

eScholarship@UMassChan

Extracellular Vesicles in Liver Diseases: Meeting Report from the International Liver Congress 2018

Item Type	Journal Article
Authors	Banales, Jesus M.;Feldstein, Ariel E.;Sanger, Hanna;Lukacs-Kornek, Veronika;Szabo, Gyongyi;Kornek, Mirosław
Citation	<p><p>Hepatol Commun. 2019 Jan 2;3(2):305-315. doi: 10.1002/hep4.1300. eCollection 2019 Feb. Link to article on publisher's site</p></p>
DOI	10.1002/hep4.1300
Rights	<p>© 2018 The Authors. Hepatology Communications published by Wiley Periodicals, Inc., on behalf of the American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>
Download date	2026-05-10 17:34:42
Item License	http://creativecommons.org/licenses/by-nc-nd/4.0/
Link to Item	https://hdl.handle.net/20.500.14038/40930

Extracellular Vesicles in Liver Diseases: Meeting Report from the International Liver Congress 2018

Jesus M. Banales,¹ Ariel E. Feldstein,² Hanna Sanger,³ Veronika Lukacs-Kornek,⁴ Gyongyi Szabo,⁵ and Miroslaw Kornek^{6,7}

Extracellular vesicles (EVs) are small and heterogeneous membrane-bound structures released by cells and found in all biological fluids. They are effective intercellular communicators, acting on a number of close and/or distant target cells. EV cargo may reflect the cell of origin as well as the specific stress that induces their formation and release. They transport a variety of bioactive molecules, including messenger RNA, noncoding RNAs, proteins, lipids, and metabolites, that can be transferred among cells, regulating various cell responses. Alteration in the concentration and composition of EVs in biological fluids is a typical hallmark of pathologies in different liver diseases. Circulating EVs can serve as biomarkers or as messengers following uptake by other cells. This review is a meeting report from the International Liver Congress 2018 (European Association for the Study of the Liver) celebrated in Paris (Symposium: Extracellular vesicles and signal transmission) that discusses the role of EVs in several liver diseases, highlighting their potential value as disease biomarkers and therapeutic opportunities. (*Hepatology Communications* 2019;3:305-315).

Extracellular vesicles (EVs) are small cell-derived structures enveloped by a double-layer membrane that are shed by cells as a mechanism of horizontal communication. First described as an outgrowth of platelet activity or sample contamination, the role of EVs remained largely unexplored^(1,2) until the early 2000s when growing enthusiasm for the field of EV biology and pathobiology resulted in

increasing numbers of new publications each year. The potential of EVs as diagnostic and prognostic tools is being increasingly recognized by the scientific community and awaits translation into human medicine.

Currently, three major types of EVs are recognized: exosomes, microvesicles (MVs), and apoptotic bodies. Despite some disagreements over their exact

Abbreviations: AH, alcoholic hepatitis; ALD, alcoholic liver disease; AMPN, aminopeptidase N; ASGPR1, asialoglycoprotein receptor 1; AUROC, area under the receiver operating characteristic curve; CA19.9, carbohydrate antigen 19-9; CCA, cholangiocarcinoma; CD, clusters of differentiation; EpCAM, epithelial cell adhesion molecule; EV, extracellular vesicle; FCN, ficolin; FIBG, fibrogenin; GPC, glypican; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; IL, interleukin; lncRNA, long noncoding RNA; LPS, lipopolysaccharide; miR, microRNA; MP, microparticle; MV, microvesicle; MVB, multivesicular body; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PIGR, polymeric immunoglobulin receptor; PPAR- γ , peroxisome proliferator-activated receptor gamma; PSC, primary sclerosing cholangitis; SEN, sensitivity; SPE, specificity; ta, tumor-associated; VNN1, vanin-1.

Received August 21, 2018; accepted November 12, 2018.

Supported by the Instituto de Salud Carlos III (grants PI12/00380, PI15/01132, and PI18/01075 to J.M.B.), the Miguel Servet Program (CON14/00129 to J.M.B.), Fondo Europeo de Desarrollo Regional, National Institute for the Study of Liver and Gastrointestinal Diseases (award CIBERehd 2018), Basque Foundation for Science, Spain (award IKERBASQUE 2018), Basque Foundation for Innovation and Health Research (award BIOEF EiTb Maratoia BIO15/CA/016/BD), Department of Health of the Basque Country (grants 2013111173 and 2017111010 to J.M.B.), Scientific Foundation of the Spanish Association Against Cancer (award AECC 2018), and the National Institutes of Health (awards R01 DK113592 and U01 AA024206 to A.E.F., and awards R01AA020744, UH2AA026970 and U01AA026933 to G.S.), the Alexander von Humboldt Foundation, Sofja Kovalevskaja Award 2012 (to V.L.-K.) and by the German Research Foundation (LU2061/4-1 to V.L.-K. and KO4103/4-1 to M.K.) and by the Deutsche Krebshilfe (111184 to M.K.).

© 2018 The Authors. *Hepatology Communications* published by Wiley Periodicals, Inc., on behalf of the American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep4.1300

Potential conflict of interest: Nothing to report.

definitions, essentially these three types of EVs differ in size and mode of production. Exosomes, which are up to 150 to 200 nm in diameter, represent the smallest type.⁽³⁾ They are produced within the endosomal membrane system of their parental cells and are transported toward the plasma membrane inside endosomal vesicles, also known as multivesicular bodies (MVBs).⁽³⁾ These MVBs merge with the cell plasma membrane and shed exosomes into the extracellular space by exocytosis.⁽⁴⁾ In contrast, MVs, also referred to as microparticles (MPs) or ectosomes, are released through a coordinated budding process of the cell plasma membrane, resulting in their membrane composition mirroring that of the parental cells.^(5,6) Characterization of MVs can be used to identify the cell type that was activated to release a particular population of MVs.⁽⁷⁾ MVs range from 0.1 to 1 μm in diameter and are characterized by a bilayer membrane containing externalized phosphatidylserine.^(8,9) In cells undergoing regulated death, apoptotic signals induce their fragmentation into apoptotic bodies,⁽¹⁰⁾ which represent the largest EVs and range from 1 to 5 μm in diameter.⁽¹¹⁾

