medium of endothelial cells pretreated with ammonia, LPS or proinflammatory cytokines causes astrocyte swelling.[91] As LPS and pro-inflammatory cytokines are known triggers for MV release by endothelial cells (Table 2), astrocyte swelling might be mediated by MVs; this hypothesis needs to be confirmed in additional studies.

Pulmonary complications

Hepatopulmonary syndrome is defined by the clinical triad of chronic liver disease, arterial hypoxaemia and intrapulmonary vascular dilatations.[92] It is characterized by dilatation of precapillary and capillary pulmonary vessels and an absolute increase in the number of dilated vessels, suggesting angiogenesis. Although the role of MVs in hepatopulmonary syndrome has not yet been assessed, it should be considered, because, theoretically, MVs modulate the main mechanisms—pulmonary vasodilation and angiogenesis—involved in the development of this syndrome. MVs affecting lung vessels could be released by the injured liver and reach the pulmonary vasculature via the hepatic veins. Alternatively, MVs from the gut could reach the lungs through portosystemic collaterals. This latter hypothesis suggests that liver detoxification is essential to prevent pulmonary vascular dilatation, which is supported by the development of hepatopulmonary syndrome in patients with type 1 Abernethy malformation (defined as a direct connection between the portal vein and the inferior vena cava and the absence of a portal vein in the liver).[92] Increasing evidence also suggests that MVs have a role in the pathophysiology of pulmonary hypertension, which might be relevant to the pathophysiology of portopulmonary hypertension, as reviewed elsewhere.[93]

Hepatorenal syndrome

Hepatorenal syndrome is characterized by functional renal failure in patients with cirrhosis and ascites. MVs from patients with advanced cirrhosis could also have a role in hepatorenal syndrome by contributing to systemic and splanchnic vasodilation (a major factor in the pathogenesis of this syndrome), as already discussed.[24] Moreover, MVs have been shown to induce renal vasoconstriction in sickle cell disease, a mechanism that could also be involved in hepatorenal syndrome.[94]

MVs and coagulation disorders

MVs are intrinsically procoagulant because they expose phosphatidylserine at their surface. Phosphatidylserine, an anionic phospholipid, interacts with positively charged domains in the clotting proteins such as factors VII, IX and X, and prothrombin,[71,72] thereby promoting the assembly of the clotting cascade. Capture assays use this property to quantify levels of MVs. MVs can also expose tissue factor (a transmembrane glycoprotein that is the primary initiator of the coagulation cascade) at their surface. Tissue-factor-bearing MVs are highly procoagulant and mostly originate from monocytes.[72]

Cirrhosis is associated with a procoagulant imbalance as shown by the results of thrombin-generation tests performed in the presence and absence of thrombomodulin.[95] In addition, clinical evidence now indicates that patients with cirrhosis are not protected from, and might even be at increased risk of, extrasplanchnic deep vein thrombosis.[96] This procoagulant state is thought to be a result of two alterations that are typically found in patients with cirrhosis: markedly increased factor VIII plasma levels (one of the most potent drivers of thrombin generation) and a concomitant decrease in protein C levels (one of the most potent anticoagulant drivers that quenches thrombin generation).[97] Even though the thrombin generation assay has been very useful to evaluate the coagulation alterations associated with cirrhosis, it has several limitations. In particular, it does not take into account the procoagulant activity of MVs as it is performed after adding exogenous phospholipids and tissue factor to plasma to trigger coagulation. Although

at low concentrations, these agents could overwhelm the endogenous procoagulant activity of MVs.

The role of phosphatidylserine in the procoagulant imbalance associated with cirrhosis might not be significant, as no difference was seen in the levels of phosphatidylserine-positive MVs between patients with cirrhosis and healthy controls in one study. However, another group found increased levels of phosphatidylserine-positive MVs in patients with cirrhosis in a smaller patient population.[20,24] Tissue-factor-positive MVs might have a more important role in the increased coagulability associated with cirrhosis than those exposing only phosphatidylserine at their surface. Indeed, levels of these highly procoagulant MVs are elevated in patients with cirrhosis potentially as a result of endotoxaemia, a common feature of cirrhosis[98] known to induce tissue factor expression in monocytic and endothelial cells and the release of tissue-factor-positive MVs.[72,99]

Although haemostatic abnormalities are an invariable feature of acute liver failure, patients rarely develop bleeding complications despite substantially elevated international normalized ratio of prothrombin times.[100] Indeed, patients with acute liver failure seem to be more prone to thrombosis than to bleeding complications, and intrahepatic thrombosis might exacerbate initial liver injury.[101] Studies in patients and in animal models suggest that MVs contribute to this surprisingly normal or hypercoagulable global haemostasis in acute liver failure.[27,29,102] The procoagulant activity of MVs due to tissue factor and phosphatidylserine in patients with acute liver injury is 38-fold and fourfold higher respectively than that in healthy controls.[27,29] Increased levels of procoagulant platelet-derived MVs have also been observed in a mouse model of sepsis, which is associated with multiorgan failure, including the liver.[102] When these MVs are reinjected into nonseptic mice, they promote thrombi formation in the liver. Interestingly, transgenic mice expressing low levels of calpain (a protein involved in MV formation) not only have decreased levels of procoagulant MVs when they are exposed to sepsis as compared with mice with normal calpain levels, but also less disseminated intravascular coagulation and improved organ function and survival.[102]

