# Cortactin enhances exosome secretion without altering cargo

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The role of cortactin, a regulator of late endosomal trafficking, in the biogenesis and secretion of exosomes is poorly understood. In this issue, Sinha et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201601025) elucidate the role of cortactin as a positive regulator of late endosomal docking and exosome secretion.

Exosomes are nanovesicles (30–150 nm in diameter) that are secreted by multiple cell types, under both physiological and pathological conditions (Gangoda et al., 2015; Tkach and Théry, 2016). The fusion of multivesicular late endosomes (MVEs) with the plasma membrane results in the release of exosomes into the extracellular space (Kalra et al., 2016). These extracellular vesicles contain DNA, RNA, proteins, lipids, and metabolites that reflect the cell of origin and hence can serve as indicators of cellular health, stress, and disease (Kalra et al., 2016). Exosomes have been implicated in cell–cell communication, both autocrine and paracrine, through the transfer of molecular cargo or via the transport of ligands to recipient cells (Gross et al., 2012; Cossetti et al., 2014). Although it is well documented that exosomes can induce both protective and pathogenic functions in recipient cells, the precise function of a specific population of exosomes largely depends on cell context (Gangoda et al., 2015). Recently, significant interest has grown in exploiting exosomes for therapeutic purposes (for instance, for the targeted delivery of drugs, inhibitors, and of miRNA and siRNA molecules) and in harnessing the healing and regenerative properties of stem cell–derived exosomes (Ohno et al., 2013; Kawikova and Askenase, 2015; Zhang et al., 2016). Although many questions remain to be answered about the safety and efficacy of using exosomes in therapeutic applications, a major technical hurdle concerning their therapeutic use would also need to be overcome. This relates to the lack of positive regulators of exosomal secretion that do not influence the cargo nor the ensuing function of these bioactive nanovesicles.

Cortactin is an actin nucleation–promoting factor that binds to and activates the Arp2/3 complex (MacGrath and Koleske, 2012). The activation of the Arp2/3 complex is critical for the nucleation of branched actin networks. Together with cortactin, it is essential for the stabilization of the branched actin. Cortactin has been implicated in cell migration, in endocytosis, and in the development and maturation of protrusive structures known as invadopodia (Kirkbride et al., 2011). Recently, the role of cortactin in regulating MVE trafficking was established (Hong et al., 2015); however, its role in the biogenesis and secretion of exosomes remains unclear. The docking and fusion of MVEs with the plasma membrane are essential for the secretion of exosomes. Hence, key regulators of this process, including Rab27a, Rab27b, and Rab35, have also been shown to govern the release of exosomes (Hsu et al., 2010; Ostrowski et al., 2010).

In this issue, Sinha et al. elucidate that cortactin expression levels positively correlate with the number of exosomes secreted by head and neck squamous carcinoma cells through gene silencing and overexpression studies. They report that cortactin knockdown or overexpression in these cells regulated the secretion of the endocytically derived exosomes. However, these approaches did not induce any significant effect on the release of ectosomes or microvesicles that are secreted as a result of plasma membrane blebbing (Keerthikumar et al., 2015). Furthermore, quantitative proteomic analysis of exosomes secreted by control and cortactin knockdown cells highlighted no significant changes in their protein cargo. These findings were further supported by Sinha et al. (2016) with the discovery that cortactin did not regulate the biogenesis of exosomes in these cells; rather, it is involved in MVE trafficking and docking and in the release of exosomes from invadopodia (Fig. 1).

Cortactin interacts with the Arp2/3 complex at its N terminus and through its acidic and repeat domain with F-actin. These interactions are critical for the stabilization of F-actin networks and for the inhibition of debranching. In contrast, coronin 1b directly antagonizes cortactin at actin branch points, thereby inducing debranching and destabilizing the F-actin branched networks. Sinha et al. (2016) were able to prove that this negative regulation of branched actin network orchestrated by coronin 1b can be overcome by Rab27a overexpression. In accordance with their initial observations, the knockdown of Rab27a increased cellular coronin 1b levels, which could be reversed by the overexpression of cortactin or by the knockdown of coronin 1b in Rab27a knockdown cells. The results corroborate that Rab27a and cortactin coordinately promote F-actin network stability, MVE docking, and exosome secretion, whereas coronin 1b antagonizes these processes (Fig. 1). Currently, it is unclear how Rab27a inhibits coronin 1b from inducing the debranching of actin networks. At this stage, the specific nature of the interactions between cortactin, coronin 1b, and Rab27a remains unclear. Further research is warranted to determine the exact domains that are involved in these interactions.