It is believed that all cell types of the human body are capable of releasing EVs either constitutively or as adaptive cellular responses.⁽⁸⁾ Based on their ability to travel through biological fluids, EVs function as messengers, communicating between distant sites while maintaining a high specificity (SPE) to their destination.⁽¹²⁻¹⁴⁾ Their cargo can comprise various types of molecules, making them ideal regulators of biological processes. EVs can transport short and long nucleic acids (microRNAs [miRs/miRNAs], small interfering RNAs, messenger RNAs,

or long noncoding RNAs [lncRNAs]), proteins (cytosolic, cytoskeletal, membrane-bound transporters and receptors, enzymes, adhesion molecules), lipids (sphingomyelin, phosphatidylserine, cholesterol, ceramide), and metabolites.⁽¹⁵⁾ Recently, their potential as vehicles for effective and site-specific drug delivery has been implemented.⁽¹⁶⁾ By fusing with a recipient cell, EVs integrate their membrane into the phospholipid bilayer of the receiving cell, thereby transferring their content into the recipient and modulating intracellular pathways.⁽⁵⁾ EVs can also be taken up by recipient cells through protein and/or lipid interactions, further leading to their endocytosis.⁽¹³⁾

In earlier years research focused on detection and quantification of EVs; however, since the mid-2000s the analysis of their composition and the study of their biological functions have become the center of attention. As EV composition and content reflect features of the parental cells, EVs represent invaluable indicators of mechanisms driving pathobiological processes.^(5,6) By isolating circulating exosomes or MVs from patient blood, it is possible to create disease-specific EV profiles with respect to their surface antigens and/or whole RNA, protein, and lipidomic content. Apart from peripheral blood, various other body fluids have been used for EV characterization, including saliva,⁽¹⁷⁾ urine,⁽¹⁸⁾ breast milk,⁽¹⁹⁾ cerebrospinal fluid,⁽²⁰⁾ and bile.⁽²¹⁾ Accordingly, numerous liver conditions have been evaluated in terms of their EV profile and pathobiological relevance, including, among others, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), alcoholic hepatitis (AH), cirrhosis, hepatocellular carcinoma (HCC),

ARTICLE INFORMATION:

From the ¹Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute, Donostia University Hospital, University of the Basque Country, CIBERehd, Ikerbasque, San Sebastian, Spain; ²Division of Pediatric Gastroenterology, Hepatology, and Nutrition, University of California San Diego, San Diego, CA; ³Department of Medicine II, Saarland University Medical Center, Saarland University, Homburg, Germany; ⁴Institute of Experimental Immunology, University Hospital of the Rheinische Friedrich-Wilhelms-University, Bonn, Germany; ⁵Department of Medicine, University of Massachusetts Medical School, Worcester, MA; ⁶Department of Oncology, Hematology and Rheumatology, University Hospital Bonn, Bonn, Germany; ⁷Department of General, Visceral, and Thoracic Surgery, German Armed Forces Central Hospital, Koblenz, Germany.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Jesus M. Banales, Ph.D.
Department of Liver and Gastrointestinal Diseases
Biodonostia Health Research Institute, Donostia University
Hospital

Paseo del Dr. Begiristain s/n, E-20014
San Sebastian, Spain
E-mail: jesus.banales@biodonostia.org
Tel.: +34-943006067

cholangiocarcinoma (CCA), and other cholangiopathies (such as primary sclerosing cholangitis [PSC]). In the liver, hepatocytes, immune cells, endothelial cells, hepatic stellate cells (HSCs), and cholangiocytes all contribute to EV production.⁽²²⁻²⁵⁾

NAFLD and NASH

Lipotoxicity, a process by which accumulation of certain toxic lipids (e.g., saturated free fatty acids) in hepatocytes triggers various molecular pathways of cell stress and eventually results in cell death, has evolved as a key event along NAFLD progression to NASH and eventually to HCC.⁽⁴⁾ During the process of lipotoxicity, hepatocytes release large numbers of EVs that may act on different target cells and contribute to key processes involved in NAFLD pathogenesis, including immune modulation, angiogenesis, and fibrosis (Fig. 1). The EVs produced and released during NAFLD progression have specific antigenic composition, reflecting the pathologic alterations typical of its progression, and

present miRs and proteins abundantly found in the liver. Additionally, the EV levels are dynamic and change over time, correlating with changes in liver histopathology.

Proteome analysis of circulating EVs from mouse models of NAFLD demonstrated that these vesicles carry a selective antigenic composition.⁽²⁶⁾ Hierarchical clustering analysis allowed for discrimination of mice with established NASH from those with isolated steatosis and normal livers, identifying a proteomic signature in serum EVs that might be used to noninvasively diagnose NASH. Notably, the level of circulating EVs released during diet-induced mouse models of NAFLD strongly correlated with histopathologic features of NAFLD (i.e., fat content, fibrosis, cell death, and pathologic angiogenesis) and were enriched in miR-122 and miR-192, two miRs highly expressed in hepatocytes. The level of these miRNAs increased in EVs and decreased in livers over time during NAFLD progression. The release of these miRs from stressed or damaged hepatocytes in EVs during NAFLD progression may provide an attractive explanation for the decreased expression

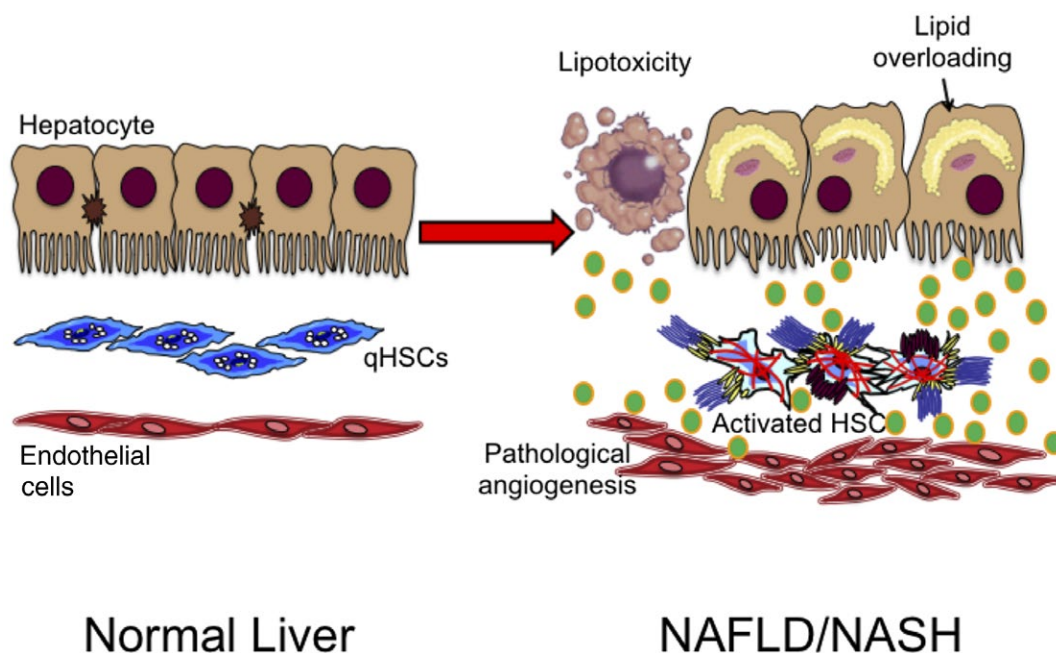


FIG. 1. Extracellular vesicles in the pathogenesis of fatty liver disease. During the process of lipotoxicity, hepatocytes release a large amount of EVs that may then act on various target cells in the local environment, contributing to key processes involved in NAFLD pathogenesis, including immune modulation, angiogenesis, and fibrosis. Additionally, EVs may be released to the systemic circulation and can be potentially used to noninvasively monitor the extent of liver injury. Abbreviation: qHSC, quiescent HSC.

