Extracellular Vesicles as Biomarkers and Therapeutics in Dermatology: A Focus on Exosomes

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Extracellular vesicles (exosomes, microvesicles, and apoptotic bodies) are ubiquitous in human tissues, circulation, and body fluids. Of these vesicles, exosomes are of growing interest among investigators across multiple fields, including dermatology. The characteristics of exosomes, their associated cargo (nucleic acids, proteins, and lipids), and downstream functions are vastly different, depending on the cell origin. Here, we review concepts in extracellular vesicle biology, with a focus on exosomes, highlighting recent studies in the field of dermatology. Furthermore, we highlight emerging technical issues associated with isolating and measuring exosomes. Extracellular vesicles, including exosomes, have immediate potential for serving as biomarkers and therapeutics in dermatology over the next decade.

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INTRODUCTION

Cell-to-cell communication is vital for human development and survival. Intercellular communication is regulated by extracellular hormones, metabolites, and cytokines or direct cell-to-cell contact. Still, there is an increasingly recognized role of extracellular vesicles (EVs) as "masters of intercellular communication" (Pitt et al., 2016b). EVs ("exosomes," "microvesicles," and "apoptotic bodies") carry complex cargo, including messenger RNAs, microRNAs, long noncoding RNAs, mitochondrial DNAs, single-stranded DNA, double-stranded DNAs, protein ligands, receptors, and transcription factors (Gross et al., 2012; Kalra et al., 2012; Keerthikumar et al., 2016; Li et al., 2014; Pitt et al., 2016b; Théry et al., 2002; Valadi et al., 2007). In this review, we highlight extracellular vesicle biogenesis, focusing on exosomes, in applications to dermatology. We discuss technical challenges in studying exosomes and future avenues for the development of EVs as biomarkers and therapeutics.

CURRENT DEFINITIONS, BIOSYNTHESIS, AND COMPOSITION OF EVs

EVs are similar to miniature versions of the parent cell, made of a lipid bilayer containing proteins and nucleic acids (Pitt et al., 2016b; Théry et al., 2001). EVs have been classically divided into broad categories: (i) exosomes, (ii) microvesicles, and (iii) apoptotic bodies (Figure 1).

Exosomes

Exosomes, mostly sized between 30 and 150 nm, are generated within multivesicular bodies (MVBs), which are endosome compartments in which "intraluminal vesicles" (ILVs) develop; these ILVs later become secreted into the extracellular space as "exosomes" (Pitt et al., 2016b). Exosomes are as durable as viruses, with the ability to deform elastically while maintaining vesicle integrity (Calo et al., 2014; Riazifar et al., 2017). Members of the Rab GTPase family modulate exosome secretion and act on different MVBs along "endosomal sorting complex required for transport" (ESCRT)-dependent and -independent pathways (Xu et al., 2016). ILVs within MVBs form when RAB5-positive early endosomes interact with the ESCRT complexes (which include ESCRT-0, -I, -II, and -III, each controlling different steps in cargo selection and exosome formation) (Henne et al., 2011; Hurley, 2008; McGough and Vincent, 2016). These specialized proteins recognize cargo marked for sorting (Piper, 2007). MVBs with ILVs can be targeted to lysosomes or transferred to the plasma membrane (involving RAB 11, RAB27, and RAB35) where SNARE proteins can promote exosome release (Cai et al., 2007; McGough and Vincent, 2016; Piper, 2007). Exosomes are highly enriched in certain tetraspanins, such as CD63, CD81, and CD9. Endosome-related protein TSG101 is a recognized marker (Raposo and Stoorvogel, 2013; van Niel et al., 2006). The protein ALIX also marks exosomes, as an adapter protein in cooperation with ESCRT complexes, contributing to ubiquitylation-independent cargo sorting into MVBs.

