論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	管玉貂 (Kuan Yu-Diao)
論文題名 Title	External stimulation induces the secretion of autophagosome-like vesicles by B cells (B細胞は外部刺激によってオートファゴソーム様小胞を分泌する)

論文内容の要旨

〔目　的(Purpose)〕

Macroautophagy/autophagy is a cellular degradation and recycling process that supports cellular homeostasis. Since an autophagosome marker, microtubule-associated protein 1A/1B-light chain 3 (LC3)-II, was found in cell-derived extracellular vesicles (EVs), autophagy may cooperate with EV secretion pathways to control unconventional secretion of intracellular molecules. Several studies have demonstrated that pharmacological inhibition of autophagic turnover and pathogen-induced endolysosomal dysfunction enhanced the secretion of autophagosome-like EVs (ALVs). However, whether external stimulation induces ALV secretion is unclear.

〔方法ならびに成績(Methods/Results)〕

Here we showed that co-stimulation with IL-4 and anti-CD40 antibody (IL-4:CD40) enhanced the secretion of SQSTM1/p62^{+}LC3-II^{+}ALVs compared to co-stimulation by IL-4 and lipopolysaccharide (IL-4:LPS) or by IL4 and anti-IgM antibody in B cells. While IL-4:LPS stimulation accelerated autophagic flux, IL-4:CD40 stimulation reduced autophagosome-lysosome fusion without affecting lysosomal function. Although both IL-4:LPS and IL-4:CD40 induced the expression of similar genes involved in vesicle fusion or transportation, IL-4:CD40 preferentially enhanced the expression of the small GTPase RAB27a compared to IL-4:LPS. Genetic disruption by the CRISPR-Cas9 system revealed that loss of RAB27a membrane-binding ability impaired LC3-II^{+}ALV secretion but not ALIX^{+}EV secretion in B-lymphoma A20 cells. Additionally, reconstitution of human wild-type RAB27A in RAB27a mutant A20 cells restored LC3-II^{+}ALV secretion, indicating that RAB27a controls autophagosome secretion. Furthermore, LC3-II^{+}ALVs were found in the sera of tumor-bearing mice and the plasma of healthy human donors.

〔総　括(Conclusion)〕

Our findings may provide a role for B-cell secretory autophagy in regulating intercellular communication under various physiological conditions, such as vaccination, pathogen infection, and B-cell lymphoma progression.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Ms. Kuan's research, focused on extracellular autophagosome-like vesicles (ALVs), which are vesicles excreted by cells that contain autophagosome markers, such as LC3-II. She showed that primary B cells and murine B lymphoma A20 cells secrete LC3-II containing ALVs after IL-4 plus anti-CD40 stimulation and direct interaction between type 2 helper T cell and B cells. These vesicles could be isolated by anti-IgG beads and anti-MHCII beads. The immuno-capturing method also allowed the isolation of LC3-II-containing ALVs from A20 lymphoma-carrying mouse serum. Such ALVs could even be detected in human plasma. IL-4 and LPS co-stimulation induced a higher autophagic flux but less ALV release than IL-4 and anti-CD40 co-stimulation, which suggests that autophagic flux counteracts ALV secretion. The GTPase RAB27a was enhanced after IL-4 and anti-CD40 co-stimulation more than after IL-4 and LPS co-stimulation. Moreover, RAB27a mutant cells lacking the CXC motif in the C-terminus released less LC3-II+ ALVs. Consistently, econstitution of WT RAB27a restored LC3-II+ ALV secretion in RAB27a mutant cells. Taken together, using immuno-capturing to measure ALV secretion could be a powerful marker for detecting or monitoring B cell-related diseases non-invasively. Although the secretome from ALV requires further investigation, Ms. Kuan speculated that ALV secretion plays diverse roles in several immune responses that require T-B interactions, including B cell maturation.