level of miR-122 found in the livers of patients with NASH.⁽²⁷⁾ In contrast to healthy individuals where miR-122 is present in circulation only in the Argonaut 2 (Ago2) complex fraction, the majority of serum miR-122 circulates in Ago2-free forms in patients with NAFLD,⁽²⁸⁾ although its specific compartment is still unknown.

Patients with NASH might also present changes in the immune-derived EV composition because increased circulating levels of leukoendothelial-derived clusters of differentiation (CD)31⁺/41⁻, pan-leukocyte-derived CD11a⁺, lymphocyte-derived CD4⁺, and erythrocyte-derived CD235a⁺ EVs occur in patients with liver cirrhosis.^(29,30) Additionally, MVs positive for inflammatory cell markers, such as CD4⁺ cells, CD8⁺ T cells, or CD14⁺ monocytes/invariant natural killer cells, are detected in plasma of patients with various liver conditions.⁽⁷⁾ However, EVs derived from inflammatory cells are not specific and may be elevated in a number of immune and inflammatory conditions associated with their activation, thus limiting their utility as biomarkers of liver disease.

At the pathobiological level, it has been shown that MPs from plasma of both mice and patients with NASH contain high levels of mitochondrial DNA and intact mitochondria that are able to trigger toll-like receptor 9 signaling, which is critical for NASH development.⁽³¹⁾ Moreover, serum EVs originated from the liver of mice with diet-induced NASH triggered migration and tubular structure formation when applied to endothelial cells *in vitro*.⁽³²⁾ The angiogenic effects of hepatocyte-derived MVs underexposed to saturated fatty acids involved a vanin-1 (VNN1)-dependent uptake of EVs by endothelial cells (i.e., an enzyme located on the surface of the vesicles). Similarly, EVs released by hepatocytes exposed to lipotoxicity are efficiently internalized into HSCs through a VNN1-dependent mechanism.⁽³³⁾ The internalization of vesicles induced a phenotypic switch of HSCs from quiescent to activated collagen-producing myofibroblasts.⁽³³⁾ These data suggest that pathologic angiogenesis and fibrosis could be reduced by preventing endothelial cells and HSCs from internalizing VNN1-positive EVs from lipotoxic hepatocytes.

On the other hand, miR-128-3p was found enriched in EVs derived from fat-laden hepatocytes in the liver of two murine diet-induced NAFLD/NASH models⁽³³⁾ and in the liver of patients with

NASH.⁽²⁷⁾ This miR is selectively transferred through hepatocyte-derived EVs into HSCs, promoting HSC activation through direct targeting of peroxisome proliferator-activated receptor gamma (PPAR- γ) expression. PPAR- γ is a member of the nuclear hormone-receptor superfamily that modulates HSC quiescence. PPAR- γ expression progressively decreases along HSC activation and is completely depleted in fully activated HSCs,⁽³⁴⁾ highlighting the important role of the EV transfer of miRs in the pathogenesis of NAFLD/NASH.

Regarding the mechanisms of EV biogenesis in NASH, mixed lineage kinase 3 was shown to mediate the release of chemokine (C-X-C motif) ligand 10 in exosomes from lipotoxic hepatocytes, and these exosomes induced macrophage chemotaxis in NASH.⁽³⁵⁾ Lipid-induced signaling can also cause the release of EVs with inflammatory potential into hepatocytes,⁽³⁶⁾ while the ceramide transport protein steroidogenic acute regulatory protein-related lipid transfer domain 11 has been recently uncovered as a novel modulator of exosome biogenesis during lipotoxic insult to hepatocytes.⁽³⁷⁾

Alcoholic Liver Disease and Alcoholic Hepatitis

AH, the clinically severe manifestation of alcoholic liver disease (ALD), represents one of the most common etiologies of progressive liver diseases leading to cirrhosis and predisposing to HCC. EVs have been explored as biomarkers for ALD and AH. Moreover, their biodistribution, cellular sources, biological effects, and therapeutic potential have been investigated (Fig. 2).

The numbers of circulating EVs were found to be increased in mouse models of ALD as well as in patients with AH.⁽²²⁾ The majority of these EVs are exosomes (40 and 200 nm) and some MVs (200 and 1,000 nm). Screening of the EV cargo revealed specific changes in miR composition in a mouse model of ALD compared to control mice. Specifically, the concentration of miR-122, miR-192, and miR-30a was found increased in EVs of alcohol-fed mice compared to controls, presenting diagnostic capacity.⁽²²⁾ Notably, the levels of these miRs were also increased in serum EVs of patients with AH, indicating the