MVs and hepatocellular carcinoma

Studies published over the past 5 years assessing levels of circulating MVs in large groups of patients with cancer did not include patients with hepatocellular carcinoma (HCC).[17,103] One pilot study investigating MV levels in a small group of patients with HCC reported associations between tumour size and plasma levels of hepatic and endothelial MVs.[20] Moreover, MV levels progressively decreased once HCC was removed by liver transplantation.[20]

Several groups have investigated the role of MVs in the development of cancer[104] and some of these findings can be tentatively extended to HCC (Figure 4). First, angiogenesis (a key feature of HCC) was demonstrated to be promoted by the transfer of oncogenic proteins by MVs.[105] *In vitro* experiments revealed that several human cancer cells lines produce MVs containing the oncogenic epidermal growth factor receptor (EGFR) that could be taken up by endothelial cells and enhance angiogenesis.[105] Moreover, in mice with human tumour xenografts, diannexin reduced the growth rate and microvascular density of tumours.[105] Second, MVs containing active oncogenes (oncosomes) might also serve as vehicles for rapid intercellular transfer of transforming activity between cells populating tumours. For instance, glioma cells expressing a truncated and ongenic form of EGFR, called EGFRvIII, release MVs containing this receptor. Such MVs can merge with plasma membranes of cancer cells lacking EGFRvIII, and so transfer the oncogenic activity.[106] Third, MVs might also favour multidrug resistance, a major cause of unsuccessful cancer treatment. Multidrug resistance is caused by overexpression of efflux transporters (such as P-glycoprotein), and MVs can transfer P-glycoprotein intercellularly from multidrug-resistant donor cells to drug-sensitive recipient cells.[107] Moreover, the expression of genes associated with vesicle shedding correlates with chemosensitivity profiles.[108]

In patients with HCC, stromal and vascular invasions contribute to tumour progression. MVs containing CD147 can be released by T cells as well as by tumour cells, thus stimulating expression of matrix metalloproteinases in fibroblasts and facilitating tumour invasion and metastasis.[22,109] Findings from a study published in 2013 indicate that MVs are involved in the acquisition of a metastatic phenotype by HCC. MVs released *in vitro* by mouse innate immune cells were taken up by a cultured mouse HCC cell line leading to tumour cell migration, invasion and attachment to the endothelium *in vitro* and to metastasis when injected into mice.[110] This effect was mediated by integrin α-M–integrin β-2 (also known as CD11b–CD18), an integrin involved in migration and cell adhesion, which was transferred from immune cells to tumour cells via MVs (Figure 5).[110] These results indicate that MVs derived from activated innate immune cells can act as a ferry to transfer innate molecules to tumour cells, leading to tumour metastasis.

MVs could also have anti-tumoral properties as MVs generated *in vitro* from human adult liver stem cells were shown to inhibit proliferation and induce apoptosis of HCC cells (HepG2 cell line and primary hepatocellular cells) both *in vitro* and in mice by delivering microRNAs.[111] This biological effect was specific as it was not observed with MVs derived from human fibroblasts even after they were modified to facilitate their incorporation into HCC cells. However, one limitation of this study was that the procedure used to isolate the MVs also isolated exosomes.

Altogether, these data suggest that MVs stimulate, more than inhibit, the development of HCC. This view is supported by the inhibition of tumour progression induced by diannexin.[105]

MVs as biomarkers for liver diseases

Portal vein thrombosis is uncommon in patients with compensated cirrhosis, but the incidence seems to rise

