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# Secretory autophagy-promoted cargo exocytosis requires active RAB37

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**ABSTRACT**

RAB37 GTPase regulates cargo exocytosis by cycling between an inactive GDP-bound form and an active GTP-bound form. We reveal that RAB37 simultaneously regulates autophagy activation and tissue inhibitor of metalloproteinase 1 (TIMP1) secretion in lung cancer cells under starvation conditions. TIMP1, an inflammatory cytokine, is a known inhibitory molecule of matrix metalloproteinases matrix metalloproteinase 9 and suppresses the mobility of lung cancer cells both in vitro and in vivo through conventional exocytosis under serum-free conditions. Notably, we disclosed that secretory autophagy participates in TIMP1 secretion in a RAB37- and Sec22b-dependent manner. Sec22b, a SNARE family protein, participates in vesicle and membrane fusion of secretory autophagy. Knockdown of Sec22b decreased TIMP1 secretion and cell motility but did not affect cell proliferation under starvation conditions. We confirmed that starvation-activated RAB37 accompanied by Sec22b is essential for secretory autophagy to further enhance TIMP1 exocytosis. We further use an off-label drug amiodarone to demonstrate that autophagy induction facilitates TIMP1 secretion and suppresses the motility and metastasis of lung cancer cells in a RAB37-dependent manner in the lung-to-lung mouse model. In conclusion, we demonstrated that the RAB37 activation plays a pivotal regulatory role in secretory autophagy for TIMP1 secretion in lung cancer.

**Abbreviations:** ATG: autophagy-related gene; GDP: guanosine diphosphate; GTP: guanosine triphosphate; LC3: microtubule-associated protein 1A/1B-light chain 3; SNARE: soluble N-ethylmaleimide-sensitive-factor attachment protein receptor; TIMP1: tissue inhibitor matrix metalloproteinase 1.

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RAB proteins, like many other small GTPases, function as a molecular switch by cycling spatiotemporally between the inactive GDP-bound and the active GTP-bound form. Their active GTP-bound RABs directly bind to specific effectors and recruit them to their locations, allowing each RAB-effector complex to act at a specific location at a specific time. RAB proteins regulate exocytosis by interacting with various effector proteins that are responsible for vesicle formation, movement, tethering, and fusion.

Autophagy regulates multiple physiological functions in cells and organisms, and has been extensively studied in diverse diseases including metabolic syndromes and cancers. Secretory autophagy is gaining more attention but most of the studies are related to cytokine secretion. However, how autophagy shifts between degradation and secretion remain elusive. Our recent report reveals that starvation activates RAB37 which not only increases autophagic activity but also guides the autophagosome to secret TIMP1 from lung cancer cells (Figure 1) [1].

The inflammatory cytokine TIMP1 has been known to suppress lung cancer cell metastasis through RAB37-mediated exocytosis under starvation conditions. Herein, we reveal that the active form RAB37 increases LC3-II amount and TIMP1 secretion in various lung cancer cells. Moreover, RAB37 activation accompanied by autophagy induction and an increase of TIMP1 expression lead to a decreased number of tumor nodules and cancer cell metastases in a lung-to-lung metastasis mouse model. Moreover, we demonstrated that the LC3 protein colocalized with either RAB37 or TIMP1, and RAB37 was detected in purified autophagosomes of the cells harboring the active form of RAB37 protein. Active RAB37 together with autophagy mediated TIMP1 secretion was abolished by genetic silencing *RAB37* expression, but only partly suppressed by silencing *ATG5*, or *ATG7* gene expression in both *in vitro* and *in vivo*. Notably, induction of autophagy with various inducers (amiodarone and Tet-D11) together with either knockdown RAB37 expression or suppress RAB37 activation could not promote TIMP1 secretion. These findings imply that RAB37 protein plays a pvital role in secretory autophagy enhanced TIMP1 exocytosis.