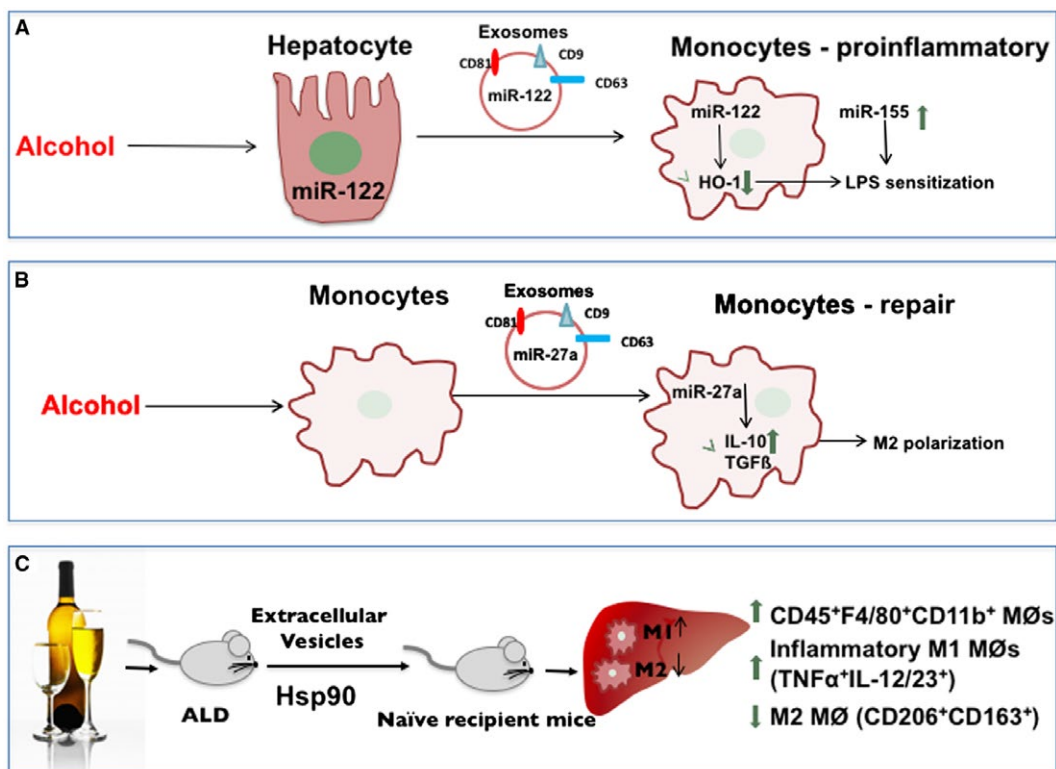


FIG. 2. Extracellular vesicles in ALD. (A) EVs are released from different cell types in the liver and provide communication between hepatocytes and immune cells as well as within immune cells, through miRs. (B) miR signals that promote M1 (classically activated) or M2 (alternatively activated) macrophage polarization contribute to the mixed M1/M2 phenotype of macrophages in the liver in ALD. (C) Circulating EVs from chronic alcohol-fed mice induce liver inflammation and macrophage polarization in alcohol-naïve recipient mice. Abbreviations: HO, Heme oxygenase; Hsp, heat shock protein; TGF β , transforming growth factor β .

potential value as disease biomarkers.⁽²²⁾ Although the mechanistic explanation for the increased levels of these miRs in EVs of ALD and AH needs to be revealed, it is important to note that miR-122 and miR-192 are highly abundant in hepatocytes and the increased levels of these miRs may be indicative of ongoing hepatocyte damage.⁽³⁸⁻⁴⁰⁾ Furthermore, miR-30a regulates different steps of the autophagy process and MVB formation.⁽⁴¹⁾ Thus, it is tempting to speculate that the miR-30a concentration in EVs may be the result of changes in autophagy and/or exosome production in ALD. Another study also found increased EV levels in a mouse model of AH.⁽⁴²⁾ This study identified mitochondrial DNA in the cargo of MVs that showed a biological effect in mediating neutrophil activation in AH.

Mass spectrometry analysis of serum EVs from a mouse model of ALD revealed specific changes in the EV protein cargo compared to controls.⁽⁴³⁾ Expression

of proteins related to immune cell movement, pattern recognition receptors, interleukin-8 (IL-8) signaling, endoplasmic reticulum stress, unfolded protein response, and hypoxia inducible factor signaling were different between ALD and control EVs. There were also specific proteins exclusively identified in ALD EVs and not in control EVs, indicating a potential disease-specific protein signature.⁽⁴³⁾ Of note, transfer of ALD EVs to naïve healthy mice resulted in recruitment of inflammatory monocytes and neutrophil leukocytes in the recipient livers, with these immune cells showing a proinflammatory phenotype.⁽⁴³⁾ Heat shock protein 90, a major regulator of multiple cellular functions, was identified as uniquely increased in ALD EVs and as a regulator of these biological effects.⁽⁴³⁾ These results indicate that circulating EVs have potential to modulate cell recruitment and function in distant organs. In a biodistribution study using miR-155 as an indicator of biodistribution, intravenously

administered EVs from wild-type mice rapidly (10 minutes) reached many organs, including the liver.⁽⁴⁴⁾ In miR-155-deficient recipient mice, miR-155 transferred in EVs was found most abundantly in the liver and adipose tissue and to a lesser extent in lung, kidney, and muscle tissue.⁽⁴⁴⁾ miR-155 transferred by EVs was taken up by specific cells in the liver, such as hepatocytes and macrophages, further regulating biological activities in these cells.⁽⁴⁴⁾ These results indicate a rapid biodistribution and cellular uptake of miRs in recipient mice and tissues *in vivo*.

The exposure of hepatocytes, liver macrophages, and Kupffer cells to alcohol was found to increase EV release in all cell types *in vitro*.^(45,46) Functionally, these EVs produced by different liver cells act as messengers on target cells. This is supported by observations where hepatocyte-derived EVs that were enriched in miR-122 were taken up by monocytes that normally have no or very low levels of miR-122, modifying the phenotype of the recipient monocytes and resulting in lipopolysaccharide (LPS) sensitization and increased LPS-dependent proinflammatory cytokine production compared to monocytes that received control EVs.⁽⁴⁵⁾ Furthermore, EVs derived from monocytes after alcohol exposure also had functional capacity to modulate naive monocyte function to result in an M2-like repair phenotype.⁽⁴⁶⁾ Alcohol exposure also increased miR-27a content in monocyte-derived EVs, and their transfer to recipient naive monocytes induced an M2-like phenotype.⁽⁴⁶⁾ EVs can be loaded with specific miR inhibitors or precursors, being potential therapeutic vehicles for liver diseases.⁽⁴⁵⁾ Alcohol was also shown to stimulate macrophage activation through hepatocyte-derived release of CD40L-containing EVs in a caspase-dependent manner.⁽²⁵⁾

