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Extracellular vesicles: Novel mediator for cell to cell communications in liver pathogenesis

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ABSTRACT

Extracellular vesicles (EVs) are membrane derived nanometer-sized vesicles. EVs are released by normal, diseased, and transformed cells *in vitro* and *in vivo*, and carry lipids, proteins, mRNAs, non-coding RNAs, and even DNA out of cells. Transferring biological information via EVs to neighboring cells and intercellular communication not only maintain physiological functions, but also involve in the pathogenesis of several diseases, including cancer. The aim of this review is to discuss the emerging role of EVs in viral hepatitis, non-alcoholic or alcoholic liver disease and liver cancers. We summarize what is known about exosome biogenesis, and role in liver disease progression, and discuss the potential clinical applications of EVs as predictive biomarkers and therapeutic modalities.

1. Introduction

In the past decade, emerging evidences observed the interest in the role of extracellular vesicles (EVs), especially exosomes, for intercellular communication. The important role of EVs for intercellular transport of trophic materials was first reported in 1980 (Trams et al., 1981). Since then, increasing evidences in the field of extracellular vesicle research has implicated the role of EVs as novel mediators of intercellular communication for both short and longer-range signaling events (Raposo and Stoorvogel, 2013; Simons and Raposo, 2009; Balaj et al., 2011; Cossetti et al., 2014; Maas et al., 2017).

EVs contain different cytosolic proteins derived from the parent cell. These proteins are particularly enriched in integrins, MHC molecules, and cytoskeletal proteins, and also express a selection of relatively vesicle-specific proteins often used as EV markers such as the tetraspanins TSG10 or CD63 (Huang-Doran et al., 2017). For EV isolation, these markers are used in immune-affinity-based techniques or for assessing the purity of the molecules after isolating using other techniques such as ultracentrifugation, density gradient separation, and polymer-based precipitation methods.

All EVs bear surface molecules that allow them to be targeted to recipient cells. Once attached to a target cell, EVs can induce signaling via receptor-ligand interaction or can be internalized by endocytosis and/or phagocytosis or even fuse with the target cell's membrane to deliver their content into its cytosol, thereby modifying the physiological state of the recipient cell. EVs can be isolated from many biological fluids, including blood, milk, saliva, malignant ascites, amniotic fluid and urine (Théry et al., 2006; Keller et al., 2011; Lässer et al., 2011). Cells can secrete different types of EVs that have been classified according to their sub-cellular origin (Colombo et al., 2014).

Liver is a multicellular organ and comprised of parenchymal (hepatocytes) and non-parenchymal cells such as Kupffer cells, hepatic stellate cells, liver endothelial cells and intrahepatic lymphocytes including T cells, natural killer T (NKT) cells, and natural killer (NK) cells (Crispe, 2009). All these cellular populations need an intercellular communication for coordination of their behaviors to function properly. More evidences suggested the role of secreted extracellular vesicles in the intracellular signaling within the liver, besides autocrine-paracrine and cell-cell contacts.

1.1. Classification of extracellular vesicles

EVs carry a battery of bioactive cargo of soluble and membrane-bound protein, lipids, metabolites, DNA, and RNA (mRNA, miRNAs,

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and other small regulatory RNAs), and represent a nonidentical subset of the contents of the parent cell of origin (Tkach and Théry, 2016; Maas et al., 2017). EVs can be divided into three categories based on the current state of knowledge of their biogenesis (Huang-Doran et al., 2017). Discrete biogenesis pathways result in subsets of EVs namely: (i) exosomes, (ii) microvesicles, and (iii) apoptotic bodies, as schematically depicted in Fig. 1.

Exosomes are complex (30–150 nm) vesicles formed by the interior budding of endosomal membranes to form large multivesicular bodies (MVBs). Exosomes play an important role in not only cellular homeostasis, but also in the pathogenesis of major human diseases. More evidence suggested that exosomes carry material from one cell to other cell for initiation of disease. Further, exosomes have been implicated for a promising source of disease-associated biomarkers, and may eventually be used as delivery vehicle for targeted biological therapies. Extracellular vesicles may be produced by budding from the extracellular membrane yielding particles from 100 to 1000 nm known as microvesicles, shedding microvesicles or microparticles. Apoptotic vesicles are formed by large-scale plasma membrane blebbing, released during apoptotic cell death and are generally larger (100–5000 nm in diameter) (Van der Pol et al., 2012). Apoptotic bodies are not the focus of this review wherein we focus on EVs released under sublethal pathophysiologic conditions. Recently, a larger size EV population (1–10 μm diameter) was identified from highly migratory cancer cells termed oncosomes (Wendler et al., 2016).

