論文内容の要旨

論文題目 : Requirement of helix 8 in leukotriene B_4 type-2 receptor for the folding to pass the quality control in the endoplasmic reticulum

和訳：ロイコトリエン B_4 第二受容体が有する Helix 8 構造の小胞体搬出における重要性についての研究

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G protein-coupled receptors (GPCRs) constitute the largest superfamily of integral membrane proteins, which are activated by specific compounds called “ligands”, and transduce signals intracellularly through heterotrimeric G proteins. GPCRs share a common topology, with seven-transmembrane domains. Three-dimensional structural analysis of several GPCRs revealed that the membrane-proximal C-terminal tail forms an additional α-helix, known as Helix 8 (H8) with amphipathic properties. To date, several studies have demonstrated the role of H8 in ligand binding and/or G protein activation. However, the role of this domain in the proper folding of the receptor for passage through the quality control mechanism of the endoplasmic reticulum (ER)

remains poorly defined.

Leukotriene B_4 (5*S*, 12*R*-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid [LTB_4]), a metabolite of arachidonic acid, is a potent lipid mediator. Two GPCRs that interact with LTB_4, a high affinity type-1 receptor (BLT1) and a low-affinity type-2 receptor (BLT2), have been cloned in our laboratory. Recently, our laboratory have reported that BLT2 recognizes 12*S*-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid (12-HHT, a cyclooxygenase product) with a higher affinity than LTB_4.

Here, I demonstrate that the lack of H8 leads to accumulation of human (h) BLT2 in the ER, suggesting the importance of this domain for the correct folding of hBLT2 during *de novo* synthesis and trafficking. Firstly, I showed that lack of H8 of the hBLT2 led to an accumulation of the receptor (hBLT2/ΔH8) in the ER by flow cytometry and immunofluorescence confocal microscopy. Similar results were obtained in two representative human GPCRs, dopamine type-1 and lysophosphatidic acid type-2 receptors, that were engineered to lack H8.

Several lines of evidence suggest that a class of compounds called “pharmacological chaperones” rescue the intracellular retention of several misfolded proteins, including GPCRs. To examine whether the accumulation of hBLT2/ΔH8 in the ER is due to an inadequate folding, I tested the effect of several ligands for hBLT2, which might behave as pharmacological chaperones. Treatment with chemical ligands or natural agonists dose-dependently and time-dependently facilitated the surface expression of hBLT2/ΔH8. These results suggest that the lack of the H8 led to the inadequate folding of the hBLT2. Additionally, I demonstrated the importance of the hydrophobic residues, Phe-Leu-x-x-Leu-Phe, in H8 for hBLT2 trafficking. Recently, several studies have analyzed the trafficking of GPCRs, *e.g*. rat (r) D1R, hV2R, hV1b/3R, hMC4R, rAT1R

and rα_{2B}-AR. These efforts to elucidate the mechanism underlying GPCR export from the ER have led to the identification of amino acid sequences in the C-terminal tail that play a crucial role in their export. Because these critical residues partially or totally constitute the putative H8 of each GPCR, the results support our hypothesis that the hydrophobic residues in H8 are important for the receptors to pass the quality control mechanism in the ER.

The surface-trafficked hBLT2/ΔH8 exhibited an agonist-evoked increase in Ca^{2+}, demonstrating that H8 is not critical for ligand binding and activation of coupled G proteins. This implies that H8-deficient hBLT2 could be a “functional mutant”. Thus, our findings suggest that some diseases caused by a deficiency of GPCR trafficking are due to mutations in H8. I conclude that the H8 region of hBLT2 plays an important role in transport-competent receptor folding. Discovery of this role of H8 in the correct folding of the receptors is useful for a better understanding of ER quality control mechanism for GPCRs.