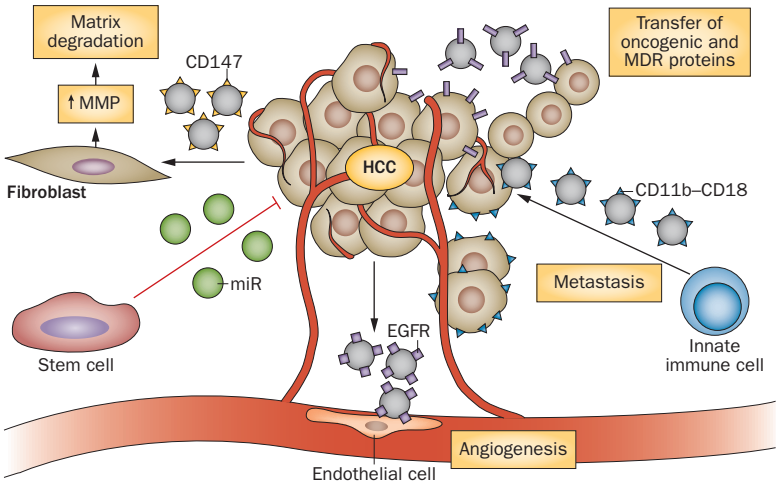

Figure 5 | Microvesicles in hepatocellular carcinoma. HCC cells might release MVs that interact with neighbouring cells to promote carcinogenesis. Tumoral stroma contains fibroblasts, which can favour tumoral progression. Tumour MVs containing CD147 induce upregulation of MMPs in fibroblasts, leading to extracellular matrix degradation and tumoral invasion. Tumour MVs can also transfer oncogenic forms of growth factor receptor or multidrug-resistant proteins to other tumour cells, thus propagating an increased proliferative, survival, and/or mitogenic capacity. In addition, EGFR, which is upregulated in HCC, could be transferred via tumour MVs to endothelial cells to promote angiogenesis to vascularize the growing tumour. Non-tumour cells can promote HCC metastasis via release of MVs: innate immune cells produce MVs harbouring CD11b–CD18, which can be taken up by HCC cells and promote their capacity of migration, invasion, attachment to the endothelium and metastasis. Non-tumour cells can also produce MVs with antitumoral properties. For instance, stem cells release MVs containing miR that inhibit proliferation of hepatic tumour cells. Abbreviations: HCC, hepatocellular carcinoma; MDR, multidrug resistant; MMP, matrix metalloproteinase; miR, microRNA; MV, microvesicle.

with the severity of liver disease.[73] The use of anticoagulants, however, decreases the risk of portal vein thrombosis.[112] Biomarkers of the risk of thrombosis could help to select patients who can benefit most from anticoagulant therapy and avoid exposing patients who have a low risk of thrombosis to the complications of this treatment. Data from patients without liver disease and the lack of increase in annexin V-positive MVs in patients with cirrhosis, suggest that phosphatidylserine-positive MVs or platelet MVs will probably not be useful as biomarkers.[24] On the other hand, MVs containing tissue factor might be more helpful.[17,99]

Levels of MVs originating from $CD4^+$ and $CD8^+$ T cells are increased in patients with chronic hepatitis C, whereas levels of MVs from $CD14^+$ and invariant natural killer T cells are augmented in patients with NAFLD.[25] This observation might be helpful in identifying the cause of liver blood test abnormalities in patients with HCV infection and the metabolic syndrome after liver transplantation. In this context, MVs could also help to exclude acute rejection as after liver transplantation, the dynamic course of circulating MVs seems to be modified by acute rejection.[20] Indeed, levels of MVs in the plasma in one patient who developed rejection remained elevated whereas levels progressively decreased after surgery in patients without rejection.[20] Other investigators have shown that miR-146a was upregulated sixfold in circulating MVs isolated from rats with acute rejection of a liver transplant.[113]

Circulating MVs are potential noninvasive markers of disease severity and activity in chronic hepatitis C, NAFLD and cirrhosis,[22] but these promising results must be validated in independent cohorts. The potential added value of measuring plasma MV levels should be compared with that of other available noninvasive tools, such as transient elastography, NAFLD-fibrosis score and MELD score, respectively.

MVs might also be valuable biomarkers for acute liver diseases. Plasma MV levels were considerably increased in 50 patients with acute liver injury or acute liver failure when compared with healthy individuals.[29] MV levels were associated with the presence of the systemic inflammatory response syndrome. Multivariate logistic regression analysis showed that MV concentrations were independently associated with death and liver transplantation in patients with acute liver failure.[29] Most of the circulating MVs found in these patients were positive for the platelet marker CD41. Thus, MVs with a prognostic value for acute liver failure are not produced by liver cells but by platelets, supporting the hypothesis that the severity of acute liver failure is related to systemic inflammatory response syndrome and coagulopathy. Other authors have reported that haematopoietic stem cell MVs are increased in patients with acute liver failure.[30]

Future directions

Marked progress in the field of MVs will be made once investigators pay close attention to both the preanalytical and analytical conditions for studying these particles. The efforts made by the International Society on Thrombosis and Haemostasis (ISTH) and International Society of Extracellular Vesicles (ISEV) over the past 3 years will hopefully lead to standardization of the isolation procedures and identification and analysis of MVs and other extracellular vesicles.[1,114] Two ongoing projects are working on this standardization: the European Metrology Research Programme (EMRP) project MetVes,[115] and the European Cooperation in Science and Technology (COST) programme 'Microvesicles and Exosomes in Health and Disease (ME-HAD).[1,161]

MVs have promise as biomarkers for diagnostic and prognostic purposes in patients with acute or chronic liver diseases as they are implicated as having roles in many stages of livers disease development and progression. In the future, investigators will have to validate these promising results in large independent cohorts of patients. Importantly, the added value of MV measurement will have to be compared with other already available and pertinent biomarkers. Investigators will also have to develop new strategies to provide routinely available MV assays, so they are no longer restricted to expert laboratories. Original approaches will probably be developed, including analysis of the cargo of MVs, such as microRNAs. From a pathophysiological point of view, *in vivo* studies are urgently needed owing to the sometimes contrasting observations made using *in vitro* MVs, as seen in fibrosis.