HCC

The correct numeration and phenotypic characterization of EVs can be regarded as tracers for the presence of specific cells, i.e., immunocytes associated with NAFLD⁽⁷⁾ or malignant tumor cells,⁽⁴⁷⁾ among others. Therefore, the tumor-associated (ta) EVs, also known as taMVs/taMPs, might help in the detection/diagnosis of cancer. Evidence suggests that EV shedding is not dependent on a metastatic

cancer phenotype.⁽⁴⁸⁾ Therefore, the use of EVs as an advanced tool for a novel kind of liquid biopsy marker eventually revealing tumor presence, entity, location, and stage/diameter might be possible. However, a main challenge is the quest for specific antigens on EVs that could help in the identification of specific EVs derived from the cancer cells (Fig. 3). A pioneering study identified glypican (GPC)-1 on the surface of pancreatic cancer-derived EVs, which showed 100% sensitivity (SEN) and SPE for the diagnosis of early and late-stage pancreatic cancer compared to healthy subjects and to patients with a benign pancreatic disease.⁽⁴⁹⁾ However, GPC1⁺ EVs were also found elevated in patients with breast cancer, suggesting that this could be a potential pan-cancer biomarker. Moreover, patients with hepatitis C virus presented a differential immune cell-derived MV pattern (i.e., from lymphocytes [CD4⁺, CD8⁺] or natural killer T cells and macrophages/monocytes [CD14⁺]) compared to patients with NAFLD.⁽⁷⁾ These data suggest that the simultaneous combination of cancer cell-derived antigens in MVs might be useful in the diagnosis of specific cancer entities. This approach resulted first in the use of a pan-cancer marker based on epithelial cell adhesion molecule (EpCAM)⁺CD147⁺MPs/MVs⁽⁴⁸⁾ that was further refined and validated for the diagnosis of hepatobiliary tumors, such as HCC and CCA.⁽⁴⁷⁾ In fact, it was confirmed that AnnexinV⁺EpCAM⁺CD147⁺ MVs/MPs were elevated in HCC and CCA as in other investigated tumor entities and that asialoglycoprotein receptor 1 (ASGPR1)⁺AnnexinV⁺EpCAM⁺CD133⁺ was more specific for hepatic disorders, such as nonmalignant cirrhosis (liver fibrosis F4 stage), HCC, and CCA. This was expected because ASGPR1 is a commonly used hepatocyte marker and therefore added hepatoma specificity to the antigen combination of the MVs. Patients with liver cancer (HCC or CCA), particularly early stage tumors, presented higher serum concentration of AnnexinV⁺EpCAM⁺ASGPR1⁺ taMPs compared to patients with nonmalignant hepatic cirrhosis (SEN, 75%; SPE, 47%),⁽⁴⁷⁾ indicating the potential value of taMPs for the early diagnosis of liver cancer in patients with cirrhosis. However, a main question is whether taEVs bear the potential to be applied as a minimally invasive liquid biopsy marker. In this regard, proteomic analysis of serum EV from patients with HCC revealed

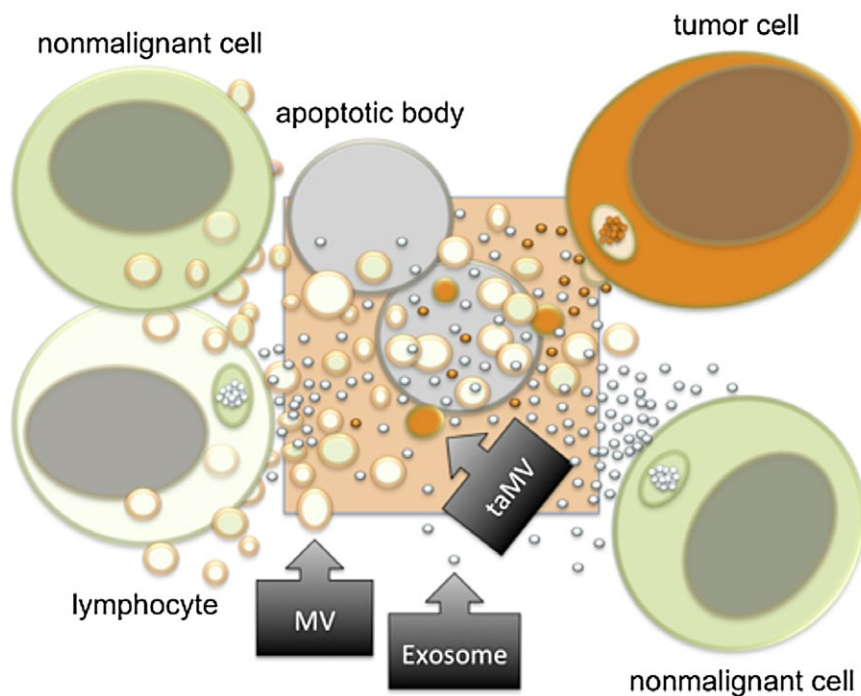


FIG. 3. Potential cellular origins of EVs. Lymphocytes and nonmalignant cells eventually secrete exosomes and MVs, whereas malignant tumor cells can also secrete taMVs/taMPs and ta-EXOs). The center box represents the typical EV content usually found in the full blood of patients with cancer, including the few taMVs/taMPs/taEXOs (inferior numbers). Abbreviation: EXO, exosome.

good potential candidates that must be investigated in future studies.⁽⁵⁰⁾

Cholangiopathies

The presence of EVs in bile was described in 2010 as a consequence of the secretory activities from both hepatocytes and cholangiocytes.⁽²¹⁾ Contrary to the initial idea in which EVs were considered as a release mechanism of waste molecules, EVs in bile regulate the biology of cholangiocytes. Under physiological conditions, bile EVs are able to bind to the primary cilium of cholangiocytes, inhibiting their proliferation and promoting the characteristic quiescent status of the biliary epithelium.⁽²¹⁾

EVs are also important in cholangiopathies, regulating different pathologic processes and containing potentially helpful biomarkers for the noninvasive diagnosis of these diseases. The value of bile and serum EVs as a source of biomarkers (e.g., miRs, lncRNAs, or proteins) for cholangiopathies has been investigated. Up-regulation of an miR panel (i.e., 191,

486-3p, 1274b, 16, 484) was described in bile EVs from patients with CCA compared to control individuals (i.e., patients with PSC, a known risk factor for CCA, biliary obstruction, or bile leak syndrome) with diagnostic significance (SEN, 67%; SPE, 96%).⁽⁵¹⁾ In addition, two lncRNAs (ENST00000588480.1 and ENST00000517758.1) were reported up-regulated in bile EVs from patients with CCA compared to biliary obstruction conditions (SEN, 83%; SPE, 59%; area under the receiver operating characteristic curve [AUROC], 0.709).⁽⁵²⁾

