論文の内容の要旨

論文題目　Lysophospholipid acyltransferases are required for the regulation of phospholipid fatty acid composition

（リゾリン脂質アシル転移酵素はリン脂質の脂肪酸組成の調節に必要である）

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Glycerophospholipids are the major components of cellular membranes, and are known to possess important structural and functional roles for cells. Not only the polar head groups, but also the fatty acid composition of glycerophospholipids are diverse. The *sn*-1 position is mainly comprised of saturated fatty acids, while the *sn*-2 position mainly contains polyunsaturated fatty acids (PUFAs). Glycerohospholipids are first synthesized by the *de novo* pathway (Kennedy pathway), and are matured through the remodeling pathway (Lands' cycle). The remodeling pathway was classically believed to be important for generating the diversity of glycerophospholipids, by incorporating PUFA, especially arachidonic acid (20:4), into the *sn*-2 position. However, no detailed studies have been performed until recently to confirm this hypothesis. I decided to investigate how the lysophosphatidic acid acyltransferase (LPAAT) enzymes and the

lysophospholipid acyltransferase (LPLAT) enzymes of the remodeling pathway contribute to the determination of the fatty acid composition of phospholipids.

First, I overexpressed LPLAT enzymes using CHO-K1 cells, and studied phosphatidylcholine (PC) acyl-chain composition of membranes, and acyl-CoA specificities of LPLAT activities. LPAAT activity towards 18:2-CoA was increased by that of LPAAT1 and LPAAT2, and LPAAT activity towards 22:6-CoA was increased by overexpression of LPAAT3. Lysophosphatidylcholine acyltransferase (LPCAT) activity towards 16:0-CoA was increased by transfection of LPCAT1, and LPCAT activity towards 18:1-CoA was increased by that of LPCAT4. I also found that overexpression of these LPLAT enzymes changed PC fatty acid profiles, which correlated with the change in activities. I also studied the correlation of acyl-CoA specificities of LPLAT activities and PC composition using various tissues. There was a correlation between LPAAT activity towards 18:2- and 22:6-CoA, with 18:2- and 22:6-containing PC, respectively. LPCAT selectivity for 16:0- and 18:1-CoA correlated with 16:0- and 18:1- containing PC, respectively. Overall, the results suggested that 18:2-PC and 22:6-PC are mainly determined through the *de novo* pathway, whereas 16:0-PC and 18:1-PC are regulated through the remodeling pathway,

which somewhat differs from the classical concept. I also newly identified an LPAAT enzyme, LPAAT4, with high specificity for polyunsaturated fatty acyl-CoA, especially 22:6-CoA. Knockdown of LPAAT4 in Neuro 2A cells decreased LPAAT activity with 22:6-CoA as a substrate, and slightly decreased the levels of some 22:6-containing species, suggesting that endogenous LPAAT4 has some enzymatic function. This supports again my speculation that 22:6-PC is mainly regulated by the *de novo* pathway. Since my studies did not clarify how 20:4-PC is synthesized, I performed further studies. I focused on LPCAT3, which has high specificity for 20:4-CoA. During differentiation of C3H10T1/2 cells, LPCAT3 mRNA was induced, concertedly with an increase in 20:4-containing glycerophospholipids. Generation of LPCAT3-deficient mice revealed that LPCAT3 deficiency decreases 20:4-containing PC and phosphatidylethanolamine (PE) *in vivo*. These data suggest that LPCAT3 is important for the regulation of 20:4-containing glycerophospholipids. My study newly revealed the mechanism of how LPLAT enzymes of the *de novo* pathway and the remodeling pathway regulate the acyl-chain composition of phospholipids in the membrane. In various diseases, aberrant acyl-chain composition is observed. This study might possibly lead to an understanding

of the pathophysiology and provide possible therapeutic tools for these diseases.