In the future, MVs might be used as therapeutic targets, such as MVs promoting liver tumorogenesis. However, specific inhibition of certain types of MVs without affecting the mother cell remains tricky. MVs are more likely to be used as therapeutic vectors, for instance to enhance liver regeneration. Indeed, MVs released by human liver stem cells *in vitro* were able to accelerate recovery of liver after hepatectomy in rats.[117] This capacity was directly related to the transfer of specific mRNA from liver stem cells to hepatocytes by MVs. Labelling of MVs with magnetic nanoparticles could be used in the future for targeting and concentrating MVs in the liver.[50]

Conclusions

MVs seem to be active mediators of intercellular communication in liver diseases. On the one hand, the injured liver releases MVs that act locally in a paracrine manner to modulate key processes such as fibrosis, angiogenesis, intrahepatic vasoconstriction and tumour development. MVs might also target remote organs such as the brain and induce hepatic encephalopathy. On the other hand, other organs and cells release MVs that might influence liver disease development and progression; for example, MVs produced by circulating cells might modulate fibrosis. Several preliminary studies have shown that MVs are promising biomarkers for liver diseases.

Review criteria

We searched PubMed for full-text publications in English that contained the terms "microvesicles", "microvesicle", "microparticles", "microparticle", "ectosomes" or "ectosome". Additional papers were identified by cross-checking the reference lists of previously identified papers. We also used our collections of published articles related to liver diseases and microvesicles. No date limitations were applied. We performed the final search on 14 October 2013.