An international web-based collection of exosome cargo from various cell types is listed at EXOCARTA (http://www.exocarta.org). Although much of the cargo is interior, some proteins may exist exteriorly. Lipophilic morphogens, such as Wnts and hedgehogs, may exist on or in exosomes; however, this is not agreed on (Gross et al., 2012; Mittelbrunn et al., 2015; Riazifar et al., 2017). The profile of lipids on different types of exosomes depends on the cell producer, and the activity of membrane lipid scramblases, flippases, and floppases (Riazifar et al., 2017). How different

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Abbreviations: AB, apoptotic body; ESCRT, endosomal sorting complex required for transport; EV, extracellular vesicle; ILV, intraluminal vesicle; MCC, Merkel cell carcinoma; MSC, mesenchymal stem cell; MVB, multivesicular body; PBMC, peripheral blood mononuclear cell

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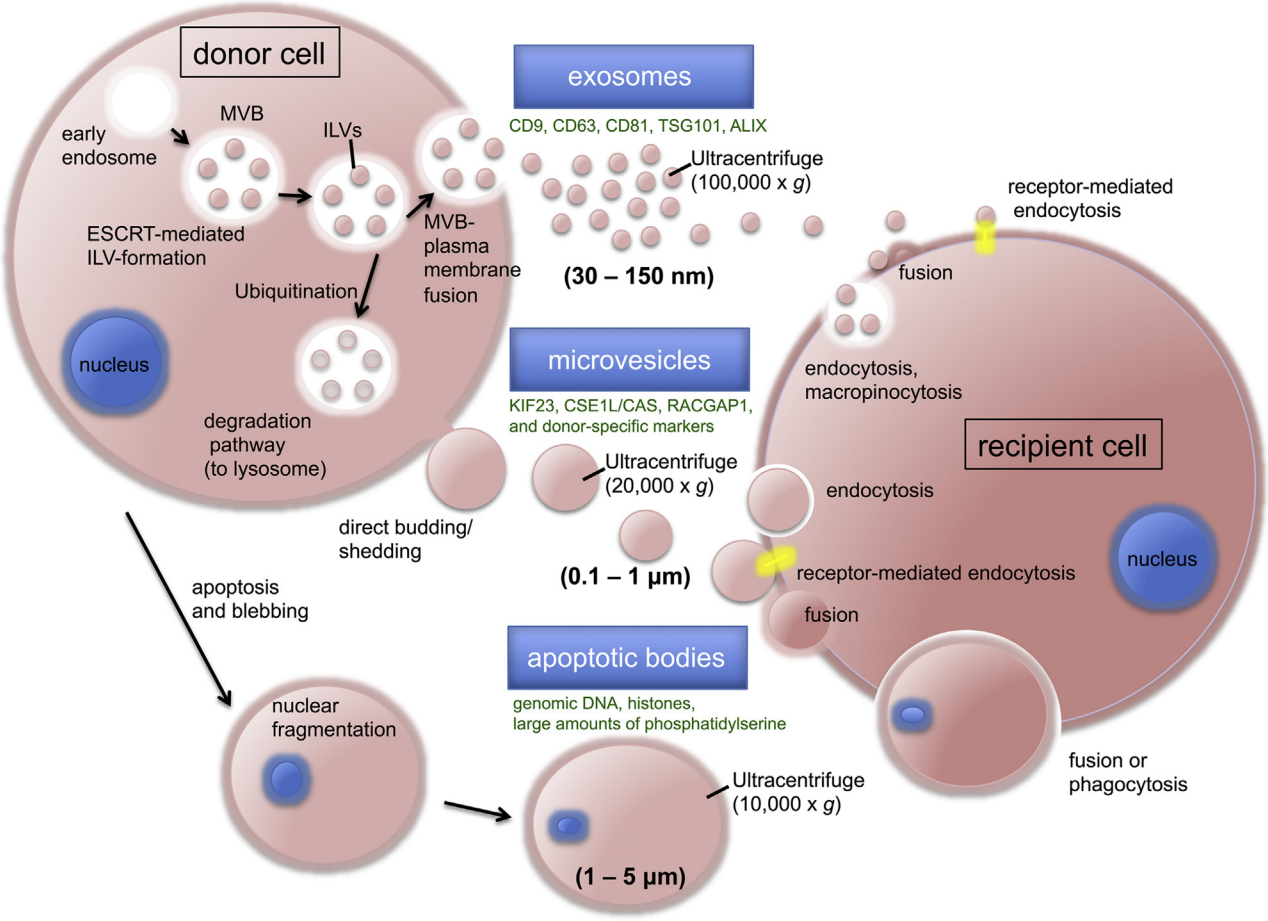

