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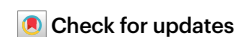
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Protrusion-derived vesicles: new subtype of EVs?

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Cellular protrusions are highly dynamic structures that facilitate cell–cell communication and are increasingly recognised for their roles in the shedding of bioactive extracellular vesicles (EVs). The intrinsic and extrinsic mechanisms that govern the shedding of EVs from cellular protrusions and their potential physiological roles are beginning to emerge.

Extracellular vesicles (EVs) are membrane-bound vesicles released by almost all cells. EVs carry a variety of components (proteins, lipids, and genetic material) that can be transferred to target cells to modulate cell fate and behaviour¹. EVs have been broadly divided into two main categories on the basis of their biogenesis and diameter: endosome-derived vesicles (including multivesicular endosome-derived exosomes and secretory autophagosome-derived EVs) (~30–150 nm), which correspond to the intraluminal vesicles of multivesicular endosomes and are secreted after fusion of such endosomes with the plasma membrane; and ectosomes (including microvesicles, Arrestin domain-containing protein 1 (ARRDC1)-mediated microvesicles (ARMMs), oncosomes, exophers, migrasomes, and enveloped viruses) (~50–15,000 nm), which are generated by the outward budding of portions of the plasma membrane.

Various cells generate cellular protrusions, such as filopodia and microvilli, at their plasma membrane. These structures are exposed to the external milieu, where external forces affect their architecture and membrane dynamics. Notably, recent reports provided evidence that membrane protrusions such as filopodia, microvilli, and cilia are often sites of EV generation¹. Although the mechanisms involved in EV shedding from protrusions are only beginning to be understood, these observations highlight the plasticity of these cellular protrusive structures and reveal a potential additional function of protrusions in cell communication through the release of bioactive EVs. In this Comment, we summarise the current understanding of EV shedding from cellular protrusions and outline gaps in our knowledge.

Membrane-shaping machinery

Filopodia and microvilli are cellular protrusive structures with highly curved membranes that are supported by the cytoskeleton. They are used by various cells to sense and interact with their environment. The formation of these protrusions involves not only a reorganisation of cytoskeleton proteins (i.e., actin, myosins, and their associated proteins) and membrane lipids, but also the generation of outward curvature of the plasma membrane, driven by Inverse Bin–Amphiphysin–Rvs

(I-BAR) domain-containing proteins. Members of the I-BAR family (including IRSp53, IRTKS, and MIM) connect membrane curvatures to actin filaments, as well as to small GTPases (i.e., Rac1 and Cdc42) and phosphoinositides². Remarkably, these proteins have recently been shown to drive EV secretion^{3,4}.

Interesting examples are the I-BAR proteins MIM and IRSp53, both of which commonly localize to the tip and shaft of filopodia. In cultured cells, MIM and IRSp53 interact with membrane phosphoinositides to promote the formation of filopodia and the subsequent release of filopodia-derived EVs under the assistance of external forces (see below)³. IRTKS, through its interaction with actin-binding proteins, is essential for microvilli biogenesis⁵. Analogous to MIM and IRSp53, IRTKS may thus promote microvilli scission to allow EV shedding, although the exact mechanisms involved remain to be determined.

The biogenesis of microvilli-derived EVs is also dependent on transmembrane proteins of the Prominin (also known as CD133) family. Prominin-1 is selectively targeted to microvilli, where it binds to cholesterol and gangliosides found in a specific subtype of cholesterol-based membrane microdomain, and interacts with the underlying actin cytoskeleton. Together with the actin cytoskeleton and cholesterol, Prominin-1 maintains microvilli architecture and promotes the release of microvilli-derived EVs⁶. In non-epithelial cells, the interaction of Prominin-1 with the actin-branching factor Arp2/3 complex favours filopodia formation⁷. It remains an interesting question whether the same molecular network as that in microvilli contributes to the regulation of filopodia-derived EV formation and in shedding from non-epithelial cells.

Microvilli and filopodia also interact with glycocalyx constituents that have been implicated in sculpting the plasma membrane architecture and in promoting EV shedding¹. Collectively, recent studies demonstrated that the protein machinery involved in the biogenesis of cellular protrusions is also implicated in the release of EVs. However, it remains to be deciphered how this protein machinery directly senses membrane curvature and promotes EV shedding.

