論文の内容の要旨

論文題目：**Mucolipin-1 positively regulates TLR7 responses in dendritic cells by facilitating single stranded RNA transportation to lysosomes**

(樹状細胞におけるMucolipin-1依存的な一本鎖RNAのリソソームへの輸送を介したTLR7応答制御機構の解明)

リ　ショウビン
李　晓冰
(Li Xiaobing)

Toll-like receptors (TLR) 7and 9 erroneously respond to self-derived nucleic acids (NAs) and cause autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Mechanisms that restrict RNA/DNA-sensing in endolysosomes avoid recognition of self-derived pathogen by TLR7 or TLR9. Aberrant transportation of self-derived RNA/DNA to endolysosomes has been shown to exacerbate autoimmunity. To limit NAs-sensing by TLR7 or TLR9 in endolysosomes, TLR7/9 transportation is tightly controlled by Unc93B1, which is a multiple transmembrane protein that is associated with TLR7/9 and transports them from ER to endolysosomes.

When compared to sensors transportation, much less is known about molecular mechanisms underlying NAs transportation. PIKfyve, a phosphatidylinositol 3-phosphate 5-kinase, is shown to be required for CpG-B translocation from early endosome to late endosomes. PIKfyve synthesizes PtdIns(3,5)P_2, suggesting a role of PtdIns(3,5)P_2 in DNA trafficking. PtdIns(3,5)P_2 is reported to directly bind to mucolipin-1 and induce Ca^{2+} release. Mucolipin-1, a member of the transient receptor potential (TRP) cation channel family, primarily localizes on the lysosome membrane and has a role in regulating lysosomal trafficking. Considering that Mucolipin-1 is a target of PtdIns(3,5)P_2 and has a role in endolysosomal trafficking, Mucolipin-1 may have a role in NAs trafficking in innate immune cells. To address this issue, TLR7 and TLR9 responses in *Mcoln1*$^{-/-}$ mice were studied.

Mcoln1$^{-/-}$ BM-cDCs and BM-pDCs were significantly impaired in the TLR7 responses to ssRNAs (RNA9.2s-DR and Poly U). The synthetic mucolipin agonist (ML1-SA1) specifically enhanced TLR7 responses to ssRNA. These results suggest that

mucolipin-1 has a role in TLR7 responses to ssRNA. Because mucolipin-1 is activated by a PtdIns(3,5)P_2, which is generated by PIKfyve, the effect of PIKfyve inhibitor YM201636 on TLR responses in BM-cDCs and BM-pDCs was next studied. TLR7 responses to RNA9.2s-DR or Poly U were diminished in the presence of PIKfyve inhibitor YM201636. These data suggest that the PtdIns(3,5)P_2 –mucolipin-1 axis has an important role in TLR7 responses to ssRNA.

Previous studies show that mucolipin-1 regulates endocytosis. However, *Mcoln1*$^{-/-}$ BM-cDCs did not show any impairment in ssRNA internalization. PIKfyve is shown to have a role in DNA transportation. To gain insight into roles of the PtdIns(3,5)P_2-mucolipin-1 axis in regulating ssRNA transportation, confocal microscopy experiments were conducted. Colocalization of ssRNA ligands with a lysosome marker was significantly impaired in *Mcoln1*$^{-/-}$ BM-cDCs. ML1-SA1 significantly enhanced the ssRNA transportation to lysosomes. Moreover, PIKfyve inhibitor drastically impaired the ssRNA transportation to lysosomes. These results demonstrated that ssRNA trafficking into lysosomes is mediated by the PtdIns(3,5)P_2-Mucolipin-1 axis.

Mcoln1$^{-/-}$ BM-cDCs and BM-pDCs showed impaired responses to small chemical TLR7 ligands. Neither mucolipin agonist nor PIKfyve inhibitor altered TLR7 responses to these small chemical ligands, suggesting that mucolipin-1 has another role in TLR7 responses in addition to the role downstream of PIKfyve. The underlying mechanisms are necessary to be further researched.

In *Mcoln1*$^{-/-}$ BM-cDCs and BM-pDCs, TLR9 responses to CpG-B were not altered, mucolipin agonist did not enhance TLR9 in responses to CpG-B. Co-localization of CpG-B with a lysosome marker was not altered in *Mcoln1*$^{-/-}$ BM-cDCs. Consistent with the previous report, PIKfyve inhibitor altered TLR9 responses to CpG-B. These results indicate that TLR9 response to CpG-B responses do not require mucolipin-1. It is possible that other downstream effectors of PtdIns(3,5)P_2 have roles in regulating TLR9 responses to CpG-B.

TLR9 responses to CpG-A were decreased in *Mcoln1*$^{-/-}$ DCs or by treatment of PIKfyve inhibitor. On the other hand, mucolipin agonist ML1-SA1 did not enhance, but significantly impaired CpG-A responses in BM-cDCs. Confocal microscopy showed that in the presence of ML1-SA1, transportation of CpG-A to lysosome is impaired. These results suggest that mucolipin family members other than mucolipin 1 negatively

regulate CpG-A trafficking into lysosomes. A role of mucolipin-1 in CpG-A responses has to be addressed in further study. PIKfyve inhibitor drastically inhibited the CpG-A transportation to lysosomes, suggesting that as yet unknown effectors downstream of PIKfyve may regulate CpG-A trafficking.

Finally, to address the immunogical relevance of mucolipin-1 in *in vivo* innate immune responses, R848 was injected into BM chimeric mice reconstituted by *Mcoln1*$^{-/-}$ bone marrow cells. Production of IL-6 and IL-12p40 showed significant impairment when compared with BM chimeric mice reconstituted with WT BM cells. These results further demonstrate the importance of mucolipin-1 in TLR7 responses *in vivo*.

In conclusion, the PtdIns(3,5)P_2-Mucolipin-1 axis has an important role in ssRNA transportation to lysosome, but not DNA transportation to lysosomes. Transportation of DNA and RNA is differentially regulated.