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学 位 論 文 名	PIG-W Is Critical for Inositol Acylation but Not for Flipping of Glycosylphosphatidylinositol-Anchor (GPI アンカーのイノシトールアシル化に必要な PIG W の同定)
論 文 審 査 委 員	(主査) 教 授 木下タロウ (副査) 教 授 谷口 直之 教 授 岡本 光弘

論 文 内 容 の 要 旨

Objective

Glycosylphosphatidylinositol (GPI), a widely conserved glycolipid that anchors many cell surface proteins, is synthesized by sequential additions of glycan components to phosphatidylinositol. In mammalian cells, the acyl chain is added to glucosaminylphosphatidyl inositol at the third step in GPI biosynthesis. The purpose of this study is to identify the responsible gene and the role of inositol acylation.

Methods and Results

Analysis of mutant cells defective in inositol acylation

Molt4 1D10 cells were derived from human T cell lymphoma Molt 4 cells and CHOPA 10.14 mutant cells were established from CHO cells. Both mutant cells were defective in surface expression of GPI anchored proteins. We analyzed GPI biosynthesis in these mutant cells by metabolic labeling with [³H]mannose in the presence of tunicamycin. Lipids were extracted from cell pellets and were separated by thin layer chromatography. In contrast to Molt4 WT cells, the Molt4-1D10 and CHOPA 10.14 accumulated abnormal manolipids which were sensitive to GPI-PLD and PI-PLC indicating that those lipids were mannosylated noninositol-acylated GPI species. These results suggested that both mutants were defective in inositol acylation of GPI and inositol acylation is important for the surface expression of GPI-anchored proteins.

By the further characterization using Jack bean α -mannosidase treatment of accumulated manolipids revealed that inositol acylation is not essential for mannosylation but is critical for addition of the bridging P-EtN to the third mannose.

Expression cloning of PIG-W cDNA

To obtain cDNAs that rescued surface expression of GPI anchored proteins on Molt 4 1D10 cells, we transfected cells with the plasmids of the rat C6 glioma cDNA library, collected CD59 positive cells by cell sorter

and recovered plasmids. Pooled plasmids were transfected into mutant cells followed by another cycle of cell sorting and plasmid recovery. Total of 960 clones were analyzed and 2 positive clones were obtained. We termed the gene *PIG-W*.

Characteristics of *PIG-W* protein

PIG-W gene encodes a 504 amino acid protein containing multiple transmembrane domains. Subcellular localization of GST-tagged *PIG-W* by sucrose density gradient centrifugation revealed that *PIG-W* was mainly detected in ER fraction. We purified tagged-*PIG-W* protein from transfected cells and analyzed enzyme activity *in vitro*. Purified *PIG-W* protein were incubated with synthetic GlcN-PI(C8) as a substrate in the presence of [³H]palmitoyl-CoA, and GlcN-acylPI(C8) was generated. Therefore, affinity-purified *PIG-W* had inositol acyltransferase activity suggesting that *PIG-W* is most likely an acyltransferase itself.

Summary

1. In this study, we have cloned *PIG-W* gene which is responsible for mutant cells defective in GPI inositol acylation.
2. *PIG-W* encodes the ER membrane protein which functions as an inositol acyltransferase of GPI.
3. Inositol acylation is critical for surface expression of GPI anchored proteins but not for flipping or mannosylation of GPI precursors.

論文審査の結果の要旨

細胞表面の GPI アンカー型蛋白の発現が、欠損している細胞変異株を樹立し、その解析により GPI アンカー生合成のうち、グルコサミン PI (GlcN-PI) のイノシトールにアシル基転移がおこらない変異体であることを確認した。この細胞株を使った発現クローニングにより、同定した human *PIG-W* は新規の膜蛋白をコードし、既知のアシル基転移酵素とはホモロジーを持たなかった。精製した蛋白に、基質として GlcN-PI、アシル基のドナーとして放射線標識をしたパルミトイル CoA を反応させると GlcN-acylPI が合成され、確かに *PIG-W* がイノシトールのアシル基転移を担うことを証明した。

GPI アンカーのイノシトールアシル化酵素を初めて同定し *in vitro* でその酵素活性を示したものであり学位に値するものと認める。