Different protein profiles were found in serum EVs from patients with CCA, PSC, HCC, or healthy individuals (control group).⁽⁵⁰⁾ Patients with HCC presented higher EV concentrations compared to the other three groups in the study. Single protein analysis provided several proteins with diagnostic value and maximum AUROC values of 0.878 for aminopeptidase N (AMPN; SEN, 91%; SPE, 66%) in CCA versus control and 0.905 for polymeric immunoglobulin receptor (PIGR; SEN, 75%; SPE, 95%) in CCA stage I-II versus control. These two AUROC values are similar to the results obtained for the nonspecific

tumor marker carbohydrate antigen 19-9 (CA19.9), commonly used to help in the diagnosis of CCA (AUROC, 0.907 and 0.916, respectively). Notably, the combination of six biomarkers (AMPN, VNN1, PIGR, C-reactive protein, gamma-glutamyltransferase 1 [GGT1], and fibrogenin [FIBG]) resulted in an AUROC value of 0.991 (SEN, 98%; SPE, 97%) in CCA versus control. Likewise, AMPN provided a maximum area under the curve value of 0.789 for the diagnosis of PSC versus control (SEN, 83%; SPE, 63%), and its combination with six additional biomarkers (ficolin [FCN]-1, nuclear export protein, PIGR, VNN1, GPC5C, immunoglobulin kappa variable 3-01) increased this value to 0.989 (SEN, 93%; SPE, 100%). A maximum AUROC value of 0.796 for FIBG was reported for CCA versus PSC, and the combination of seven biomarkers (alpha-1-acid glycoprotein 1, S100A8, A10A9, small archaeal modifier proteins, GGT1, FCN2, immunoglobulin heavy constant alpha 1) increased this value to 0.956 (SEN, 88%; SPE, 93%) compared to 0.819 of CA19.9. Moreover, the stratification of patients with CCA resulted in a maximum AUROC value of 0.956 for FCN2 in CCA

stage I-II versus patients with PSC compared to the 0.736 value of CA19.9. When comparing HCC versus control, galectin-3-binding protein provided a maximum AUROC value of 0.904 (SEN, 97%; SPE, 72%) compared to 0.802 for alpha-fetoprotein (AFP; commonly used to help in the diagnosis of HCC). Furthermore, FIBG provided a maximum AUROC value of 0.894 (SEN, 83%; SPE, 90%) for the diagnosis of intrahepatic CCA versus HCC compared to CA19.9 (0.801) and AFP (0.753) values. Remarkably, although these biomarkers are present in EVs, they can be equally detected by immunoblot using total serum, which could be helpful for future validation studies and potential translation of the results into a clinical setting.⁽⁵⁰⁾

EVs in serum can be secreted by multiples cells, including cancer cells. An orthotopic mouse model of human CCA showed the presence of specific human proteins in serum EVs of these mice, pointing out the role of tumor-derived EVs for biomarker discovery and as potential regulators of disease progression (i.e., metastasis, immune regulation).⁽⁵⁰⁾ Furthermore, total EV concentration could also help in the diagnosis of

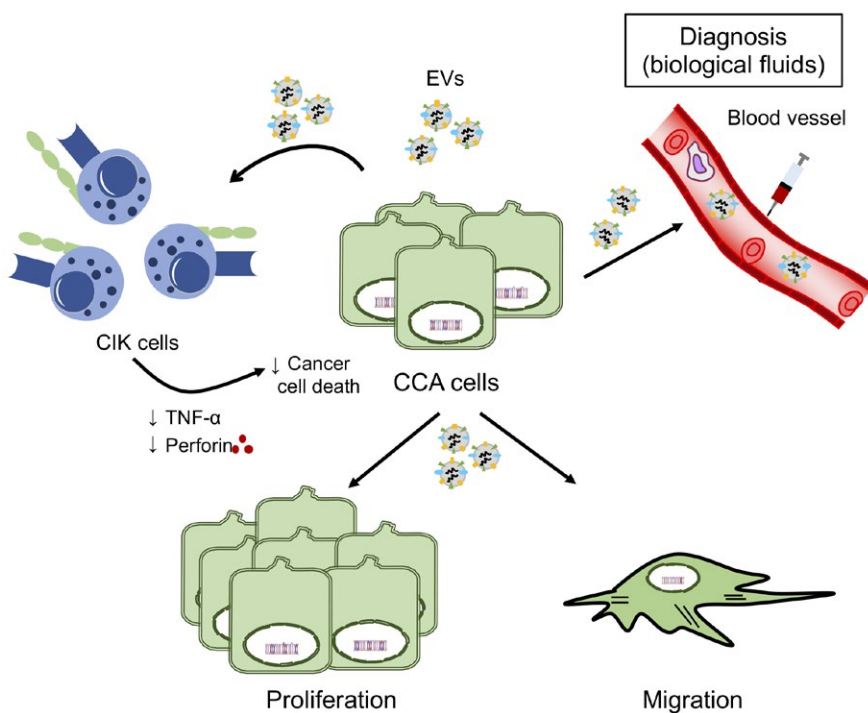


FIG. 4. Cholangiocarcinoma-derived EVs regulate tumor growth and the microenvironment and are a source of biomarkers in biological fluids. Abbreviations: CIK, cytokine-induced killer; TNF, tumor necrosis factor.

malignant common bile duct stenosis (i.e., pancreatic cancer and CCA) compared to nonmalignant conditions (i.e., chronic pancreatitis or biliary stones) as a higher concentration of EVs was reported in bile (AUROC, 1.000) and serum (AUROC, 0.813) of the malignant group.⁽⁵³⁾

EVs may also regulate the pathogenesis of biliary diseases (Fig. 4). EVs secreted by human CCA cells contain oncogenic proteins (i.e., epidermal growth factor receptor, integrin- β 4, agrin, disintegrin, and metalloproteinase domain-containing protein 10, among others) involved in the promotion of cancer cell proliferation, survival, and migration.^(50,54,55) These cancer EVs are able to regulate the tumor microenvironment. CCA-derived EVs promote the differentiation of mesenchymal stem cells into fibroblasts and the secretion of IL-6, which ultimately stimulates CCA proliferation.⁽⁵⁶⁾ CCA-derived EVs are also able to inhibit the antitumor capacity of cytokine-induced killer cells by down-regulating the secretion of tumor necrosis factor alpha and perforin.⁽⁵⁷⁾ On the other hand, the serum concentration of the cholangiocyte-derived exosomal lncRNA H19 increases under certain cholestatic conditions, further promoting hepatocellular injury and fibrosis.⁽⁵⁸⁾

Conclusions and Future Directions

Increasing evidence points to the significant role of EVs in liver pathobiology, participating in intercellular and interorgan communications, and emerging as a new opportunity for biomarker discovery and therapy. It is now evident that EVs participate in the development and progression of certain liver diseases, including NAFLD/NASH, ALD/AH, HCC, CCA, and other cholangiopathies, which are being postulated as potential targets for therapy. However, EVs also participate in the pathogenesis of other types of liver diseases/conditions not discussed in the EV Symposium of the International Liver Conference 2018 (e.g., drug-induced liver injury, viral hepatitis, and liver fibrosis, among others). Several inhibitors of EV biogenesis, release, and/or uptake are available, but their safety, efficacy, and selectivity need to be further evaluated at the preclinical and clinical level. The different concentration and/or composition of

EVs in certain liver diseases provide a unique opportunity for biomarker discovery. Moreover, all studies involving EVs require their adequate experimental characterization⁽⁵⁹⁾ as indicated by the International Society of Extracellular Vesicles. In sum, EVs represent a new and promising field of research in liver diseases that deserves future investigation.