1. Witwer, K. W. *et al.* Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J. Extracell. Vesicles* **2**, 20360 (2013).
2. van der Pol, E., Boing, A. N., Harrison, P., Sturk, A. & Nieuwland, R. Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol. Rev.* **64**, 676–705 (2012).
3. Connor, D. E., Exner, T., Ma, D. D. & Joseph, J. E. The majority of circulating platelet-derived microparticles fail to bind annexin V, lack phospholipid-dependent procoagulant activity and demonstrate greater expression of glycoprotein Ib. *Thromb. Haemost.* **103**, 1044–1052 (2010).
4. Mause, S. F. & Weber, C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ. Res.* **107**, 1047–1057 (2010).
5. Rautou, P. E. & Mackman, N. Deletion of microvesicles from the circulation. *Circulation* **125**, 1601–1604 (2012).
6. Bernimoulin, M. *et al.* Differential stimulation of monocytic cells results in distinct populations of microparticles. *J. Thromb. Haemost.* **7**, 1019–1028 (2009).
7. Leroyer, A. S. *et al.* CD40 ligand microparticles from human atherosclerotic plaques stimulate endothelial proliferation and angiogenesis a potential mechanism for intraplaque neovascularization. *J. Am. Coll. Cardiol.* **52**, 1302–1311 (2008).
8. Berckmans, R. J., Sturk, A., van Tienen, L. M., Schaap, M. C. & Nieuwland, R. Cell-derived vesicles exposing coagulant tissue factor in saliva. *Blood* **117**, 3172–3180 (2011).
9. Rood, I. M. *et al.* Comparison of three methods for isolation of urinary microvesicles to identify biomarkers of nephrotic syndrome. *Kidney Int.* **78**, 810–816 (2010).
10. Witek, R. P. *et al.* Liver cell-derived microparticles activate hedgehog signaling and alter gene expression in hepatic endothelial cells. *Gastroenterology* **136**, 320–330 (2009).
11. Boilard, E. *et al.* Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* **327**, 580–583 (2010).
12. Chahed, S. *et al.* Increased vitreous shedding of microparticles in proliferative diabetic retinopathy stimulates endothelial proliferation. *Diabetes* **59**, 694–701 (2010).
13. Franz, C. *et al.* Procoagulant tissue factor-exposing vesicles in human seminal fluid. *J. Reprod. Immunol.* **98**, 45–51 (2013).
14. Leroyer, A. S. *et al.* Microparticles from ischemic muscle promotes postnatal vasculogenesis. *Circulation* **119**, 2808–2817 (2009).
15. Baron, M. *et al.* PPARα activation differently affects microparticle content in atherosclerotic lesions and liver of a mouse model of atherosclerosis and NASH. *Atherosclerosis* **218**, 69–76 (2011).
16. Amabile, N., Rautou, P. E., Tedgui, A. & Boulanger, C. M. Microparticles: key protagonists in cardiovascular disorders. *Semin. Thromb. Hemost.* **36**, 907–916 (2010).
17. Rautou, P. E. & Mackman, N. Microvesicles as risk markers for venous thrombosis. *Expert Rev. Hematol.* **6**, 91–101 (2013).
18. Zahra, S., Anderson, J. A., Stirling, D. & Ludlam, C. A. Microparticles, malignancy and thrombosis. *Br. J. Haematol.* **152**, 688–700 (2011).
19. Fusegawa, H. *et al.* Platelet activation in patients with chronic hepatitis C. *Tokai J. Exp. Clin. Med.* **27**, 101–106 (2002).
20. Brodsky, S. V. *et al.* Dynamics of circulating microparticles in liver transplant patients. *J. Gastrointestin. Liver Dis.* **17**, 261–268 (2008).
21. Sayed, D., Amin, N. F. & Galal, G. M. Monocyte-platelet aggregates and platelet micro-particles in patients with post-hepatitic liver cirrhosis. *Thromb. Res.* **125**, e228–e233 (2010).
22. Kornek, M., Popov, Y., Libermann, T. A., Afdhal, N. H. & Schuppan, D. Human T cell microparticles circulate in blood of hepatitis patients and induce fibrolytic activation of hepatic stellate cells. *Hepatology* **53**, 230–242 (2011).
23. Ogasawara, F. *et al.* Platelet activation in patients with alcoholic liver disease. *Tokai J. Exp. Clin. Med.* **30**, 41–48 (2005).
24. Rautou, P. E. *et al.* Abnormal plasma microparticles impair vasoconstrictor responses in patients with cirrhosis. *Gastroenterology* **143**, 166–176 (2012).
25. Kornek, M. *et al.* Circulating microparticles as disease-specific biomarkers of severity of inflammation in patients with hepatitis C or nonalcoholic steatohepatitis. *Gastroenterology* **143**, 448–458 (2012).
26. Esch, J. S. *et al.* Platelet activation and increased tissue factor expression on monocytes in reperfusion injury following orthotopic liver transplantation. *Platelets* **21**, 348–359 (2010).
27. Agarwal, B. *et al.* Evaluation of coagulation abnormalities in acute liver failure. *J. Hepatol.* **57**, 780–786 (2012).
28. Agarwal, B. *et al.* Hemostasis in patients with acute kidney injury secondary to acute liver failure. *Kidney Int.* **84**, 158–163 (2013).
29. Stravitz, R. T. *et al.* Role of procoagulant microparticles in mediating complications and outcome of acute liver injury/acute liver failure. *Hepatology* **58**, 304–313 (2013).
30. Schmelzle, M. *et al.* Increased plasma levels of microparticles expressing CD39 and CD133 in acute liver injury. *Transplantation* **95**, 63–69 (2013).
31. Chen, Y., Davis-Gorman, G., Watson, R. R. & McDonagh, P. F. Platelet CD62p expression and microparticle in murine acquired immune deficiency syndrome and chronic ethanol consumption. *Alcohol* **38**, 25–30 (2003).
32. Meckes, D. G. Jr & Raab-Traub, N. Microvesicles and viral infection. *J. Virol.* **85**, 12844–12854 (2011).
33. Diamant, M. *et al.* Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes mellitus. *Circulation* **106**, 2442–2447 (2002).
34. Esposito, K. *et al.* Endothelial microparticles correlate with endothelial dysfunction in obese women. *J. Clin. Endocrinol. Metab.* **91**, 3676–3679 (2006).
35. Ferreira, A. C. *et al.* Postprandial hypertriglyceridemia increases circulating levels of endothelial cell microparticles. *Circulation* **110**, 3599–3603 (2004).
36. Navasiolava, N. M. *et al.* Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers. *Am. J. Physiol. Heart Circ. Physiol.* **299**, H248–H256 (2010).
37. Guicciardi, M. E. & Gores, G. J. Apoptosis as a mechanism for liver disease progression. *Semin. Liver Dis.* **30**, 402–410 (2010).
38. Violi, F. *et al.* Patients with liver cirrhosis suffer from primary haemostatic defects? Fact or fiction? *J. Hepatol.* **55**, 1415–1427 (2011).

