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Exosomes in the pathogenesis, diagnostics and therapeutics of liver diseases

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Summary

Exosomes are small (30–100 nm in diameter) extracellular membrane-enclosed vesicles released by different cell types into the extracellular space or into biological fluids by exocytosis as a result of fusion of intracellular multivesicular bodies with the plasma membrane. The primary function of exosomes is intercellular communication with both beneficial (physiological) and harmful (pathological) potential outcomes. Liver cells are exosome-releasing cells as well as targets for endogenous exosomes and exosomes derived from cells of other organs. Despite limited studies on liver exosomes, initial observations suggest that these vesicles are important in liver physiology and pathophysiology. In this review, we briefly summarize the recent findings on liver exosomes, their functions and significance for novel diagnostic and therapeutic approaches.

Introduction

A wide variety of cells release small (30–100 nm in diameter) membrane-enclosed vesicles termed exosomes into the extracellular milieu. Over the past several years, there has been increasing interest in the biological properties and pathophysiological relevance of these extracellular vesicles derived from liver cells, principally hepatocytes and cholangiocytes. In this review, we briefly summarize the recent findings on liver exosomes, their functions and significance for novel diagnostic and therapeutic approaches.

Formation, composition, and functions of exosomes

Exosomes are derived from the internal vesicles of multivesicular bodies (MVBs). While some MVBs are degraded in lysosomes, other MVBs fuse with the plasma membrane and release their content of microvesicles in an exocytic manner into biological fluids such as blood, urine, bile, saliva *in vivo*, or into culture medium *in vitro* [1–3] (Fig. 1). The number of exosomes present in biological fluids is high; in serum, it is estimated to be about 3 million exosomes per microliter [3].

Morphologically by electron microscopy, exosomes typically are vesicles with distinctive cup- or "deflated football"-shaped morphology (Fig. 1); the shape may reflect a collapse of vesicles during sample preparation, because cryo-electron and scanning electron microscopy show a perfectly rounded shape of isolated exosomes (Fig. 1) [4]. Biochemically, exosomes contain common "marker" proteins (e.g., tetraspanins such as CD9, CD10, CD26, CD53, CD63, CD81, CD82; endosome-associated proteins that are involved in MVB biogenesis, Alix and TSG101; cytoplasmic heat shock proteins, Hsc70 and Hsp90), and cell-type specific proteins and nucleic acids including mRNAs, microRNAs (miRNAs) and other non-coding RNAs, the composition of which depends on the functional state of the cells (e.g., rested, stimulated, stressed, transformed, etc.) [1,3]. The estimated total cargo per exosome is about 100 proteins and 10,000 net nucleotides [3]. As of January 2012, 11,261 proteins, 2375 mRNAs and 764 miRNAs have been identified in association with exosomes suggesting their great heterogeneity and uniqueness [5].

The primary function of exosomes is intercellular communication; i.e., to shuttle various signaling molecules between neighboring and distant cells [1–3]. Exosomes can interact with target cells via unknown receptors, including receptors localized to primary cilia, and activate downstream intracellular signaling pathways [1–3,6]. They can also directly fuse with the cell membrane integrating exosomal membrane proteins into the plasma membrane or be endocytosed delivering their cargo into the cytoplasm of the recipient cells [1,3,4] (Fig. 2).

Exosomes are heterogenous in size and content and demonstrate different biological effects and targeting specificities. They can influence the immune system, act as signaling complexes, transfer receptors from one cell to another, convey specific mRNAs, miRNAs, and proteins into the cytoplasm of recipient cells, and, as a result, impact numerous physiological processes [2–4]. Exosomes also facilitate viral transport, spread cell damage, and stimulate malignant transformation [4,7].

Exosomes in liver physiology and disease

Both types of liver epithelia (i.e., hepatocytes and cholangiocytes), natural killer T (NKT) cells, hepatic stellate cells, adult liver

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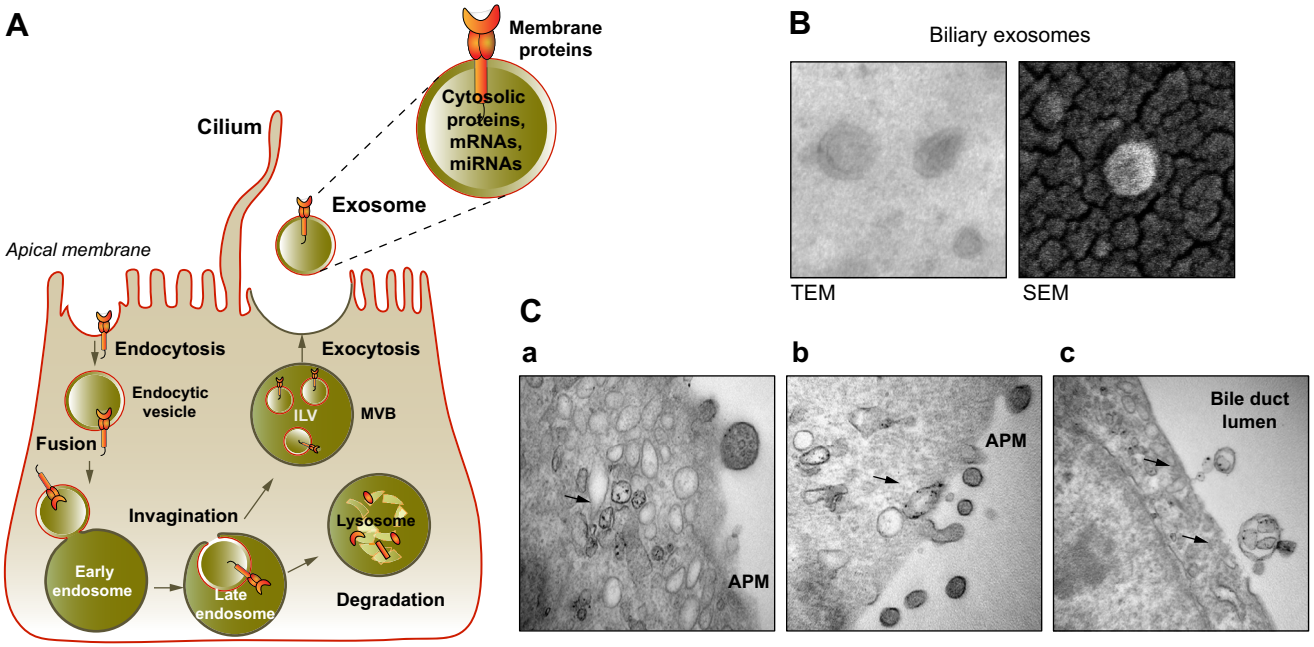