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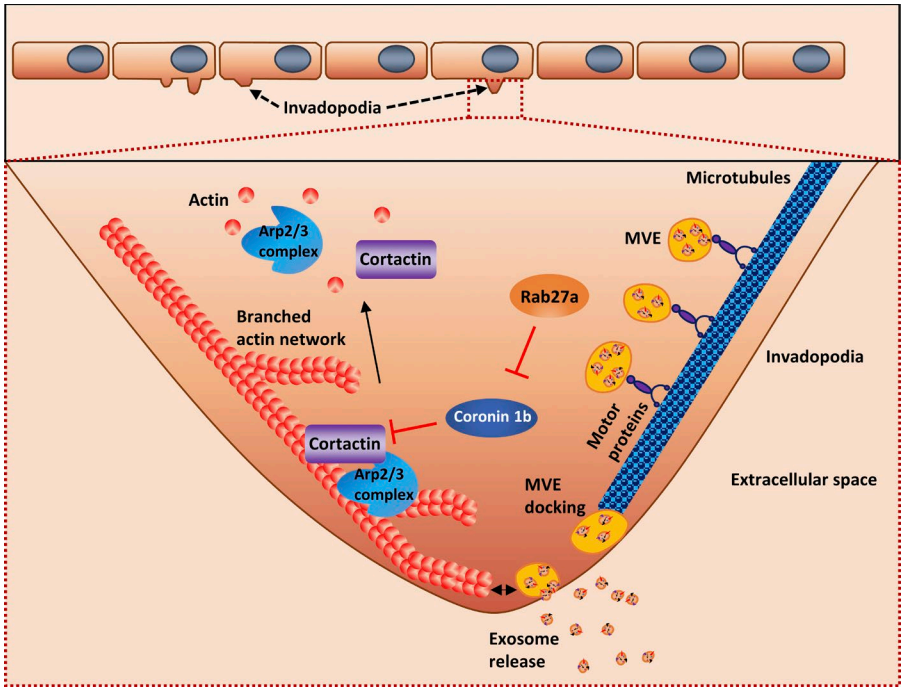


Figure 1. **Cortactin, Rab27a, and coronin 1b regulate branched actin network, MVE docking, and exosome secretion.** In invadopodia, cortactin binds to the Arp2/3 complex and promotes actin polymerization. Activated Arp2/3 protein complex causes the nucleation of branched actin networks. The interaction between cortactin and Arp2/3 stabilizes the branched actin networks and inhibits debranching. However, actin-binding protein coronin 1b antagonizes cortactin and induces the debranching of actin networks. Rab27a can inhibit coronin 1b activity, thereby restoring the stabilization of branched actin networks. This coordinated stabilization of actin networks by cortactin and Rab27a allows for more docking sites near the plasma membrane. As a result, the fusion of MVE to the plasma membrane is enhanced and the secretion of exosomes is augmented.

Cortactin is overexpressed in many types of cancer, including ovarian, melanoma, gastric, colorectal, and liver. Additionally, cortactin is also amplified in breast cancer and in head and neck squamous carcinoma (MacGrath and Koleske, 2012). As cortactin promotes cell migration, invasion, and metastasis, its overexpression positively correlates with tumor aggressiveness. Interestingly, Sinha et al. (2016) were able to attribute exosomes to some of the cortactin-associated cancer phenotypes. Exosomes were able to rescue the phenotype of cortactin and Rab27a knockdown cells in the context of serum-independent growth and invasion. As exosomes contain hundreds of proteins and RNA, it is unclear as to which proteins and/or RNA orchestrated this phenotypic rescue. As reported by Sinha et al. (2016), the matrix metalloproteinase MT1-MMP contained within exosomes was not the key regulator of exosome-mediated serum-independent growth and invasion. It is possible that cortactin contained within the exosomes might have compensated for its loss in the cortactin knockdown cells upon transfer. According to the ExoCarta (http://www.exocarta.org) and Vesiclepedia (http://www.microvesicles.org) databases, cortactin is secreted via exosomes by at least six different cell types. Further research is needed, perhaps with cortactin-negative exosomes, to validate this speculation.