39. Miyoshi, H. *et al.* Calpain activation in plasma membrane bleb formation during tert-butyl hydroperoxide-induced rat hepatocyte injury. *Gastroenterology* **110**, 1897–1904 (1996).
40. Cazzaniga, M. *et al.* Increased flow-mediated vasodilation in cirrhotic patients with ascites: relationship with renal resistive index. *Liver Int.* **28**, 1396–1401 (2008).
41. Tazi, K. A. *et al.* Role of shear stress in aortic eNOS up-regulation in rats with biliary cirrhosis. *Gastroenterology* **122**, 1869–1877 (2002).
42. Rasaratnam, B., Kaye, D., Jennings, G., Dudley, F. & Chin-Dusting, J. The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann. Intern. Med.* **139**, 186–193 (2003).
43. Nomura, S., Nakamura, T., Cone, J., Tandon, N. N. & Kambayashi, J. Cytometric analysis of high shear-induced platelet microparticles and effect of cytokines on microparticle generation. *Cytometry* **40**, 173–181 (2000).
44. Abid Hussein, M. N. *et al.* Cell-derived microparticles contain caspase 3 *in vitro* and *in vivo*. *J. Thromb. Haemost.* **3**, 888–896 (2005).
45. Essayagh, S. *et al.* Microparticles from apoptotic vascular smooth muscle cells induce endothelial dysfunction, a phenomenon prevented by β3-integrin antagonists. *Thromb. Haemost.* **94**, 853–858 (2005).
46. Szotowski, B. *et al.* Antioxidative treatment inhibits the release of thrombogenic tissue factor from irradiation- and cytokine-induced endothelial cells. *Cardiovasc. Res.* **73**, 806–812 (2007).
47. Liu, M. L., Reilly, M. P., Casasanto, P., McKenzie, S. E. & Williams, K. J. Cholesterol enrichment of human monocyte/macrophages induces surface exposure of phosphatidylserine and the release of biologically-active tissue factor-positive microvesicles. *Arterioscler. Thromb. Vasc. Biol.* **27**, 430–435 (2007).
48. Li, D. *et al.* TLR4 signaling induces the release of microparticles by tumor cells that regulate inflammatory cytokine IL-6 of macrophages via microRNA let-7b. *Oncoimmunology* **1**, 687–693 (2012).
49. Povero, D. *et al.* Lipid-induced toxicity stimulates hepatocytes to release angiogenic microparticles that require vanin-1 for uptake by endothelial cells. *Sci. Signal.* **6**, ra88 (2013).
50. Al Faraj, A. *et al.* Endothelial cell-derived microparticles loaded with iron oxide nanoparticles: feasibility of MR imaging monitoring in mice. *Radiology* **263**, 169–178 (2012).
51. Willekens, F. L. *et al.* Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors. *Blood* **105**, 2141–2145 (2005).
52. Gomez, F., Ruiz, P. & Schreiber, A. D. Impaired function of macrophage Fc γ receptors and bacterial infection in alcoholic cirrhosis. *N. Engl. J. Med.* **331**, 1122–1128 (1994).
53. Chen, L., Pan, D. D., Zhou, J. & Jiang, Y. Z. Protective effect of selenium-enriched *Lactobacillus* on CCl4-induced liver injury in mice and its possible mechanisms. *World J. Gastroenterol.* **11**, 5795–5800 (2005).
54. Rimola, A. *et al.* Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology* **4**, 53–58 (1984).
55. Tanimoto, A. *et al.* Superparamagnetic iron oxide-mediated hepatic signal intensity change in patients with and without cirrhosis: pulse sequence effects and Kupffer cell function. *Radiology* **222**, 661–666 (2002).
56. Dasgupta, S. K., Le, A., Chavakis, T., Rumbaut, R. E. & Thiagarajan, P. Developmental endothelial locus-1 (Del-1) mediates clearance of platelet microparticles by the endothelium. *Circulation* **125**, 1664–1672 (2012).
57. Distler, J. H. *et al.* The induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts stimulated with immune cell microparticles. *Proc. Natl Acad. Sci. USA* **102**, 2892–2897 (2005).
58. Taraboletti, G. *et al.* Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am. J. Pathol.* **160**, 673–680 (2002).
59. Gasser, O. *et al.* Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. *Exp. Cell. Res.* **285**, 243–257 (2003).
60. Szabo, G. & Bala, S. MicroRNAs in liver disease. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 542–552 (2013).
61. Bartel, D. P. MicroRNAs: target recognition and regulatory functions. *Cell* **136**, 215–233 (2009).
62. Diehl, P. *et al.* Microparticles: major transport vehicles for distinct microRNAs in circulation. *Cardiovasc. Res.* **93**, 633–644 (2012).
63. Cantaluppi, V. *et al.* Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia-reperfusion injury by microRNA-dependent reprogramming of resident renal cells. *Kidney Int.* **82**, 412–427 (2012).
64. Thabut, D. & Shah, V. Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: new targets for the treatment of portal hypertension? *J. Hepatol.* **53**, 976–980 (2010).
65. Mause, S. F. *et al.* Platelet microparticles enhance the vasoregenerative potential of angiogenic early outgrowth cells after vascular injury. *Circulation* **122**, 495–506 (2010).
