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RESEARCH HIGHLIGHT

An exosome pathway without an ESCRT

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Exosome biogenesis occurs via multiple mechanisms, but how these pathways are controlled remains poorly understood. In a recent study published in *Cell Research*, Wei et al. identify Rab31 GTPase as a regulator of lipid-mediated exosome biogenesis independent of other commonly-recognized ESCRT and tetraspanin pathways.

Exosomes are 30–150 nm vesicles that mediate intercellular crosstalk by transferring bioactive cargos in normal physiology and disease. Exosomes originate in the multivesicular body (MVB) of endosomes; inward membrane budding produces intraluminal vesicles (ILVs) contained within MVBs that carry membrane cargos and molecules from the surrounding cytoplasm. MVBs fuse with the plasma membrane, secreting ILVs as exosomes (Fig. 1).

Mechanisms controlling cargo selection and ILV budding into MVBs include regulation by endosomal sorting complex required for transport (ESCRT), lipids, and tetraspanins.[1] ESCRTs are complexes regulating cargo sequestration and ILV budding. Ceramides reside at endosomal membrane microdomains and are cone-shaped, inducing ILV budding. Tetraspanins cluster membrane-associated molecules at domains prone to ILV formation. ESCRTs are best-characterized, with specific mechanisms coupling cargo recognition with ILV formation defined, whereas less is understood about mechanisms of these steps in other pathways. Another essential feature of exosome biogenesis involves trafficking events ensuring that ILVs are secreted as exosomes.[1] As MVBs also fuse with lysosomes, mechanisms dictating MVB fate are also critical but have remained unclear.

Wei et al.[2] report that the Rab GTPase, Rab31, modulates exosome biogenesis by promoting ILV formation independently of the common ESCRT pathway and by blocking MVB–lysosome fusion (Fig. 1). The authors monitored epidermal growth factor receptor (EGFR) exosomal packaging to screen for Rab GTPases with unknown roles in exosome biogenesis. Rab GTPases are membrane-associated proteins involved in virtually all intracellular vesicular trafficking. Rab27a/b regulates fusion of MVBs at the plasma membrane for exosome release,[3] but Rab functions upstream of this step are less established. By overexpressing constitutively active forms of 62 different Rabs and employing colocalization analysis, the authors identified active Rab31 ($Rab31^{Q65L}$) as an inducer of EGFR MVB targeting. The researchers further scrutinized localization of EGFR at MVBs by immunoelectron microscopy and discovered that $Rab31^{Q65L}$ increased ILV production and the capture of EGFR into these ILVs. Accordingly, analysis of exosomes by particle tracking and exosome marker expression indicated that $Rab31^{Q65L}$ enhanced exosome production and EGFR packaging. These results are consistent with studies showing that endogenous Rab31, which undergoes cycles of activation and inactivation, targeted EGFR to late endosomes but promoted EGFR lysosomal degradation,[4] suggesting that $Rab31^{Q65L}$ uniquely supports EGFR exosomal targeting. Notably, Rab31 overexpression has been reported in cancer,[4] indicating that Rab31-mediated exosome biogenesis may be important in this context.

Wei et al. explored the mechanism of Rab31, focusing on determining whether it intersects with established pathways. Rab31-dependent ILV budding and exosome production were not altered by ESCRT or tetraspanin depletion; rather, inhibition of ceramide and cholesterol synthesis attenuated ILV budding, sequestration of EGFR into ILVs, and production of $EGFR^{+}$ exosomes. These effects were phenocopied by loss of flotillin proteins, which are exosome markers and associate with ceramide- and cholesterol-rich membranes.[5] Additionally, $Rab31^{Q65L}$ and EGFR interacted and colocalized with flotillins at MVBs. These results suggest that $Rab31^{Q65L}$ is recruited to ceramide- and cholesterol-containing membranes through interaction with flotillins to stimulate ILV budding and EGFR packaging. Intriguingly, recent work defined the autophagy-related protein MAP1LC3B (LC3) as a mediator of ceramide-induced ILV formation;[6] thus, regulation by LC3 and Rab31 may represent distinct ceramide-dependent pathways. Because LC3 captures cytoplasmic constituents and Rab31 may sequester receptors, these pathways may coordinate production of exosomes harboring various cargos.

