Catching filopodia: Exosomes surf on fast highways to enter cells

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The mechanisms of exosomal uptake and their intracellular itinerary are not understood. In this issue, Heusermann et al. (2016. *J Cell. Biol.* http://dx.doi.org/10.1083/jcb.201506084) show that exosomes surf filopodia and are endocytosed in a process reminiscent to virus entry. Intraendosomal exosomes travel to the ER and are distributed to lysosomal compartments.

Exosomes are small extracellular vesicles, which are released by many cell types, and were initially described as vehicles for the disposal of superfluous cellular content. Increasing evidence points toward their functions in cell–cell communication and transcellular delivery of cargo such as RNA (Simons and Raposo, 2009; Tkach and Théry, 2016). Exosomes play important roles in diverse processes including immune regulation, tumor formation, and pathogen spreading (Raposo and Stoorvogel, 2013; Tkach and Théry, 2016). Despite these far-reaching functions, there is little mechanistic insight into the regulation of target cell tropism, uptake, intracellular trafficking, and cargo release from exosomes. Unraveling the process of exosome uptake and cargo unloading has become increasingly important because exosomes are regarded as potential vehicles for the delivery of therapeutic agents in patients (Johnsen et al., 2014; Leoni et al., 2015).

Similarities between exosomes and viruses have been pointed out before, because of their shared mechanisms of biogenesis and release (Wurdinger et al., 2012; van Dongen et al., 2016). Some scientists have proposed that exosomes are the evolutionary descendants of ancient retroviruses as they can carry viral nucleic acids that are capable of controlling gene expression in target cells (Wurdinger et al., 2012; Narayanan et al., 2013). Others have put forward the Trojan hypothesis that states that retroviruses use the preexisting, nonviral exosome pathways for the formation and uptake of infectious particles (Gould et al., 2003; Izquierdo-Useros et al., 2011). In this issue, Heusermann et al. use single particle tracking analysis to study the internalization of exosomes and show that exosomes and viruses can enter cells by a common route.

To track individual exosomal entry events, the researchers first generated exosomes that contain CD63 fused to either GFP or mCherry by overexpression in HEK293 cells. Heusermann et al. (2016) find that these exosomes are very efficiently and quickly taken up by primary human fibroblasts and that the vast majority of exosomes is internalized by endocytosis at the base of filopodia (Fig. 1). Exosomal uptake was preceded and facilitated by their surfing on filopodia (25% of exosomes) and, less frequently, laterally moving filopodia appear to pull (3%) or grab (1%) exosomes. Actin- and myosin-dependent filopodia surfing is a well-known mechanism of virus entry, particularly for viruses that need to cross mucosal surfaces for host infection (Lehmann et al., 2005). Analysis of the trajectories and velocities of exosomes in proximity to filopodia indicated that they move in parallel with F-actin–dependent retrograde flow. The role of filopodia in exosome entry was further supported by perturbation with the small molecule inhibitor SMIFH2, which blocks formin-dependent actin polymerization at the barbed end of filopodia. Loss of filopodia from the cell surface by SMIFH2 treatment led to a vast reduction in the rate of exosome uptake. Integrins and other cell adhesion molecules have previously been described as coupling receptors for retrograde filopodial virus flow (Lehmann et al., 2005) and it would be interesting to investigate whether these molecules are also required for exosomal filopodia surfing. Importantly, Heusermann et al. (2016) detected differences in exosomal and liposomal entry routes. Liposomes accumulated at the cell surface with low uptake levels over the first hours. In contrast, uptake of exosomes was highly efficient and occurred within minutes, which further corroborates the notion that exosomes use viral entry routes.

