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A Dedicated ATG9A Vesicle Pathway for Selective Unconventional Protein Secretion

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Abstract: Autophagy-related proteins are increasingly recognized for functions beyond canonical autophagosome-mediated degradation. Our recent work identifies ATG9A-positive vesicles as a dedicated, autophagy-independent pathway for selective unconventional protein secretion (UcPS). This route is essential for the extracellular release of tandem-repeat galectins, including galectin-9, galectin-4 and galectin-8, but is dispensable for other UcPS cargos such as IL-1β, galectin-3 or FGF2. Mechanistically, galectins are physically enclosed within ATG9A vesicles in a TMED10-dependent manner. ATG9A vesicles traffic from the Golgi via an AP-4–RUSC2 module and fuse with the plasma membrane through a STX13–SNAP23–VAMP3 SNARE complex. This pathway is mechanistically distinct from secretory autophagy, exosome release and LC3-dependent extracellular vesicle secretion. Together, these findings establish ATG9A vesicles as selective carriers in UcPS and expand the functional landscape of ATG proteins beyond degradation.

Keywords: ATG9A vesicles; galectins; unconventional protein secretion (UcPS)

Autophagy has long been regarded as a fundamentally degradative pathway, responsible for the sequestration and lysosomal breakdown of intracellular components. Over the past three decades, the identification of autophagy-related genes (ATGs) has shaped a conceptual framework in which these proteins are primarily viewed through the lens of autophagosome biogenesis and turnover. However, as the field matures, it has become increasingly clear that ATG proteins participate in cellular processes that extend well beyond canonical autophagy.

Unconventional protein secretion (UcPS) allows proteins that lack signal peptides to exit cells independently of the ER-Golgi pathway. Galectin-9 is a β-galactoside-binding lectin involved in immune regulation that has attracted particular attention as a biomarker and therapeutic target in cancer, infection and inflammatory disorders. Yet the unconventional secretion route responsible for galectin-9 release has remained elusive. Our recent work identified an autophagy-independent role for ATG9A-positive vesicles as selective carriers for galectin-9 [1].

Knockdown or knockout of ATG9A markedly reduces extracellular galectin-9 levels, whereas knockdown of ULK1, knockout of FIP200, or simultaneous depletion of ATG2A and ATG2B does not diminish galectin-9 secretion, despite effectively suppressing canonical autophagy. LC3 lipidation (via ATG5, ATG7 and LC3) is essential for galectin-9 release; however, the ATG9A-dependent route is distinct from secretory autophagy, the exosome pathway and LC3-dependent extracellular vesicle loading and secretion (LDELS), indicating that it represents a separate branch of UcPS.

Galectin-9 is physically enclosed within ATG9A-positive vesicles, gaining membrane protection during transport, and these vesicles are actively delivered to the plasma membrane. Vesicle trafficking depends on the adaptor protein complex AP-4 and the kinesin-associated factor RUSC2, which together direct ATG9A vesicles from the Golgi apparatus toward the cell periphery. Transmembrane emp24 domain-containing protein 10 (TMED10),

a channel protein for unconventional secretion cargos, is crucial for the entry of galectin-9 into ATG9A vesicles by mediating its passage across the vesicle membrane. Membrane fusion at the plasma membrane is mediated by a specific SNARE complex comprising STX13, SNAP23 and VAMP3, thereby enabling galectin-9 release into the extracellular milieu.

ATG9A vesicle-mediated unconventional secretion is observed in multiple cell types, including HeLa, THP-1 and HEK293T cells, indicating that this pathway is conserved across different cellular contexts. Moreover, this ATG9A-dependent route exhibits marked cargo selectivity. It participates in the unconventional secretion of a diverse array of proteins, including galectin-9, galectin-4, galectin-8 and annexin A6, while having no effect on IL-1β, galectin-3 or fibroblast growth factor 2 (FGF2), a type I UcPS cargo that undergoes direct plasma membrane translocation. Notably, all ATG9A-dependent galectins (galectin-4, -8 and -9) belong to the tandem-repeat type with two carbohydrate recognition domains (CRDs), whereas galectin-3, which contains only a single CRD, is not regulated by ATG9A. This pattern suggests that the tandem-repeat architecture is critical for engagement with this pathway and points to a finely tuned cargo-sorting mechanism.