1.2. Biogenesis and cellular release of extracellular vesicles

The process of distinguishing exosomes and microvesicles are based on their biogenesis and release into extracellular milieu. However, this process is still not well understood and many studies suggest that the mechanisms of exosome biogenesis can be cell specific and pathological or physiological condition of the cells, the stimuli triggering their release, and the different pathways of EV biogenesis (Van der Pol et al., 2012; Kourembanas, 2015). Exosomes are mainly secreted by two different mechanisms, constitutive release via the *trans*-Golgi network and inducible release (Perez-Hernandez et al., 2013; Record et al., 2014). In the vesicle generation process, the coordination of endosomal sorting complex proteins (ESCRTs) plays important roles. ESCRT0 ubiquitinates proteins for MVB delivery and recruits ESCRTI to endosomal membrane, which in turn recruits ESCRTII and ESCRTIII. Polymeric filaments formation are mediated by ESCRTIII which leads to membrane invaginations and eventually results in intraluminal vesicle (ILV) formation (Katzmann et al., 2001; Babst et al., 2002; Wollert et al., 2009; Tamai et al., 2010; Kowal et al., 2014). The presence of ESCRT components in exosomes was identified using high throughput protein analysis methods. Downregulation of key components of ESCRT system abrogates ILV formation and release of exosomes (Tamai et al., 2010).

Exosome release is also controlled by Rab guanosine triphosphatases. For example, Rab11 associates with vesicles derived from the *trans*-Golgi network, promoting MVB formation and subsequent plasma membrane fusion (Hirsova et al., 2016). We observed that knockdown of Rab27a inhibits generation of intracellular and extracellular infectious hepatitis C virus particles (Shrivastava et al., 2015). Further, silencing of Rab27a decreases exosome release in the culture supernatant without altering the exosome protein content (Ostrowski et al., 2010; Shrivastava et al., 2015). The process of biogenesis and exosome secretion is described elsewhere (Kowal et al., 2014; Hirsova et al., 2016; Maas et al., 2017) and summarized in Fig. 2.

Methods of exosomes isolation are summarized below. Culture supernatants from control or virus infected hepatocytes were collected and centrifuged at 300 × g at 4 °C for 5 min. Without disturbing the cell pellet, supernatants were transferred to new tube. Supernatants then sequentially centrifuged at 2000×*g* for 10 min at 4 °C, 26,500 × g for 30 min at 4 °C followed by at 110,000 × g for 90 min at 4 °C. After discarding the supernatant, and the exosome pellet was washed two times with PBS by centrifugation at 110,000 × g for 60 min at 4 °C. The final pellet was

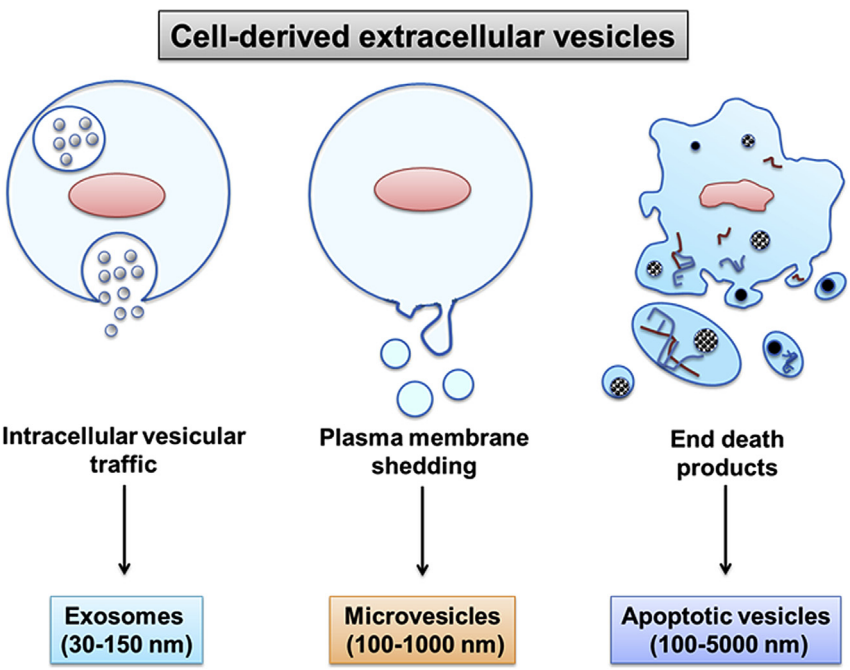

Fig. 1. Classification of extracellular vesicles according to the mechanism of generation. Exosomes are generated intracellularly from multivesicular bodies. Microvesicles are produced by budding from the extracellular membrane. Apoptotic vesicles are released upon cell fragmentation during apoptotic cell death. Representative sizes are shown at the bottom.

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