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Hepatology Snapshot:

Extracellular vesicles, the liquid biopsy of the future

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MICROVESICLES
Membrane budding

100-800 nm

EXOSOMES
Exocytosis of MVB content (ILV)

30-150 nm

MVB

ILV

EXTRACELLULAR VESICLES (EVs)

EVs are cell-derived nano- and micro-size entities containing a specific cargo from the cell of origin.
They are abundant in body fluids, emphasizing their potential utility in novel non-invasive liquid biopsies.
Based on their origin, EVs are currently classified into 3 main categories: exosomes, microvesicles and apoptotic bodies.

Recent studies have shown that by identifying specific molecular signatures, EVs from body fluids can be used in the diagnosis of liver disease.

MONITORING CIRCULATING EVs
for diagnosis and staging liver disease by hepatocytes and other liver cells, contributing to liver injury

BLOOD

BILE

NASH	ALD	Fibrosis	HCV	Cholangiocarcinoma
CD14* Ceramide* S1P* Cyp2e1 miR-122 miR-192 ASGPR1 CXCL10 Vanin	CD40L* MIF* miR-122 miR-155 miR-192* Let7f* miR-29a* miR-340*	PDGFRα* S1P SK1 CTGF miR-214	CD4*, CD8* miR-1274b* miR-197* miR-1974* miR-21* miR-34a* miR-451* miR-548d-5p* miR-760* miR-767-3p*	miR-126* miR-618* miR-31* miR-222* miR-16* miR-486-3p* miR-484* miR-19b* miR-19a* miR-191* miR-1274b*

* potential EV-associated biomarkers for diagnosis of liver diseases in patients

CIRCULATING EV PURIFICATION
Characterisation and quantification

1 Differential ultracentrifugation
2 Size exclusion chromatography
3 Density gradient
4 Immunoaffinity

Magnetic bead
EVs

EV composition	Whole EVs	
• Protein profile via ELISA, WB, proteomics	• Transmission electron microscopy (characterization)	• Dynamic light scattering (characterization)
• RNA profile via miRNA-seq, mRNA-seq, qPCR	• Nanoparticle tracking analysis (characterization and quantification)	• Flow cytometry (quantification/characterization with CCD camera)
• Lipid profile via lipidomics		

EV RELEASE
by hepatocytes and other liver cells, contributing to liver injury

BILE DUCT | SPACE OF DISSE | SINUSOID

Endothelial cell
Kupffer cell/ monocyte
NK cell
Chemotaxis and migration
Angiogenesis and migration
Antitumor response
Activation
HSC
Activation
Virus transmission
Activation
Proliferation
Proliferation
Proliferation
Cholangiocytes
Hepatocytes

APOPTOTIC BODIES
Membrane blebbing/cellular disassembly in apoptotic cell

200-5,000 nm

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Introduction

Extracellular vesicles (EVs) are cell-derived nano- and micro-size entities containing a specific cargo from the cell of origin. They are abundant in body fluids, emphasizing their potential utility in novel non-invasive liquid biopsies. Based on their specific molecular signature, EVs from body fluids can be used to diagnose liver diseases. This snapshot summarizes the current knowledge on EV classification, characterization, origin and cargo, while reviewing the potential of EVs as novel biomarkers in the context of liver diseases.

EV classification

Based on their origin, EVs are currently classified into 3 main categories: exosomes, microvesicles and apoptotic bodies.[1] Exosomes originate from multivesicular bodies which fuse with the plasma membrane and release their intraluminal vesicles in the extracellular space. Their size ranges from 30 to 150 nm and they are enriched for markers such as CD81, CD63 and CD9. Microvesicles, which can be positive for CD81, CD63 and Annexin V, derive from plasma membrane budding and are between 100–800 nm in size. Apoptotic bodies originate from membrane blebbling and cellular disassembly from cells undergoing programmed cell death. They are heterogeneous in size with a diameter ranging from 200 to 5,000 nm and are enriched for Annexin V.[1,2]

Circulating EV purification, characterization and quantification

Many soluble factors are present in the non-EV fraction of the circulation. Thus, careful EV purification is an important first step towards their characterization and analysis. The most widely used method for EV purification is differential ultracentrifugation which eliminates several types of contaminants including large serum proteins. Other methods for EV purification include density gradient, size exclusion chromatography[3] and magnetic bead immunoaffinity. Each technique varies with increased specificity traded for decreased sensitivity.[4] The characterization of EVs is based on their size and concentration. The size of circulating EVs can be characterized using transmission electron microscopy, dynamic light scattering analysis, nanoparticle tracking analysis (NTA) and flow cytometry (FC). Moreover, NTA and FC can also measure EV concentration, which may be important in the context of disease.[4] EV composition is another crucial aspect that can be explored in disease diagnosis. EVs are composed of proteins, RNAs and lipids. Protein cargo can be identified by ELISA, western blot and proteomics/mass spectrometry. RNAs can be measured using qPCR, mRNA-seq and miRNA-seq. Lipid content of EVs can be explored using lipidomics/mass spectrometry.

EV release by hepatocytes and other liver cells and contribution to liver injury

In the last decade, increasing interest has focused on EVs as important particles for cell-to-cell communication. In the liver, EVs are released from hepatocytes and nonparenchymal cells and may act as key mediators of various pathological responses contributing to the progression of liver diseases. EVs from injured hepatocytes (Hep-EVs) can target several cell types, in an autocrine or paracrine manner, and modulate their behavior. Hep-EVs induce hepatic stellate cell (HSC) activation,[5] Kupffer cell/infiltrating macrophage recruitment and activation,[6,7] anti-tumor responses by natural killer cells, and endothelial cell migration and angiogenesis.[8] Hep-EVs can also act on the surrounding hepatocytes to induce cell proliferation and liver regeneration[9] or transmit viral particles.[10] From non-parenchymal cells, activated HSC-EVs promote neighboring HSC migration and regulate fibrogenesis.[11,12] In the bile, Hep-EVs and cholangiocyte-derived EVs are mediators of cholangiocyte proliferation.[13] These studies demonstrate that liver cell-derived EVs may be important contributors to hepatic regeneration, inflammation, angiogenesis and fibrosis. However, methods to specifically identify an EV's cell of origin remain an unmet need.

Monitoring EVs from body fluids for diagnosis and staging liver disease

Several studies emphasize the potential of EV content as biomarkers of specific diseases. In the context of liver diseases, EVs have been collected from blood or bile. Blood EVs have been studied in non-alcoholic steatohepatitis, alcohol-related liver disease, cirrhosis and hepatitis C virus infection. They are reported to contain proteins and microRNAs important for disease progression.[2,8,11,12,14–16] Bile EVs have been examined in the context of cholangiocarcinoma, with microRNAs being the center of attention of most of these investigations.[2] Some of these EV-associated markers have been studied in patients with liver disease. Taken together, these studies suggest that a combination of several EV molecules could represent a signature of a specific liver disease in a liquid biopsy.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

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Author names in bold designate shared co-first authorship

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