While examining EGFR localization, the authors noted diminished EGFR-containing MVB/late endosome trafficking to lysosomes. Therefore, they investigated whether $Rab31^{Q65L}$ may alter MVB-lysosome fusion. Highlighting Rab7 as crucial for this step, they found that $Rab31^{Q65L}$ appeared to inhibit Rab7 activity at MVBs by recruiting the inhibitor TBC1D2B, whereas Rab31 depletion decreased TBC1D2B-Rab7 association. While these findings indicate that Rab31-mediated inhibition of Rab7 promotes exosome secretion, further study is necessary to verify how this Rab31-TBC1D2B-Rab7 pathway impacts MVB-plasma membrane fusion and exosome release. Surprisingly, this result contrasts with prior findings showing that Rab7 promotes exosome secretion,[7] suggesting that this role of Rab7 depends on Rab31 activity. Nevertheless, it underscores the importance of the lysosome in influencing exosome secretion by suggesting that disruptions in lysosomal fusion or function lead to re-routing of MVBs to the plasma membrane. Support for this has come from work showing that impairment of lysosomal acidification enhanced exosome production.[8]

Exosome heterogeneity is currently under intense study. Wei et al. reinforce the importance of this concept by demonstrating that cells expressing $Rab31^{Q65L}$ dictate biogenesis of an exosome subtype driven by flotillins and lipids and enriched in membrane cargos. It remains unclear into which exosome subtype of the recently identified large exosome, small exosome, and

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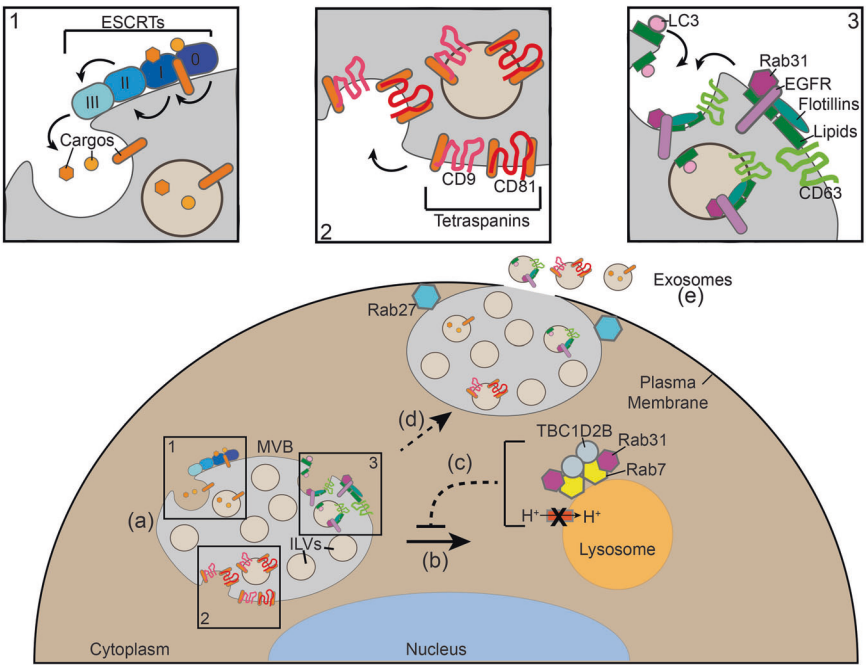

Fig. 1 Exosome biogenesis. (a) Exosomes form in MVBs through three primary pathways: (1) ESCRTs involve four complexes, ESCRT-0, -I, -II, and -III. ESCRT-0 and -I recruit cargos and ESCRT-II, which then recruits ESCRT-III. ESCRT-III promotes ILV budding. (2) Tetraspanins cluster membrane cargos and reside at membrane domains that promote ILV budding. (3) Ceramide and cholesterol lipid domains recruit factors, including flotillins and the autophagy-related protein LC3. Flotillins recruit Rab31, promoting enrichment of EGFR in CD63-containing MVBs. (b) MVBs fuse with lysosomes for degradation. (c) Decreased Rab7 activity due to TBC1D2B recruitment by Rab31 or impaired lysosomal acidification may block MVB–lysosome fusion. (d) Rab27 promotes fusion of MVBs with the plasma membrane. (e) ILVs are secreted as a heterogeneous mixture of exosomes.

exomere classes these Rab31 exosomes are categorized, but small exosomes package flotillins, suggesting that Rab31 regulates this population.[9] Moreover, a recent proteomics study of hundreds of human cancer and healthy patient samples identified new exosome biomarkers for diagnosis and treatment.[10] Remarkably, this extensive characterization showed that the traditional exosome marker CD63 was rarely detected in human exosomes, whereas Wei et al. demonstrated that Rab31 potentiated $CD63^{+}$ exosome biogenesis, highlighting the contribution of Rab31 to exosome heterogeneity. Finally, Rab31 also elicited production of an exosome subtype harboring therapy-resistant EGFR, corroborating the importance of characterizing patient exosomes for therapy. Overall, further characterization, alongside additional work defining the contexts in which Rab31-dependent exosomes are produced, will establish their relevance to cancer.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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