SnapShot: Extracellular Vesicles

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(1 and 2) What are Extracellular Vesicles (EVs)?

Extracellular Vesicles (EVs) are structures released into their environment by all cells. They are delimited by a lipid-bilayer and contain components from the cells that release them. EVs can form first as intraluminal vesicles (ILVs) in multivesicular compartments (generally of endocytic nature), and be secreted upon fusion of these compartments with the plasma membrane (exosomes). Other EVs can be released directly from the plasma membrane (ectosomes, microvesicles, microparticles, large oncosomes, apoptotic bodies). Because prokaryotic cells do not have internal compartments, their EVs can only originate from their limiting membrane. Enveloped viruses that highjack membranes from the infected cell for release can also be considered as a type of EV. EVs have the same membrane orientation as the cells: they expose at their surface lipids and the extracellular domains of transmembrane proteins, and enclose mainly cytoplasmic components, including proteins and nucleic acids. Since they enclose components from the originating cells, EVs are also explored as sources of circulating biomarkers in biological fluids. Several comparative proteomic studies have recently provided lists of proteins that could be specific for determined EV subtypes. However, because of the difficulty in finding consistent markers across studies, specific EV subtype marker proteins are not yet clearly identified.

(3) What do we know about the mechanisms of biogenesis of EVs?

Several mechanisms of EVs budding can take place, involving either one or a combination of the following machineries: ESCRT complex, tetraspanins, sphingomyelinases generating cone-shaped lipids (ceramide), relocalization of phospholipids toward the leaflet of the membrane where budding takes place, depolymerization of the actin cytoskeleton. Some of these mechanisms have been described specifically in exosome or ectosome formation but their potential involvement in the budding of both cannot be excluded. Therefore, specific biogenesis mechanisms are not yet clearly known.

(4) What do we know about the function of EVs?

EVs have been identified as a means, for the secreting cell, of disposing harmful or useless intracellular components, but also as important mediators of communication with other cells. EVs carry various cargoes that can deliver signals to induce physiological changes in recipient cells. For example, EVs expose ligands that can bind to cognate receptors on the target cells mediating signaling cascades. Internalized EVs can be degraded, becoming a source of nutrients for recipient cells. EVs' content can also be transferred into and present activity in the cytoplasm of target cell. Transfer mechanisms for non-viral EVs are yet unclear. EVs can mediate the interaction of secreting cells with the surrounding extracellular matrix (ECM). Moreover, EVs deposited in ECM can also serve as indirect communication to nearby cells. EVs also function in long-distance communication, as they can be released into blood or lymphatic vessels and deliver their content to distant target cells.

(5) How can we separate different types of EVs and co-isolated components?

EVs are isolated from conditioned medium of cultured cells, or from biological fluids, after elimination of cells by low-speed centrifugation. The different types of EVs display overlapping biophysical properties. The diameter of ILVs of endocytic compartments is around 100 nm: exosomes are

therefore in the diameter range of 50-150 nm. EVs formed at the plasma membrane can be of this size range, or larger (up to 5 μm). Other extracellular nanoparticles (ENPs) with unknown site of origin and mode of formation, for instance “exomeres”, can be found together with EVs. Exomeres contain proteins, nucleic acids and lipids and are in the lowest range of EV sizes (around or below 50nm). Various non-vesicular lipoproteins released by some cell types and in biological fluids also overlap with EVs in size and density. Because of these overlapping properties, most EV isolation methods co-isolate to various degrees some of the other extracellular structures, or do not separate the different EV subtypes. Here, we present some of the most commonly used techniques, ranking them in a recovery versus specificity grid. Specificity can be considered either for EVs (as opposed to other ENPs), or for a specific EV subtype.

Global concentration methods by filtration (FC) or polymer-based precipitation (P) (e.g. with PEG, *polyethylene glycol*) can recover EVs, ENPs and a majority of other secreted products. EVs can be separated from soluble components and some lipoproteins by size-exclusion chromatography (SEC: soluble components and the smallest lipoproteins remain longer in the column bed). In these settings, EV subtypes display slightly different but overlapping properties, and therefore separation of EV subpopulations is inefficient. Separation of EV subtypes can be performed based on size and/or weight using differential centrifugation (dUC) with increasing g force/time. This approach enriches for (but does not purify) EVs of a selected range of sizes, and ENPs as well as soluble components are non-specifically co-isolated, especially with the smallest EVs. Further separation of EV subtypes and ENPs can be achieved by bottom-up flotation into a density gradient (DG): non-lipidic structures do not float upwards. Alternatively, they can be separated by top-down DG or density cushions: soluble components do not enter the gradient. Separation of EV subtypes and ENPs can also be achieved based on size via Asymetric flow field-flow fractionation (AF4). Finally, the most specific separation can be achieved by immunoprecipitation (IP) using antibodies specific to a given surface protein of EVs. In general, a combination of techniques is necessary to first concentrate EVs and then achieve better specificity of isolation.

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