Together, these findings establish ATG9A-positive vesicles as a previously unrecognized, autophagy-independent carrier for UcPS. By coupling ATG9A with TMED10, RUSC2, and a STX13–SNAP23–VAMP3 SNARE module, cells construct a dedicated route for exporting tandem-repeat galectins (Figure 1). This study defines ATG9A-positive vesicles for the first time as active cargo carriers and identifies their specific protein cargoes, thereby revealing new molecular mechanisms and therapeutic targets for galectin-related immune regulation. Future studies will be required to delineate the upstream signals that allocate ATG9A vesicles between degradative and secretory fates, to identify the cofactors that cooperate to regulate their precise trafficking, and to determine how this pathway contributes to immune regulation, tissue homeostasis, and disease.

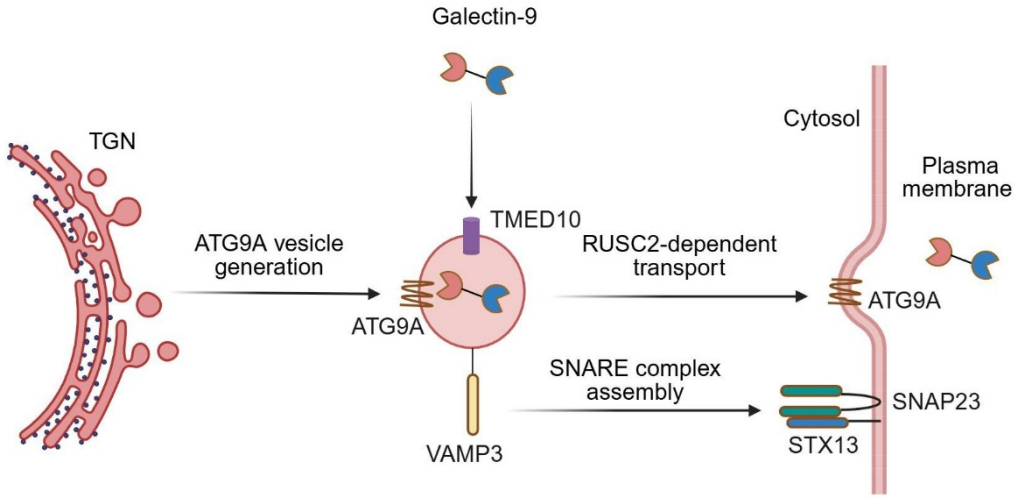

Figure 1. Model of the ATG9A-mediated unconventional secretion pathway.

ATG9A vesicles originating from the Golgi capture galectin-9 via TMED10-mediated entry. These vesicles are transported to the cell periphery in a RUSC2-dependent manner and fuse with the plasma membrane through the STX13–SNAP23–VAMP3 SNARE complex. This figure was created with BioRender.

Author Contributions

Y.W.: conceptualization, validation, writing—original draft, writing—review & editing; H.Z.: conceptualization, validation, writing—original draft, writing—review & editing; H.H.: supervision, writing—review & editing; J.W.: conceptualization, funding acquisition, project administration, supervision, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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This article is a Point-type manuscript and does not report original research or generate new data. Therefore, no new datasets were generated or analyzed during the current study.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used AI-assisted tools for language editing and polishing of specific sentences. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Acronyms

ATG	Autophagy-related genes
CRD	Carbohydrate recognition domain
ER	Endoplasmic reticulum
LDELS	LC3-dependent extracellular vesicle loading and secretion
SNARE	Soluble N-ethylmaleimide–sensitive factor attachment protein receptor
UcPS	Unconventional protein secretion

Reference

1. Zhang, W.; Ji, C.; Li, X.; et al. Autophagy-independent role of ATG9A vesicles as carriers for galectin-9 secretion. *Nat. Commun.* **2025**, *16*, 4259.