[課程―2]

審査の結果の要旨

氏名 李 晓冰 (Li Xiaobing)

The present study shows that a PtdIns(3,5)P_2-muclipin-1 axis has a role in ssRNA transportation to lysosome in bone marrow dendritic cells. The following results were shown in this thesis.

1. *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 PtdIns(3,5)P_2, which is generated by the PIKfyve kinase, 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.

2. 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 demonstrate that ssRNA trafficking into lysosomes is mediated by the PtdIns(3,5)P_2-Mucolipin-1 axis.

3. *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 need further study.

4. In *Mcoln1*$^{-/-}$ BM-cDCs and BM-pDCs, TLR9 responses to CpG-B were not altered, mucolipin agonist did not enhance TLR9 responses to CpG-B. Co-localization of CpG-B with a lysosome marker was not altered in *Mcoln1*$^{-/-}$ BM-cDCs. These results indicate that TLR9 responses to CpG-B 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.

5. 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. PIKfyve inhibitor drastically inhibited the CpG-A transportation to lysosomes, suggesting that as yet unknown effectors downstream of PIKfyve may regulate CpG-A trafficking.

6. 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 was significantly impaired 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, this thesis has revealed an important role of the PtdIns(3,5)P_2-Mucolipin-1 axis in ssRNA transportation to lysosome, but not DNA transportation to lysosomes. The thesis contributes to the understanding of molecular mechanisms underlying the transportation of RNA and DNA. Regarding the discovery reported in the thesis, Li Xiaobing is awarded doctor of philosophy by the University of Tokyo.