REFERENCES

- 1) Chargaff E, West R. The biological significance of the thromboplastic protein of blood. *J Biol Chem* 1946;166:189-197.
- 2) Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol* 1967;13:269-288.
- 3) Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002;2:569-579.
- 4) Hurley JH, Boura E, Carlson LA, Rozycki B. Membrane budding. *Cell* 2010;143:875-887.
- 5) Kornek M, Schuppan D. Microparticles: modulators and biomarkers of liver disease. *J Hepatol* 2012;57:1144-1146.
- 6) Marzese AM, Janich P, Wilsch-Brauninger M, Dubreuil V, Langenfeld K, Corbeil D, et al. Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells. *J Cell Sci* 2005;118:2849-2858.
- 7) Kornek M, Lynch M, Mehta SH, Lai M, Exley M, Afdhal NH, et al. Circulating microparticles as disease-specific biomarkers of severity of inflammation in patients with hepatitis C or nonalcoholic steatohepatitis. *Gastroenterology* 2012;143:448-458.
- 8) Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci* 2011;68:2667-2688.
- 9) Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol* 2009;9:581-593.
- 10) Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972;26:239-257.
- 11) Hristov M, Erl W, Linder S, Weber PC. Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells *in vitro*. *Blood* 2004;104:2761-2766.
- 12) Maas SLN, Breakefield XO, Weaver AM. Extracellular vesicles: unique intercellular delivery vehicles. *Trends Cell Biol* 2017;27:172-188.
- 13) Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. *J Extracell Vesicles* 2014; 4:3.
- 14) Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borrás FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015;4:27066.
- 15) Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res* 2010;107:1047-1057.
- 16) Vader P, Mol EA, Pasterkamp G, Schiffelers RM. Extracellular vesicles for drug delivery. *Adv Drug Deliv Rev* 2016;106:148-156.
- 17) Kim J, Shin H, Park J. RNA in salivary extracellular vesicles as a possible tool for systemic disease diagnosis. *J Dent Res* 2017;96:938-944.
- 18) Lytvyn Y, Xiao F, Kennedy CR, Perkins BA, Reich HN, Scholey JW, et al. Assessment of urinary microparticles in normotensive patients with type 1 diabetes. *Diabetologia* 2017;60:581-584.
- 19) Zonneveld MI, Brisson AR, van Herwijnen MJ, Tan S, van de Lest CH, Redegeld FA, et al. Recovery of extracellular vesicles

