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Microparticles: Modulators and biomarkers of liver disease

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Emerging role of microparticles

Microparticles (MP) have gained increasing attention as biomarkers for various diseases. First described as platelet dust, MP were regarded as unspecific debris [1]. However, it has become apparent that cell derived MP or ectosomes represent a novel route of horizontal communication between cells. MP are between 100–1000 nm in size and generated through cell membrane shedding (ectocytosis), a process that can be triggered by the activation of the complement C5b-9 complex, as shown for platelet derived MP, or by inhibition of flippase activity through Ca^{2+} influx, which facilitates collapse of the plasma membrane phospholipids asymmetry [2,3]. This process involves regulated sorting of membrane proteins into the shed MP and flipping of phosphatidylserine from the inner to the outer membrane during cellular activation or early apoptosis [2,4,5]. MP resemble their parental cells on a smaller scale and share with them many characteristics, such as surface receptors, integral membrane and certain cytosolic proteins, some mRNAs and miRNAs [5]. Notably, earlier studies focused mostly on platelet MP [1,2,4–12], except for more recent studies on endothelial cell and leukocyte derived MP in cardiovascular diseases [13]. An elevation of platelet (CD41+) MP in the blood indicates the presence of inflammatory disease or its progression. Thus, an increase of platelet MP in synovial fluid or blood plasma is found in rheumatoid arthritis [8], HIV [9], the polycystic ovary syndrome [11], malaria [10] or inflammation in general [12]. Importantly, MP are not simply surrogate markers of platelet activation or disease activity, but can also modulate neighbouring cells, e.g., by transferring functional cell surface receptors that may trigger novel pathways in the recipient cell, by exchange of mRNA or miRNA, or by transfer of antigen via MHC class II molecules and other surface molecules [13]. For example, the phenotype of cells deficient in CXCR4 and CD81 was rescued by transfer of MP carrying these surface receptors [5,7,14]. Moreover, recent studies have demonstrated that apart from platelets, other cells release functional MP upon activation or early apoptosis, such as from T cells that via transfer of the metalloproteinase inducer CD147 (EMMPRIN) induce fibrolytic activation of hepatic stellate cells [15], or from leukocytes of cirrhotic patients that affect microvessels by compromising vasoconstrictor responses and decreasing systemic blood pressure [16] (Fig. 1).

Microparticles as liver disease biomarkers

As outlined before, platelet MP were considered a shared marker of disease activity, precluding their utility as disease-specific biomarker. Only recently, the concept and technology to quantitate different MP populations from plasma or serum have been established, allowing to assess the presumed extent to which a specific (immune cell) compartment is activated in a given disease. Notably, this is illustrated in chronic hepatitis C (CHC), and non-alcoholic fatty liver (NAFLD) [15,17], which show disease-specific MP profiles/signatures. Thus patients with CHC, a disease that is dominated by CD4+ and CD8+ hepatic T-cell infiltration, have primarily elevated circulating CD4+ and CD8+ T cell MP, whereas patients with NAFLD, a disease that is characterized by invariant NKT (iNKT) cells and CD14+ macrophages, monocytes, and dendritic cells (which play a key role in fatty tissue inflammation and the metabolic syndrome) have a unique signature of circulating iNKT and CD14+ MP [15,17]. Notably, these MP elevations correlate with the extent of histological inflammation in paired biopsies, though this correlation is not perfect, likely due to the biopsy's sampling variability rather than incorrectness of MP diagnostics.

However, it must be stressed that exact MP functional studies and diagnostics heavily depend on proper purification and characterization of MP fractions, which have not been done in many prior studies. Several groups showed that MP are annexin V positive on their external surface, since they cannot maintain the phospholipid asymmetry of the plasma membrane, which requires energy and various enzymes such as a phospholipid flippase, which is largely absent or inactive in MP [4,18]. Upon cell activation resulting in increased intracellular Ca^{2+}, the flippase becomes inactivated and a floppase activated, leading to collapse of the energy consuming lipid asymmetry and resulting in membrane budding and release of annexin V positive MP [4]. Generation of annexin V

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Abbreviations: ALT, alanine aminotransferase; CHC, chronic hepatitis C; NAFL, non-alcoholic fatty liver; MP, microparticle.