Heusermann et al. (2016) then used their single particle tracking analysis protocol to find out where the internalized exosomes go after entering cells. They show that exosomes are transported as intact vesicles within endosomes and, surprisingly, that the majority of the internalized exosomes moved toward lysosomal compartments, which opens up the question of how exosomal cargo could be released in a functional active form rather than being directed to lysosomal degradation. It is possible that a subset of exosomes may fuse with the plasma membrane, which would allow the direct transfer of exosomal content to the cytosol (Montecalvo et al., 2012). Heusermann et al. (2016) did not observe fusion of exosomes with the plasma

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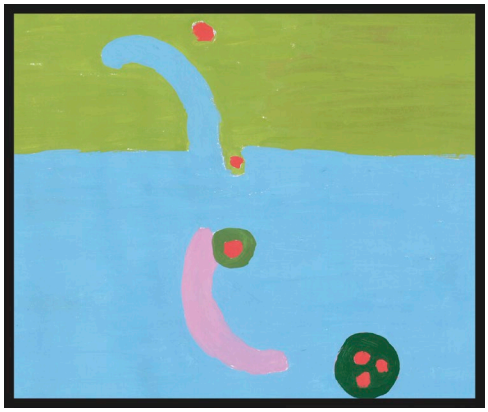

Figure 1. **Painting showing how exosomes are taken up from the extracellular space (green) into the cell (blue).** Exosomes (red) move along filopodia (blue), enter the cell at the base of the filopodia, travel within endosomes (light green) along the ER (pink), and are finally distributed to the lysosomal compartment (dark green). We thank Johanna Simons for painting the figure.

membrane in their study; however, rapid fusion events after cell surface binding might be difficult to detect with the methods used. Another possible mechanism of cargo delivery could be back-fusion of exosomes with the limiting membrane of the endolysosomal compartment. Of note, previous studies reported that exosome fusion with membranes was favored by an acidic pH (Parolini et al., 2009; Montecalvo et al., 2013), a mechanism that is also used by many viruses to enter the cytosol.

The observation by Heusermann et al. (2016) that the internalized exosomes move along the mesh-like structures of the rough ER before they are trafficked to the lysosomal compartments suggests another possible depot for cargo delivery. The close proximity and stalled movements of exosome-containing endosomes along the ER are important findings and suggests a potential role of the ER in cargo unloading or sorting, which warrants further research. The ER possesses a retro-translocation machinery, which is utilized for the delivery of misfolded proteins from the ER into the cytosol where they are ubiquitinated and degraded by the proteasome. Several viruses and bacterial pathogens, such as the polyomavirus, use the ER retro-translocation pathway to enter the cytosol (Inoue and Tsai, 2013). It is tempting to speculate that a subset of exosomes may reach the cytosol via the ER as part of their productive route, whereas others are directed to lysosomes for degradation. In light of the recent observation that RNA-induced silencing complex loading and mRNA silencing occur at the rough ER membrane (Li et al., 2013; Stalder et al., 2013), an ER-cytosol–mediated egress of exosomal cargo could explain how microRNA delivered by exosomes modulates gene expression in recipient cells. Further research is needed to elucidate whether exosomes indeed interact with the ER and whether the ER is the exit site for the cytosolic release of exosomal cargo.

Although the study by Heusermann et al. (2016) does not solve the chicken-and-egg question of whether exosomes or retroviruses came first, it emphasizes how much exosomes and viruses have in common. The exosomal field still has a long way to go; however, it is moving at a fast pace and we have reached a point at which we can state that exosomes cannot simply be regarded as inactive cellular debris but instead represent active vesicles with efficient uptake mechanisms. It is not clear whether the entry pathway described by Heusermann et al. (2016) with exosomes surfing on filopodia, internalization via filopodial endocytic hot spots, intra-endosomal trafficking to the ER, and subsequent distribution to the lysosomal compartments holds true for all exosomes regardless of their cellular origin or target tissue. The diverse functions of exosomes likely require several different mechanisms of biogenesis, cell entry, and cargo release. Understanding these processes is not only important to enhance our knowledge of exosomal biology, but will also help researchers in the field design novel strategies to use exosomes as nanocarriers for the delivery of therapeutic cargo.

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