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LAMTOR2/LAMTOR1 complex is required for the TAX1BP1-mediated xenophagy

（LAMTOR2/LAMTOR1 複合体は TAX1BP1 を介したゼノファジーを制御する）

京都大学大学院医学研究科博士課程

医学専攻　微生物感染症学分野

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Background

Autophagy is a conserved intracellular recycling process, which regulates macromolecular fusion to lysosomes and degrades autophagosomal contents in response to environmental stress such as nutrient starvation [1]. Aside from nutrient acquisition to maintain cell survival, autophagy also plays a critical role in mediating intracellular defense response to pathogens, including bacteria and virus [2, 3]. Pathogenic invasion induces autophagosome formation, and these autophagosomes fuse with lysosome to degrade the entrapped bacteria [2]. To recognize targets, autophagy receptors play pivotal roles by assisting autophagosome-specific targets to ubiquitin-tagged microorganisms [4]. A previous study identified LAMTOR2 as one of the NBR1 ligands and suggested its involvement in autophagy. LAMTOR2 may perform other unknown functions [5]. So far, the role of LAMTOR2 in autophagy to protect against pathogenic invasion has not been studied.

Materials and Methods

Group A *Streptococcus* (GAS) strain JRS4 (M6+ F1+) and *Salmonella enterica* Typhimurium strain LT2 were cultured in Todd-Hewitt broth (BD Diagnostic Systems, 249240) containing 0.2% yeast extract. HeLa cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco) and 50 μg/mL gentamicin (Nacalai Tesque). After infection of cells with GAS or *Salmonella*, immunofluorescence microscopy was performed to examine protein localization. Protein-protein interaction was evaluated with immunoprecipitation and western blotting. Bacteria survival rate was examined using bacterial invasion and viability assays.

Result

In non-infected cells, mCherry-tagged LAMTOR2 (mCherry-LAMTOR2) was localized in the cytosol but colocalized with EmGFP-galectin 3-positive bacteria in response to GAS infection. At 1 h after infection, about 5% cells showed LAMTOR2-positive GAS, but this number increased to about 50% at 4 h. LAMTOR2 was not recruited to SLO-deficient GAS at 4 h post-infection. After LLOMe treatment,

mCherry-LAMTOR2 formed puncta and clearly colocalized with EmGFP-galectin 3 puncta. Although LAMTOR2 expression knockout had no marked effect on the invasion rate of GAS into HeLa cells, the percentage of cells carrying galectin 3-positive GAS was significantly decreased at 2 and 4 h after infection. Furthermore, both the number of cells with GAS-containing autophagosomes and the average number of GAS-containing autophagosomes were lower among LAMTOR2-knockout cells than in wild-type cells during GAS infection. Autophagosomes positive for the lysosome marker LAMP1 (i.e., autolysosomes) were significantly decreased following LAMTOR2 expression knockout.

EmGFP-LAMTOR2 co-precipitated with FLAG-NBR1 as well as LAG-TAX1BP1 and FLAG-p62. In LAMTOR2 knockout cells, the recruitment of NBR1 and TAX1BP1 to autophagosomes markedly diminished at 2 h and at both 2 and 4 h after infection, respectively. In HeLa cells infected with *S. typhimurium*, TAX1BP1 recruitment to *Salmonella*-containing autophagosomes significantly decreased at 4 h.

Immunoprecipitation results revealed the critical role of the amino acid residues 62−81 in LAMTOR2 for interaction with TAX1BP1. The deletion of LIR, CC1, CC3, or Zn region resulted in the impairment in the interaction between TAX1BP1 and LAMTOR2. While the autophagosomes from wild-type cells colocalized with LAMP1, those from TAX1BP1-knockout cells showed reduced LAMP1 signal (Fig. 7B). Quantification revealed that TAX1BP1 knockout resulted in a 17.1% reduction in LAMP1-positive autophagosomes (Fig. 7C), suggestive of the involvement of TAX1BP1 in autolysosome formation in response to GAS invasion. Here, we also tested whether TAX1BP1 deficiency affects intracellular GAS proliferation, and found that GAS survival rate was considerably higher in TAX1BP1-knockout cells. These findings indicate that TAX1BP1 recruited to GAS through LAMTOR2 facilitates autolysosome formation and functions in the defense mechanism against the invading GAS.