The exciting, novel findings reported by Sinha et al. (2016) have profound implications in advancing the field of exosome research. Positive regulators of exosomal release are of critical importance. Researchers culture millions of cells in the presence of liters of conditioned media to obtain micrograms of exosomes. As a result, an overarching question in the field of exosome research has been: How can we increase the release of exosomes? Previously, monensin, a membrane-permeable $Na^+$ ionophore, had been shown to augment exosome secretion (Savina et al., 2003). However, as this ionophore increases intercellular $Ca^{2+}$ levels, it also induces apoptosis (Ketola et al., 2010), and hence it can presumably modify the cargo of exosomes. Sinha et al. (2016) highlight that the overexpression of cortactin might be an ideal strategy to increase the yield of exosomes. This potential use of cortactin is further exemplified by the demonstration that its overexpression or knockdown did not change the protein cargo of exosomes nor did these interventions affect cell proliferation rates. Because exosomes are implicated in regenerative medicine, in tissue engineering, and as therapeutic delivery vehicles, overexpressing cortactin in the cell of interest might augment the secretion of exosomes. Based on the observations of Sinha et al. (2016), it is tempting to speculate that cortactin knockdown or overexpression might also not alter the RNA cargo of exosomes; however, further research is needed to validate this. Functional studies to compare the exosomes secreted by control and cortactin-overexpressing cells are also warranted to confirm that the biological functions of exosomes also remain unaltered.

Another important implication of these findings is the potential use of cortactin to reduce exosome secretion. To attribute a specific function to exosomes, the current state-of-the-art method is to block their release with inhibitors or by targeting proteins that regulate exosomal biogenesis or release (Peinado et al., 2012). Ceramide inhibitors are most commonly used, at least in vitro, for blocking exosome release. However, analogous with many drugs, these inhibitors might also have off-target effects and are known to induce apoptosis in certain cell types (Colombo et al., 2014). Molecular targets such as Rab27a and Tsg101 have also been exploited to attenuate the release of exosomes (Peinado et al., 2012). In the case of Rab27a, however, its protein is present at low levels in many cell types and hence it is not a universal option for reducing exosome release. In contrast, Tsg101 is critical for cell proliferation and is implicated in exosomal biogenesis; it can also alter the protein cargo of exosomes when its expression levels are regulated. Hence, the down-regulation of cortactin might be an ideal way to circumnavigate such issues when attenuating the secretion of exosomes. As cortactin is generally expressed at higher levels in most cell types, and given that neither its knockdown nor overexpression affect cell proliferation rates nor exosomal protein cargo, it could be a viable target to reduce the secretion of exosomes.

In addition to advancing our knowledge of the biogenesis of exosomes and their secretion, the study by Sinha et al. (2016) has stimulated many follow up questions that will need to be addressed by biomedical researchers. Although cortactin is important for the formation of invadopodia that are associated with MVE docking sites, the precise role of cortactin in regulating the secretion of exosomes in noninvasive cells is unclear. Even though Sinha et al. (2016) were able to prove that cortactin-mediated regulation of exosomal secretion is conserved in at least two different cancer cell types, it remains unclear whether cortactin regulates exosomal secretion in other normal cells. As protrusive structures similar to invadopodia are found in normal cells, such as macrophages, osteoclasts, dendritic, endothelial, and vascular smooth muscle cells that cross tissue barriers or remodel the extracellular matrix (Revach and Geiger, 2014), it is an intriguing possibility that cortactin-regulated exosomes could aid in this process. In addition, as cortactin is overexpressed in many cancer types, these cells presumably would secrete more exosomes. Further research is needed to assess whether cortactin expression and the amount of exosomes correlate with tumor invasiveness. It is intriguing how cortactin-overexpressing cells meet the cell's energy demands given the high levels of energy required for enhanced MVE docking and fusion with plasma membrane. Lastly, it remains unclear whether MVE docking and the secretion of exosomes can occur without both Rab27a and cortactin. Regardless of these intriguing questions that require further investigation, Sinha et al. (2016) provide novel insights on how cortactin controls exosome secretion and open up exciting avenues by which cortactin could regulate cell migration and extracellular matrix remodeling via exosomes.


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