Fig. 1. Exosome release. (A) Exosomes containing membrane and cytosolic proteins, mRNAs, and miRNAs, are derived from the multivesicular body (MVB) sorting pathway. Membrane proteins are oriented in a fashion (extracellular region out) that permits profound biological autocrine and paracrine effects. (B) Exosomes isolated from rat bile have a cup- or "deflated football"-shaped morphology by transmission electron microscopy (TEM), but they have a perfectly round shape by scanning electron microscopy (SEM). (C) In cholangiocytes of mouse liver, MVBs containing exosomes (arrows) (a) move to the apical plasma membrane (APM) (b), and release exosomes into the bile duct lumen by exocytosis (c). (B and C adapted from [6], with permission).

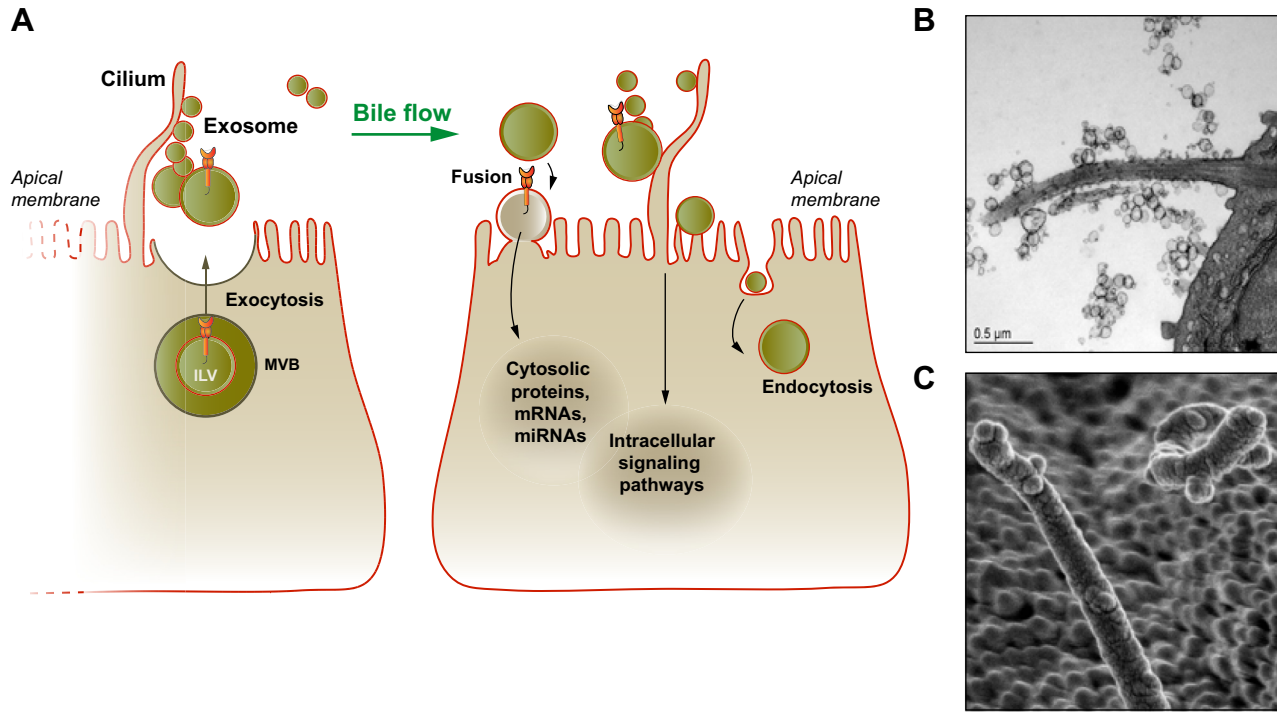

Fig. 2. Exosomes in intercellular signaling. (A) In the liver, exosomes derived from hepatocytes and cholangiocytes are transported by bile flow to target cholangiocytes with which they may interact via several mechanisms depending on their cargo and biological properties. They can fuse with the plasma membrane and deliver their content into the cytoplasm of a target cell; interact with receptors on the apical plasma and ciliary membrane inducing intracellular signaling; and endocytosed for recycling. (B and C) Biliary exosomes surround and attach to cholangiocyte cilia in mouse liver as viewed by TEM (B) and SEM (C), supporting the involvement of exosomes and cilia in mechanisms of intercellular signaling. (B and C adapted from [6], with permission).