66. Benameur, T., Soleti, R., Porro, C., Andriantsitohaina, R. & Martinez, M. C. Microparticles carrying Sonic hedgehog favor neovascularization through the activation of nitric oxide pathway in mice. *PLoS ONE* **5**, e12688 (2010).
67. Deregibus, M. C. *et al.* Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood* **110**, 2440–2448 (2007).
68. Friedman, S. L. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol. Rev.* **88**, 125–172 (2008).
69. Thabut, D. *et al.* High-density lipoprotein administration attenuates liver proinflammatory response, restores liver endothelial nitric oxide synthase activity, and lowers portal pressure in cirrhotic rats. *Hepatology* **46**, 1893–1906 (2007).
70. Aram, G., Potter, J. J., Liu, X., Torbenson, M. S. & Mezey, E. Lack of inducible nitric oxide synthase leads to increased hepatic apoptosis and decreased fibrosis in mice after chronic carbon tetrachloride administration. *Hepatology* **47**, 2051–2058 (2008).
71. Nieuwland, R. *et al.* Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood* **95**, 930–935 (2000).
72. Owens, A. P. 3rd & Mackman, N. Microparticles in hemostasis and thrombosis. *Circ. Res.* **108**, 1284–1297 (2011).
73. Valla, D. C. Thrombosis and anticoagulation in liver disease. *Hepatology* **47**, 1384–1393 (2008).
74. Teoh, N. C. *et al.* Diannexin, a novel annexin V homodimer, provides prolonged protection against hepatic ischemia–reperfusion injury in mice. *Gastroenterology* **133**, 632–646 (2007).
75. Garcia-Pagan, J. C., Gracia-Sancho, J. & Bosch, J. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. *J. Hepatol.* **57**, 458–461 (2012).
76. Lemoinne, S. *et al.* Portal myofibroblasts promote liver angiogenesis through the release of microparticles and interaction with cholangiocytes. *Hepatology* **56**, 304A–305A (2012).
77. Schwartz, D. *et al.* Inhibition of constitutive nitric oxide synthase (NOS) by nitric oxide generated by inducible NOS after lipopolysaccharide administration provokes renal dysfunction in rats. *J. Clin. Invest.* **100**, 439–448 (1997).
78. Mostefai, H. A. *et al.* Circulating microparticles from patients with septic shock exert protective role in vascular function. *Am. J. Respir. Crit. Care Med.* **178**, 1148–1155 (2008).
79. Melgar-Lesmes, P. *et al.* Vascular endothelial growth factor and angiopoietin-2 play a major role in the pathogenesis of vascular leakage in cirrhotic rats. *Gut* **58**, 285–292 (2009).
80. Densmore, J. C. *et al.* Endothelium-derived microparticles induce endothelial dysfunction and acute lung injury. *Shock* **26**, 464–471 (2006).
81. Perez-Casal, M. *et al.* Microparticle-associated endothelial protein C receptor and the induction of cytoprotective and anti-inflammatory effects. *Haematologica* **94**, 387–394 (2009).
82. Tripodi, A. & Mannucci, P. M. The coagulopathy of chronic liver disease. *N. Engl. J. Med.* **365**, 147–156 (2011).
83. Press, J. Z. *et al.* Microparticles from ovarian carcinomas are shed into ascites and promote cell migration. *Int. J. Gynecol. Cancer* **22**, 546–552 (2012).
84. Ginestra, A., Miceli, D., Dolo, V., Romano, F. M. & Vittorelli, M. L. Membrane vesicles in ovarian cancer fluids: a new potential marker. *Anticancer Res.* **19**, 3439–3445 (1999).
85. Choi, D. S. *et al.* Proteomic analysis of microvesicles derived from human colorectal cancer ascites. *Proteomics* **11**, 2745–2751 (2011).
86. Cejudo-Martin, P. *et al.* Hypoxia is an inducer of vasodilator agents in peritoneal macrophages of cirrhotic patients. *Hepatology* **36**, 1172–1179 (2002).
87. Jimenez, W. *et al.* Nitric oxide production and inducible nitric oxide synthase expression in peritoneal macrophages of cirrhotic patients. *Hepatology* **30**, 670–676 (1999).
88. Morales-Ruiz, M. *et al.* Ascites from cirrhotic patients induces angiogenesis through the phosphoinositide 3-kinase/Akt signaling pathway. *J. Hepatol.* **43**, 85–91 (2005).
89. Wright, G. & Jalan, R. Ammonia and inflammation in the pathogenesis of hepatic encephalopathy: Pandora's box? *Hepatology* **46**, 291–294 (2007).
90. Wright, G. *et al.* Endotoxemia produces coma and brain swelling in bile duct ligated rats. *Hepatology* **45**, 1517–1526 (2007).
91. Jayakumar, A. R., Tong, X. Y., Ospel, J. & Norenberg, M. D. Role of cerebral endothelial cells in the astrocyte swelling and brain edema associated with acute hepatic encephalopathy. *Neuroscience* **218**, 305–316 (2012).
92. Rodriguez-Roisin, R. & Krowka, M. J. Hepatopulmonary syndrome—a liver-induced lung vascular disorder. *N. Engl. J. Med.* **358**, 2378–2387 (2008).
93. Amabile, N. *et al.* Cellular microparticles in the pathogenesis of pulmonary hypertension. *Eur. Respir. J.* **42**, 272–279 (2013).