- from human breast milk is influenced by sample collection and vesicle isolation procedures. *J Extracell Vesicles* 2014; 14:3.
- 20) Tietje A, Maron KN, Wei Y, Feliciano DM. Cerebrospinal fluid extracellular vesicles undergo age dependent declines and contain known and novel non-coding RNAs. *PLoS One* 2014;9:e113116.
 - 21) Masyuk AI, Huang BQ, Ward CJ, Gradilone SA, Banales JM, Masyuk TV, et al. Biliary exosomes influence cholangiocyte regulatory mechanisms and proliferation through interaction with primary cilia. *Am J Physiol Gastrointest Liver Physiol* 2010;299:G990-G999.
 - 22) **Momen-Heravi F, Saha B**, Kodys K, Catalano D, Satishchandran A, Szabo G. Increased number of circulating exosomes and their microRNA cargos are potential novel biomarkers in alcoholic hepatitis. *J Transl Med* 2015;13:261.
 - 23) Szabo G, Bala S. microRNAs in liver disease. *Nat Rev Gastroenterol Hepatol* 2013;10:542-552.
 - 24) Kostallari E, Hirsova P, Prasnicka A, Verma VK, Yaqoob U, Wongjarupong N, et al. Hepatic stellate cell-derived platelet-derived growth factor receptor-alpha-enriched extracellular vesicles promote liver fibrosis in mice through SHP2. *Hepatology* 2018;68:333-348.
 - 25) **Wang R, Ding Q**, Yaqoob U, de Assuncao TM, Verma VK, Hirsova P, et al. Exosome adherence and internalization by hepatic stellate cells triggers sphingosine 1-phosphate-dependent migration. *J Biol Chem* 2015;290:30684-30696.
 - 26) Povero D, Eguchi A, Li H, Johnson CD, Papouchado BG, Wree A, et al. Circulating extracellular vesicles with specific proteome and liver microRNAs are potential biomarkers for liver injury in experimental fatty liver disease. *PLoS One* 2014;9:e113651.
 - 27) Cheung O, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, et al. Nonalcoholic steatohepatitis is associated with altered hepatic microRNA expression. *Hepatology* 2008;48:1810-1820.
 - 28) Pirola CJ, Fernandez Gianotti T, Castano GO, Mallardi P, San Martino J, Ledesma Mora Gonzalez Lopez, M, et al. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2015;64:800-812.
 - 29) Rautou PE, Bresson J, Sainte-Marie Y, Vion AC, Paradis V, Renard JM, et al. Abnormal plasma microparticles impair vasoconstrictor responses in patients with cirrhosis. *Gastroenterology* 2012;143:166-176.e166.
 - 30) Schmelzle M, Splith K, Andersen LW, Kornek M, Schuppan D, Jones-Bamman C, et al. Increased plasma levels of microparticles expressing CD39 and CD133 in acute liver injury. *Transplantation* 2013;95:63-69.
 - 31) Garcia-Martinez I, Santoro N, Chen Y, Hoque R, Ouyang X, Caprio S, et al. Hepatocyte mitochondrial DNA drives non-alcoholic steatohepatitis by activation of TLR9. *J Clin Invest* 2016;126:859-864.
 - 32) Povero D, Eguchi A, Niesman IR, Andronikou N, de Mollerat du Jeu X, Mulya A, et al. Lipid-induced toxicity stimulates hepatocytes to release angiogenic microparticles that require vanin-1 for uptake by endothelial cells. *Sci Signal* 2013;6:ra88.
 - 33) Povero D, Panera N, Eguchi A, Johnson CD, Papouchado BG, de Araujo Horcel L, et al. Lipid-induced hepatocyte-derived extracellular vesicles regulate hepatic stellate cell via microRNAs targeting PPAR-gamma. *Cell Mol Gastroenterol Hepatol* 2015;1:646-663.e644.
 - 34) Hazra S, Xiong S, Wang J, Rippe RA, Krishna V, Chatterjee K, et al. Peroxisome proliferator-activated receptor gamma induces a phenotypic switch from activated to quiescent hepatic stellate cells. *J Biol Chem* 2004;279:11392-11401.
 - 35) Ibrahim SH, Hirsova P, Tomita K, Bronk SF, Werneburg NW, Harrison SA, et al. Mixed lineage kinase 3 mediates release of C-X-C motif ligand 10-bearing chemotactic extracellular vesicles from lipotoxic hepatocytes. *Hepatology* 2016;63:731-744.
 - 36) Hirsova P, Ibrahim SH, Krishnan A, Verma VK, Bronk SF, Werneburg NW, et al. Lipid-induced signaling causes release of inflammatory extracellular vesicles from hepatocytes. *Gastroenterology* 2016;150:956-967.
 - 37) Fukushima M, Dasgupta D, Mauer AS, Kakazu E, Nakao K, Malhi H. StAR-related lipid transfer domain 11 (STARD11)-mediated ceramide transport mediates extracellular vesicle biogenesis. *J Biol Chem* 2018;293:15277-15289.
 - 38) Bandiera S, Pfeffer S, Baumert TF, Zeisel MB. miR-122—a key factor and therapeutic target in liver disease. *J Hepatol* 2015;62:448-457.
 - 39) **Lin Y, Ding D**, Huang Q, Liu Q, Lu H, Lu Y, et al. Downregulation of miR-192 causes hepatic steatosis and lipid accumulation by inducing SREBF1: novel mechanism for bisphenol A-triggered non-alcoholic fatty liver disease. *Biochim Biophys Acta Mol Cell Biol Lipids* 2017;1862:869-882.
 - 40) Satishchandran A, Ambade A, Rao S, Hsueh YC, Iracheta-Velvet A, Tornai D, et al. microRNA 122, regulated by GRLH2, protects livers of mice and patients from ethanol-induced liver disease. *Gastroenterology* 2018;154:238-252.e237.
 - 41) **Zhu H, Wu H**, Liu X, Li B, Chen Y, Ren X, et al. Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. *Autophagy* 2009;5:816-823.
 - 42) Cai Y, Xu MJ, Koritzinsky EH, Zhou Z, Wang W, Cao H, et al. Mitochondrial DNA-enriched microparticles promote acute-on-chronic alcoholic neutrophilia and hepatotoxicity. *JCI. Insight* 2017;2:pii:92634.
 - 43) Saha B, Momen-Heravi F, Furi I, Kodys K, Catalano D, Gangopadhyay A, et al. Extracellular vesicles from mice with alcoholic liver disease carry a distinct protein cargo and induce macrophage activation through heat shock protein 90. *Hepatology* 2018;67:1986-2000.
 - 44) Bala S, Csak T, Momen-Heravi F, Lippai D, Kodys K, Catalano D, et al. Biodistribution and function of extracellular miRNA-155 in mice. *Sci Rep* 2015;5:10721.
 - 45) **Momen-Heravi F, Bala S**, Bukong T, Szabo G. Exosome-mediated delivery of functionally active miRNA-155 inhibitor to macrophages. *Nanomedicine* 2014;10:1517-1527.
 - 46) **Saha B, Momen-Heravi F**, Kodys K, Szabo G. MicroRNA cargo of extracellular vesicles from alcohol-exposed monocytes signals naive monocytes to differentiate into M2 macrophages. *J Biol Chem* 2016;291:149-159.
 - 47) **Julich-Haertel H, Urban SK, Krawczyk M, Willms A**, Jankowski K, Patkowski W, et al. Cancer-associated circulating large extracellular vesicles in cholangiocarcinoma and hepatocellular carcinoma. *J Hepatol* 2017;67:282-292.
 - 48) Willms A, Muller C, Julich H, Klein N, Schwab R, Gusgen C, et al. Tumour-associated circulating microparticles: a novel liquid biopsy tool for screening and therapy monitoring of colorectal carcinoma and other epithelial neoplasia. *Oncotarget* 2016;7:30867-30875.
 - 49) **Melo SA, Luecke LB, Kahlert C**, Fernandez AF, Gammon ST, Kaye J, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 2015;523:177-182.
 - 50) Arbelaiz A, Azkargorta M, Krawczyk M, Santos-Laso A, Lapitz A, Perugorria MJ, et al. Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. *Hepatology* 2017;66:1125-1143.
 - 51) Li L, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, et al. Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. *Hepatology* 2014;60:896-907.
 - 52) **Ge X, Wang Y, Nie J, Li Q**, Tang L, Deng X, et al. The diagnostic/prognostic potential and molecular functions of long non-coding RNAs in the exosomes derived from the bile of human cholangiocarcinoma. *Oncotarget* 2017;8:6995-70005.

- 53) Severino V, Dumonceau JM, Delhay M, Moll S, Annessi-Ramseyer I, Robin X, et al. Extracellular vesicles in bile as markers of malignant biliary stenoses. *Gastroenterology* 2017;153:495-504.e498.
- 54) Dutta S, Reamtong O, Panvongsa W, Kitdumrongthum S, Janpipatkul K, Sangvanich P, et al. Proteomics profiling of cholangiocarcinoma exosomes: a potential role of oncogenic protein transferring in cancer progression. *Biochim Biophys Acta* 2015;1852:1989-1999.
- 55) **Chaiyadet S, Sotillo J**, Smout M, Cantacessi C, Jones MK, Johnson MS, et al. Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. *J Infect Dis* 2015;212:1636-1645.
- 56) Haga H, Yan IK, Takahashi K, Wood J, Zubair A, Patel T. Tumour cell-derived extracellular vesicles interact with mesenchymal stem cells to modulate the microenvironment and enhance cholangiocarcinoma growth. *J Extracell Vesicles* 2015;4:24900.
- 57) Chen JH, Xiang JY, Ding GP, Cao LP. Cholangiocarcinoma-derived exosomes inhibit the antitumor activity of cytokine-induced killer cells by down-regulating the secretion of tumor necrosis factor-alpha and perforin. *J Zhejiang Univ Sci B* 2016;17:537-544.
- 58) Li X, Liu R, Huang Z, Gurley EC, Wang X, Wang J, et al. Cholangiocyte-derived exosomal long noncoding RNA H19 promotes cholestatic liver injury in mouse and humans. *Hepatology* 2018;68:599-615.
- 59) Lotvall J, Hill AF, Hochberg F, Buzas EI, Di Vizio D, Gardiner C, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *J Extracell Vesicles* 2014;3:26913.

Author names in bold designate shared co-first authorship.