論文の内容の要旨

論文題目　Characterization of the non EDG family lysophosphatidic acid receptors
和訳　　非 EDG 型リゾホスファチジン酸受容体群の機能解析

指導教員　清水 孝雄 教授
東京大学大学院医学系研究科
平成 18 年 4 月進学
医学博士過程
分子細胞生物学専攻

柳田　圭介

Lysophosphatidic acid (LPA) is a bioactive lipid mediator with diverse physiological and pathological actions on many types of cells. LPA has been considered to elicit its biological functions through three types of endothelial differentiation gene (EDG) family of G protein-coupled receptors (GPCRs), LPA_{1-3}. However, even after the identification of these LPA receptors, the existence of an additional LPA receptor(s) has been implied by several studies. In 2003, our group identified p2y9/GPR23 as a fourth LPA receptor, LPA_4, which is structurally distant from EDG family LPA receptors. The expressed sequence tag cDNA encoding LPA_4 was originally isolated from human brain, and LPA_4 is expressed in many kinds of mouse/rat neural cells. However, only very limited information is available on its neurological functions.

To assess the functions of LPA_4 in neuronal cells, I used rat neuroblastoma B103 cells that appear to lack endogenous responses to LPA. Unlike B103 cells transfected with control vector, B103 cells stably expressing LPA_4 (B103-LPA_4 cells) showed contracted cell shapes and formed aggregates in a serum-containing medium, which is rich in LPA. In B103-LPA_4 cells, I observed $G_{q/11}$-dependent calcium mobilization, whereas LPA did not affect adenylyl cyclase activity, indicating that LPA_4 does not couple to G_s and $G_{i/o}$ protein. I also found that LPA induced dramatic morphological changes of B103-LPA_4 cells, *i.e.*, neurite retraction, cell aggregation, and N-cadherin-dependent cell adhesion, which are consistent with the characteristic appearance of B103-LPA_4 cells in a serum-containing medium. The morphological changes were inhibited by the treatment with a Rho-associated kinase (ROCK) inhibitor, Y27632. On the other hand, the treatment with $G_{i/o}$ inhibitor pertussis toxin and $G_{q/11}$ inhibitor YM-254890 did not inhibit the morphological changes, suggesting the role of $G_{12/13}$-dependent Rho/ROCK activation in morphological changes in neuronal cells.

In 2006, the orphan GPCR GPR92 was identified as a fifth LPA receptor (LPA_5). GPR92 is closely related to LPA_4, with a 28% amino acid identity in human. Of note, another orphan receptor, p2y5 shares the highest amino acid sequence homology (59%) with LPA_4 among all GPCRs, suggesting that p2y5 is also an LPA receptor. Although I could not detect p2y5-dependent response to LPA in cyclic AMP assay, Ca^{2+} mobilization assay, or reporter gene assay, I noticed that

B103 cells stably expressing p2y5 (B103-p2y5 cells) formed aggregates in a serum-containing medium. This similar morphology of B103-p2y5 cells to B103-LPA_4 cells led me to hypothesize that LPA activates Rho through p2y5 in these cells. This hypothesis was confirmed by following experiments using "LPA receptor-null" RH7777 and B103 cells exogenously expressing p2y5: [^{3}H]-LPA binding, LPA-induced [^{35}S]-GTPγS binding, Rho-dependent alternation of cellular morphology, and cyclic AMP accumulation via a $G_{s/13}$ chimeric protein. Consistently, LPA-induced contraction of human umbilical vein endothelial cells was suppressed by siRNA knockdown of endogenously expressed p2y5. I also found that 2-acyl-LPA had higher activity to p2y5 than 1-acyl-LPA.

In conclusion, I revealed that Rho is an important effector of LPA_4, and p2y5 is a novel LPA receptor (LPA_6), which activates the $G_{12/13}$-Rho signaling pathways. These findings will help better understand the physiological and pathological roles of "non EDG" family LPA receptors.