94. Camus, S. M. *et al.* Erythrocyte microparticles can induce kidney vaso-occlusions in a murine model of sickle cell disease. *Blood* **120**, 5050–5058 (2012).
95. Tripodi, A. *et al.* An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology* **137**, 2105–2111 (2009).
96. Tripodi, A., Anstee, Q. M., Sogaard, K. K., Primignani, M. & Valla, D. C. Hypercoagulability in cirrhosis: causes and consequences. *J. Thromb. Haemost.* **9**, 1713–1723 (2011).
97. Tripodi, A., Primignani, M., Lemma, L., Chantarangkul, V. & Mannucci, P. M. Evidence that low protein C contributes to the procoagulant imbalance in cirrhosis. *J. Hepatol.* **59**, 265–270 (2013).
98. Wiest, R. & Garcia-Tsao, G. Bacterial translocation (BT) in cirrhosis. *Hepatology* **41**, 422–433 (2005).
99. Rautou, P.-E., Vion, A.-C., Luyendyk, J. P. & Mackman, N. Microparticle tissue factor activity is increased in patients with cirrhosis. *Hepatology* (in press).
100. Stravitz, R. T. *et al.* Minimal effects of acute liver injury/acute liver failure on hemostasis as assessed by thromboelastography. *J. Hepatol.* **56**, 129–136 (2012).
101. Ganey, P. E. *et al.* Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. *Hepatology* **46**, 1177–1186 (2007).
102. Zafrani, L. *et al.* Calpastatin controls polymicrobial sepsis by limiting procoagulant microparticle release. *Am. J. Respir. Crit. Care Med.* **185**, 744–755 (2012).
103. Thaler, J. *et al.* Microparticle-associated tissue factor activity, venous thromboembolism and mortality in pancreatic, gastric, colorectal and brain cancer patients. *J. Thromb. Haemost.* **10**, 1363–1370 (2012).
104. Rak, J. Microparticles in cancer. *Semin. Thromb. Hemost.* **36**, 888–906 (2010).
105. Al-Nedawi, K., Meehan, B., Kerbel, R. S., Allison, A. C. & Rak, J. Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. *Proc. Natl Acad. Sci. USA* **106**, 3794–3799 (2009).
106. Al-Nedawi, K. *et al.* Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat. Cell Biol.* **10**, 619–624 (2008).
107. Jaiswal, R. *et al.* Microparticle-associated nucleic acids mediate trait dominance in cancer. *FASEB J.* **26**, 420–429 (2012).
108. Shedden, K., Xie, X. T., Chandaroy, P., Chang, Y. T. & Rosania, G. R. Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. *Cancer Res.* **63**, 4331–4337 (2003).
109. Sidhu, S. S., Mengistab, A. T., Tauscher, A. N., LaVail, J. & Basbaum, C. The microvesicle as a vehicle for EMMPRIN in tumor–stromal interactions. *Oncogene* **23**, 956–963 (2004).
110. Ma, J. *et al.* Innate immune cell-derived microparticles facilitate hepatocarcinoma metastasis by transferring integrin α(M)β(2) to tumor cells. *J. Immunol.* **191**, 3453–3461 (2013).
111. Fonsato, V. *et al.* Human liver stem cell-derived microvesicles inhibit hepatoma growth in SCID mice by delivering antitumor microRNAs. *Stem Cells* **30**, 1985–1998 (2012).
112. Villa, E. *et al.* Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* **143**, 1253–1260 (2012).
113. Hu, J. *et al.* Plasma microRNA, a potential biomarker for acute rejection after liver transplantation. *Transplantation* **95**, 991–999 (2013).
114. Lacroix, R. *et al.* Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop. *J. Thromb. Haemost.* **11**, 1190–1193 (2013).
115. European Association of National Metrology Institutes (EURA-MET). Metrological characterisation of micro-vesicles from body fluids as non-invasive diagnostic biomarkers [online], http://www.metves.eu (2014).
116. European Cooperation in Science and Technology (COST). *European Network on Microvesicles and Exosomes in Health and Disease (ME-HAD)* [online], http://www.cost.eu/domains_actions/bmbs/Actions/BM1202 (2012).
117. Herrera, M. B. *et al.* Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomized rats. *J. Cell. Mol. Med.* **14**, 1605–1618 (2010).

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