Figure 1. Three types of extracellular vesicles (EVs). Exosomes form in structures called multivesicular bodies (MVBs) derived from endosomes, and are typically marked by CD63, CD9, CD81, TSG101, and ALIX. Exosomes form inside of MVBs as intraluminal vesicles (ILVs) via ESCRT proteins sorted via RAB-mediated processes to be released at the plasma membrane, a process regulated by SNAREs. Microvesicles are released directly from the plasma membrane (direct shedding), which might be regulated, at least in part, by ESCRT-I, ADP-ribosylation factor 6 (ARF6), and/or acid sphingomyelinase (aSMase). Microvesicles may be marked by kinesin-like protein (KIF23), exportin-2/chromosome segregation like-1 protein (CSE1L/CAS), and Rac GTPase-activating protein 1 (RACGAP1). Apoptotic bodies are the result of apoptosis-driven cell fragmentation, containing genomic DNA and histones, and can contain the types of inner vesicle cargo that exosomes and microvesicles contain. Once released into the extracellular space, EVs can carry nucleic acids, proteins, lipids, and other associated molecules to cause broad effects in the recipient cells, including activation of cell signaling at the surface or regulation of molecules inside the recipient cell. Recipient cells uptake vesicles via fusion, endocytosis of all types, including macropinocytosis and receptor-mediated endocytosis. The biological functions of EVs are numerous, and largely depend on the cargo delivered to the recipient cell. Not shown: the "recipient cell" can become "donor cell," establishing a crosstalk in the other direction, consisting of vesicle exchanges that mediate complex biological processes (see Supplementary Table S1 online). ESCRT, endosomal sorting complex required for transport.

exosome-lipid compositions might affect the affinity for different lipophilic proteins is unknown. Cholesterol and sphingolipids enable tight lipid packaging and structural rigidity of exosomes (Yanez-Mo et al., 2015). It will be important to study how lipid compositions differ in various exosome populations and what, if any, effects these differences may have on functional outcomes in dermatologic studies.

Microvesicles

Microvesicles are formed by direct shedding from the plasma membrane via outward invaginations. Microvesicles contain plasma membrane proteins, cytosolic proteins, nucleic acids, and other small molecules (Riazifar et al., 2017). Their sizes average approximately 200 nm, but range from 50 to 1500 nm (Pitt et al., 2016b; Raposo and Stoorvogel, 2013; Xu et al., 2016). Much is still not known regarding microvesicle biogenesis, although ARF6, acid sphingomyelinase activity, and ESCRT proteins are thought to be involved (Xu et al., 2016). Because microvesicles originate from plasma membrane pinching, microvesicles "pick up" cytosolic cargo on the periphery of the cell before secretion, whereas exosomes contain cytosolic contents accumulated near the formation of ILVs in MVBs (Riazifar et al., 2017).

Apoptotic bodies

Apoptotic bodies (ABs) are released during cell apoptosis, resulting in vesicles composed of organelle and plasma membranes with nuclear and cytoplasmic contents (Riazifar et al., 2017). ABs are larger than exosomes and microvesicles, typically ranging from 1,000 to 5,000 nm. Phagocytes usually take up ABs after being shed and eliminated rapidly from the human circulation, but can interact with many recipient cells before phagocytosis (Pitt et al., 2016b; Raposo and Stoorvogel, 2013; Xu et al., 2016). Uptake of