Moreover, we reveal the role of Sec22b in RAB37 and autophagy-mediated TIMP1 secretion including that Sec22b positively associates with LC3-II level, LC3 puncta number, RAB37-LC3 colocalization, and TIMP1 secretion. Moreover,

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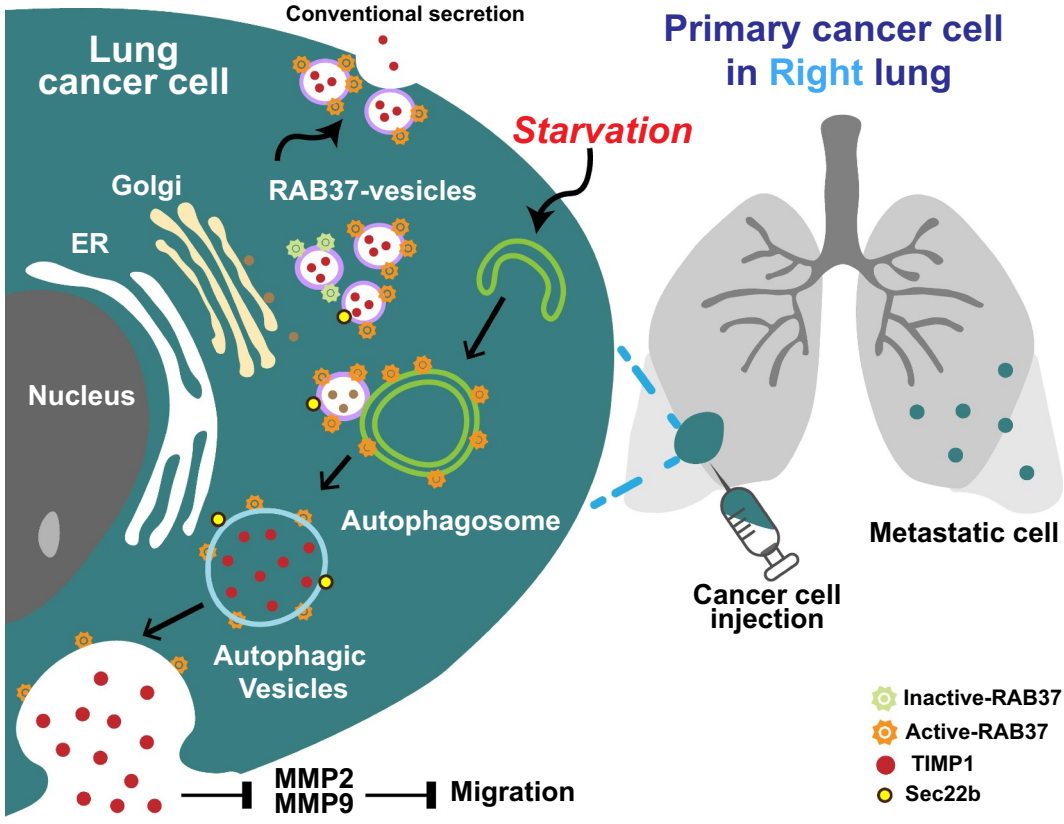


**Figure 1.** A schematic hypothetical diagram of activated-RAB37 participates in secretory autophagy that promotes TIMP1 secretion under starvation in lung cancer cell models.

knockdown of Sec22b expression decreased TIMP1 secretion together with decreased cell motility, however, the underlying mechanism that Sec22b participates in the disruption of RAB37 and LC3 colocalization and TIMP1 secretion warrants further exploration. Altogether, our exciting findings provide compelling evidence that secretory autophagy plays a promoting role in RAB37-controlled exocytosis of TIMP1 both *in vitro* and *in vivo*. However, the specific guanosine exchange factor activating RAB37 and the effectors participating in the secretion of TIMP1 remains to be determined.

In conclusion, our novel findings indicate that the mechanism of RAB37 activation and interaction with its effectors may be a useful target for the development of therapeutic agents aimed at suppressing lung cancer cell metastasis.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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