EmGFP-LAMTOR2 interacts with both galectin 3 and TRIM16. Moreover, LAMTOR2 was shown to interact with galectin 8 and LC3. Endogenous LAMTOR1 was detected around SLO-deficient GAS and found to be colocalized with LC3-surrounded GAS. LAMTOR1 plays a role in autophagosome-lysosome fusion by recruiting LAMTOR2 and TAX1BP1. We further investigated whether TAX1BP1 recruitment to autophagosomes was affected in LAMTOR1-knockout cells. The results showed that TAX1BP1 recruitment was impeded and its colocalization with autophagosomes was markedly decreased. Furthermore, TAX1BP1 recruitment was impaired in LAMTOR1-knockout cells even during *Salmonella* infection. Thus, the LAMTOR1/LAMTOR2 complex is essential for the targeting of TAX1BP1 to autophagosomes.

Discussion

In this study, we revealed the role of LAMTOR2 in the response of non-phagocytic cells to bacterial invasion, wherein it functions as a selective autophagy regulator by mediating autophagosome-lysosome fusion. LAMTOR2 was recruited to and colocalized with galectin 3 in LLOMe-treated and bacterium-infected cells, suggestive of its involvement in response to vacuolar damage. To date, several galectins, including galectin 3, 8, and 9, have been reported to be accumulated in damaged endosomal/lysosomal vacuoles [6-8]. Galectin 3 and 8 have been found to regulate autophagy initiation by recruiting the E3 ligase TRIM16 and the autophagy adaptor NDP52 to the membrane, respectively. Our results demonstrate that LAMTOR2 associated with LC3 and membrane-damage markers, including galectin 3 and TRIM16. Thus, the galectin 3/TRIM16 complex and LAMTOR2 may co-operatively function as scaffold proteins in xenophagy and lysophagy.

Deficiency of the LAMTOR2-MP1 complex results in aberrant endosomal trafficking and subcellular distribution [9]. The percentage of LAMP1-positive autophagosomes was substantially lower in LAMTOR2-knockout cells. Our results suggest that this decrease was attributed to the perturbation in intracellular trafficking and lysosome distribution in knockout cells. In LAMTOR2-knockout cells, the GAS-targeting autophagosome-lysosome fusion was impaired, but no domain related to membrane anchorage has been yet identified in LAMTOR2. LAMTOR2 plays a crucial role in overall subcellular distribution, and therefore, the perturbation in autophagosome-lysosome fusion may not be a direct consequence of LAMTOR2 deficiency.

We failed to observe any difference in LC3 recruitment to galectin 3-positive GAS between wild-type and LAMTOR2-knockout cells; however, the proportion of cells with galectin 3-positive GAS was substantially diminished among LAMTOR2-knockout cells, suggesting that the vacuole-membrane damage decreased in knockout cells. Among LAMTOR2-knockout cells, those containing SLO signal-positive GAS markedly decreased, indicative of the impediment in the escape of GAS into the cytosol. Further investigation is warranted to reveal the reason underlying the requirement of the LAMTOR1/LAMTOR2 complex to mediate the escape of GAS into the cytosol.

LAMTOR1 colocalized with GAS-containing autophagosome-like vacuoles and LC3-surrounded GAS, suggestive of the role of LAMTOR1 in the autophagy response to bacterial invasion. Although LAMTOR1 depletion failed to impair the formation of autophagosomes against galectin 3-positive GAS, autolysosome formation was potently disrupted. As LAMTOR1 functions as a key component involved in the fusion of autophagosomes and lysosomes [10], the defective autophagosome-lysosome fusion observed in LAMTOR1-knockout cells was expected. The most plausible explanation for the impaired recruitment of TAX1BP1 to autophagosomes in LAMTOR1-knockout cells is the perturbation of LAMTOR2. These results identify a critical role of LAMTOR1 in autolysosome formation in response to GAS invasion.

Taken together, our results demonstrate that LAMTOR2 is a key modulator of the TAX1BP1-regulated autophagy against bacterial invasion. We demonstrate the requirement of LAMTOR1 for autophagosome-lysosome fusion in the defense response against GAS invasion. This study has provided a new insight into the distinct function of LAMTOR2 in selective autophagy. Our results imply that the rag-regulator complex may be involved in autophagy triggered upon pathogenic invasion.

References

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