Mechanical input

There seem to be three mechanical inputs for scission of the protrusions: the endosomal sorting complex required for transport (ESCRT), which

“recent studies demonstrated that the protein machinery involved in the biogenesis of cellular protrusions is also implicated in the release of EVs”

severs the membrane tubules in an ATP-dependent manner; actomyosin contractility; and external forces.

Viral buds are thin protrusions that are shed and released from the plasma membrane in an ESCRT-dependent manner, similar to EVs. Interestingly, IRSp53 is required for the progression of HIV-1 membrane curvature and for the release of HIV-1 vesicles⁸. The release of small plasma membrane vesicles containing ARRD1, called ARMMs, requires the ESCRT complex¹. But the interplay between ARRD1, ESCRT, and IRSp53 or any BAR domain proteins in the biogenesis of HIV-1 and ARMMs is unclear.

EVs can be released from the plasma membrane by actomyosin contractility. In tumour cells, the actomyosin contraction responsible for EV release is regulated in part by the small GTPase ADP-ribosylation factor 6 through the formation of bleb-like protrusions¹. As another example, the release of EVs from enterocyte microvilli is triggered by the motor protein myosin, which is distributed along microvilli tips and presumably exerts directed force on the apical membrane¹. Yet it remains to be determined whether shedding is mediated by mechanical contractile forces or is promoted by unknown molecules with membrane scission activity. In addition, actin filament bundling must be finely tuned to enable EV shedding, as an excess of Fascin-mediated actin bundles disfavours EV secretion by increasing the force required for vesiculation³. Actomyosin contractility also mediates EV release from the tip of the primary cilia⁹, where Prominin-1 is also found. Because Prominin-1-containing EVs are released from primary cilia⁹, both actomyosin and Prominin-1 could facilitate the shedding of microvilli- and cilia-derived EVs.

External forces also facilitate the shedding of protrusion-derived EVs. The flow of fluid surrounding cells generates shear stress, which contributes to EV release from protrusions, most likely through friction-mediated scission. Although these processes require further elucidation, we hypothesize that filopodia can be cleaved in vivo by forces generated by naturally occurring pressures, such as those present in blood capillaries or the extracellular matrix³.

Together, these findings indicate that protrusions could be sources of EV production, whose shedding is mediated by mechanical forces. How the breakpoints in protrusions are determined or regulated will require future studies. However, the boundaries of membrane protrusions that result from the embedding of the I-BAR domain-containing proteins or Prominin-1 into specific lipid-rich microdomains could represent major breakpoints sites.

Perspectives for EV classification

Given the increasing diversity of EV subtypes reported, the criteria for discriminating among the different subpopulations of exosomes and ectosomes need to be adapted. The previous criteria, based on biogenesis, size, and associated components of EVs, do not enable the distinction of protrusion-derived EVs from previously defined EV subtypes. First, the diameter of EVs derived from these thin membrane protrusions overlaps that of small EVs such as endosome-derived EVs. Second, the proteins involved in their biogenesis, of which the representative protein is ESCRT, may also be shared by both protrusion- and

endosome-derived EV subpopulations. Third, the tetraspanins CD9, CD63, and CD81, considered to be standard EV markers, localise not only to the endosomal compartment but also to the plasma membrane, including that of protrusions. Interestingly, it was recently demonstrated that EVs carrying CD9 or CD81 but not CD63 bud from the plasma membrane, whereas EVs enriched in CD63 with little CD9 correspond to endosome-derived EVs¹⁰. The use of a particular combination of proteins (Prominin-1, I-BAR domain-containing proteins, CD9, CD81) could be considered to discriminate between different EV populations; e.g., plasma membrane- and protrusion-derived EVs. Although these proteins are promising markers, more-precise identification of specific proteins is needed to consolidate an updated nomenclature of EVs. Protrusions and their derived EVs, in addition to exosomes and ectosomes, extend the repertoire of EVs and the ability of donor cells to exchange with their neighbours and the environment.

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Competing interests

The authors declare no competing interests.