Hepatology Snapshot

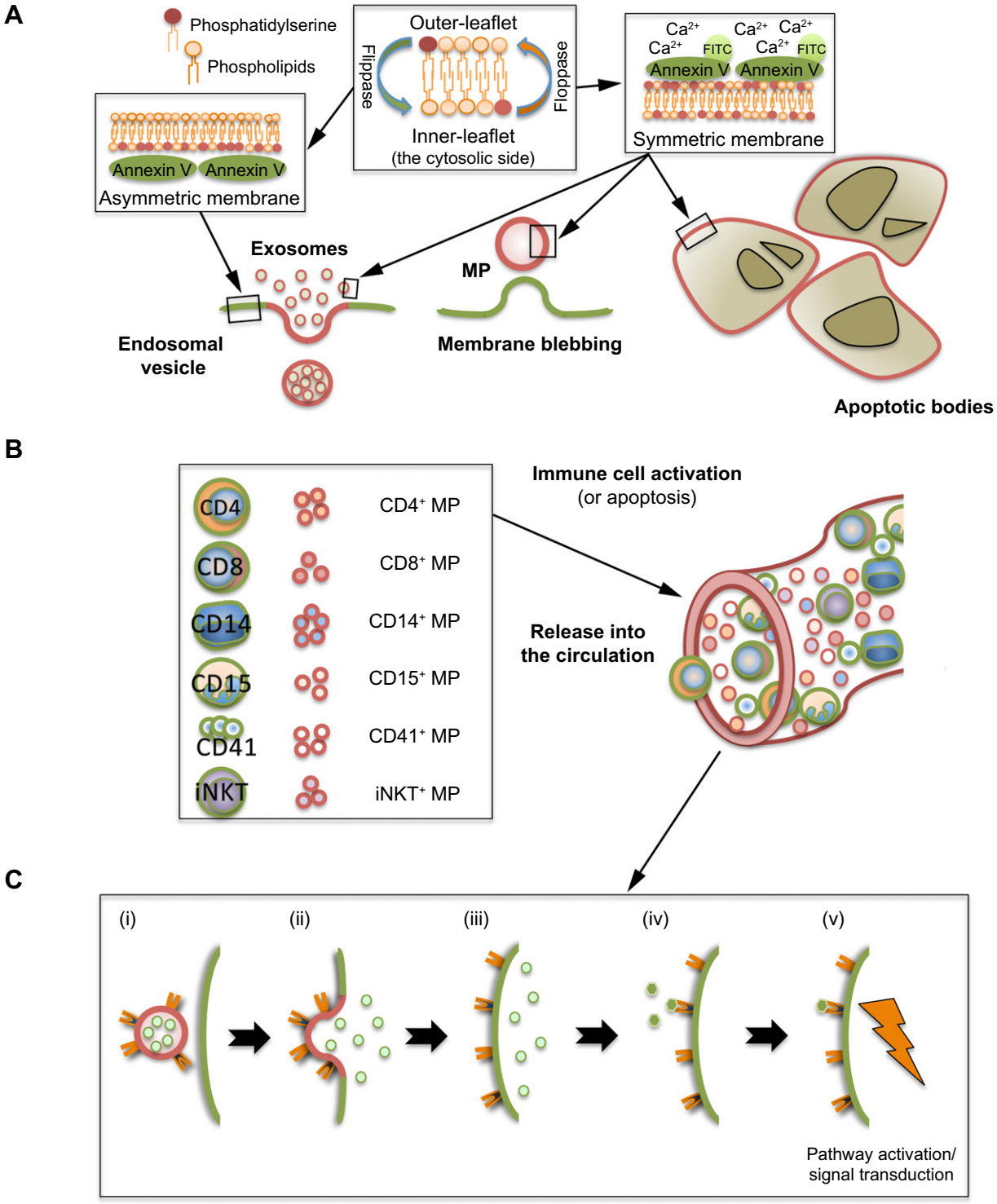

Fig. 1. Microparticles: origin, characteristics and biological effects. (A) Characteristics of exosomes, microparticles (MP), apoptotic bodies and living cells regarding phosphatidylserine (PS, annexin V) orientation within their membranes. PS is found preferentially in the inner leaflet of the cell membrane of living cells. Exosomes, MP and apoptotic bodies are incapable to maintain the asymmetry of their bilayer cell membrane due to lack of flippase activity, permitting their detection with antibodies to annexin V or dye labelled recombinant annexin V in FACS analysis. Exosomes are formed intracellularly, stored within endosomal vesicles and released by an energy-consuming process, while MP are released by plasma membrane blebbing resulting in vesicles equipped with parts of the parental cell membrane. (B) Simplified overview of the most recently studied cell types and accompanying MP populations as found in the peripheral blood [4,8,10,11,15–17]. MP are released from multiple cell types and fuse with plasma membranes of other cells, including hepatic stellate and vascular endothelial cells. Insert (C) shows how cytosolic proteins, mRNA, microRNA or other components stored in the lumen of MP can be transferred to a recipient cell (i–iii). In addition, membrane fusion can transfer receptors from MP altering receptor-mediated signal transduction in the recipient cells.

positive MP during early apoptosis may follow another yet ill-defined mechanism during generation of apoptotic bodies [4]. However, apoptotic bodies are no MP. They are characterized by a larger diameter (>1000 nm) and contain nuclear material [4]. Such material sediments at 10,000*g* as opposed to MP sedimenting between 10,000 and 100,000*g* [8,15,17]. Another "contaminant" of shedded vesicles are exosomes, a class of phospholipid bilayer vesicles that are stored intracellularly and are either annexin V negative or only slightly positive [13]. They are smaller in size (<100 nm) and sediment at 100,000*g* in sucrose gradients [4,6]. All these 'undesired' particles have to be removed physically by differential centrifugation or gated out during the process of fluorescent-activated cell sorting (FACS). Only such rigorous separation permits an exact MP quantification [15–17].

Prospects

Based on current knowledge, we suggest that circulating MP will likely become novel, highly attractive biomarkers for cell and disease specific (immune) activation, and that MP promise to become useful markers for diagnosis and especially monitoring of acute and chronic liver diseases. Importantly, MP profiling may allow for a better on-target, e.g., inflammatory cell-specific assessment of therapies directed to subsets of cells that are involved in disease pathogenesis, such as certain T cells in viral or autoimmune hepatitis, endothelial cells in cirrhosis, or iNKT cells and macrophages in NASH. To this aim, state of the art methodology for MP isolation and quantification is mandatory. Finally, the biological properties of MP as cell–cell signaling modalities may be exploited for an improved cell-specific delivery of drugs and biologicals in liver and other diseases.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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