論文の内容の要旨

論文題目

ALTERED GLYCOLIPID METABOLISM AND ITS CELL BIOLOGICAL IMPACT IN PEROXISOMAL BIOGENESIS DISORDERS

（ペルオキシソーム形成異常症における糖脂質代謝変化と細胞機能への影響）

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Introduction

Zellweger syndrome [OMIM#214100] (ZS) is an autosomal recessive disorder characterized by multi-organic clinical phenotypes: severe neurologic dysfunction, craniofacial abnormalities and liver dysfunction. It is characterized biochemically by the absence of peroxisomes. The absence of peroxisomes in ZS patients results in various abnormalities in the metabolism of lipids including fatty acids, plasmalogens, cholesterol and bile acids. Tissues and cells originated from ZS patients show accumulation of very long chain fatty acids (VLCFAs) such as lignoceric (C24:0) or hexacosanoic (C26:0) acids, and branched chain fatty acids such as pristanic and homophytanic acids.

Neurological deficits in ZS are caused by severe disturbance in brain development, characterized by impaired neuronal migration and hypomyelination. Clinical symptoms are profound hypotonia, seizures and severe motor and mental developmental delay. The neuropathology of ZS indicates the importance of peroxisomal function in the maturation of the central nervous system (CNS). The molecular basis of ZS neuropathology remains to be elucidated. It is unknown how the accumulation of VLCFAs and/or defect of plasmalogen associate with the pathogenesis of ZS brain. It has been suggested that peroxisomal dysfunction affects mitochondrial function and enhances oxidative stress. VLCFAs accumulation alone is not the sole cause for neuronal deficit in ZS, since normalization of the VLCFAs level in brain of *pex* knockout mice do not lead to a full restoration.

In this study, I hypothesized that secondary metabolic alteration may be involved in the pathological brain development. Accumulation of glycolipids has previously been noted in the cerebral grey matter, fibroblasts of ZS, as well as in peroxisome deficient mutant Z65 cells. Based on this finding, I focused on the secondary abnormalities in the metabolism of glycolipids, which are abundant in CNS and are involved in cellular proliferation, differentiation and recognition. I speculated that glycolipids metabolism alternation could affect cellular signaling in neuronal migration process.

I examined ZS patients' tissues to confirm the accumulation of glycolipids. To elucidate how

the accumulation of VLCFAs and/or the defect of plasmalogen is associated with the altered glycolipids metabolism, I analyzed the expression of glycolipid metabolizing enzymes using RNAi of peroxisomal enzymes. In order to investigate the effects of accumulated glycolipids on cellular function, I treated Z65 with a UDP-glucose glucosyltransferase inhibitor, D-PDMP. The objective of the present study is to elucidate how accumulated glycolipids affect cellular function in Z65.

Control and patient samples

Six patients (five with ZS and one with peroxisomal D-bifunctional protein (DBP) deficiency) and six control (C1-C6) samples were examined in this study.

Materials and methods

Thin layer chromatography was used to analyze crude lipid extracted from controls' and patients'tissue samples. Then RNAi of peroxisomal acylCo-A oxidase (*ACOX1*) and glyceronephosphate O-acyltransferase (*GNPAT*) was performed using cultured neural cells to evaluate enzyme regulation. For cellular function study, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), an inhibitor of UDP-glucose glycosyltransferase, was used. CHO-K1 and Z65 cells were exposed to PDMP treatment and were subjected to assays for proliferation, cell sensitivity towards H_2O_2, and cell adhesion. To investigate the difference of the expression of adhesion related molecules between CHO-K1 and Z65 cells, Western blotting forβ1-integrin was performed.

Results

ZS cerebellum samples contained a higher amount of sphingomyelin with shorter chain fatty acids compared to normal controls. The amount of phosphatidylethanolamine (PE) was smaller than a half of that in controls, with the absence of the PE-type of plasmalogen. Gangliosides were accumulated in the brain and fibroblasts of ZS patients. In neuronal F3-Ngn1 cells, ACOX1 and GNPAT silencing up-regulated ceramide galactosyltransferase (UGT8) mRNA expression, and down-regulated UDP-glucose ceramide glucosyltransferase (UGCG). Incubating Z65 cells with D-PDMP reduced the amount of GluCer in Z65 cells and suppressed cellular growth to a degree comparable to wild type CHO-K1 cells. D-PDMP aggravated fragility to H_2O_2 exposure, the extent of which being larger in Z65 cells than in CHO-K1 cells; L-PDMP had no effects. TLC analysis showed that the content of ganglioside GM3 and other a-series gangliosides were decreased in Z65 cells by D-PDMP. Cell adhesion assay revealed that Z65 cells were more tightly bound to laminin or collagen pretreated plates than CHO-K1 cells. Treatment of D-PDMP induced a weaker binding of Z65 cells. The amount of β1-integrin from total

cell lysate in Z65 cells expressed a smaller amount than CHO-K1 under no treatment, consistent with the results of Western blotting.

Discussion

Molecular species of SM modulate the stability of sphingolipid-enriched membrane microdomains that plays a role in the sorting and trafficking of membrane proteins. In two cerebella with ZS, the ratio of SM1 to SM2 was elevated, suggesting that membrane protein sorting and trafficking may be affected in ZS. The decrease in PE fraction in patients'specimens may reflect the decrease of PE-type plasmalogen. Nervous system, kidney, and testis contain relatively high levels of PE-type plasmalogen. Phosphatidylcholine (PC)-type plasmalogen is abundant in the heart and skeletal muscles. The liver has a very small amount of plasmalogens. I detected both types of plasmalogens in normal control liver, while PE-type plasmalogen was absent in patient tissues. Plasmalogens play a role in affecting membrane fluidity, mediate signal transduction and protect against oxidative stress. More detailed analysis of the phospholipid molecular species in the liver is necessary in the future.

The results of my *ACOX1* and *GNPAT* shRNA study were inconsistent between neuronal F3-Ngn1 and astrocytic HTB-14 cells. A plausible explanation is that the regulation of glycolipid metabolism is different depending on the cellular type. Increased CMH in the frontal lobe grey matter of ZS, but not in the white matter, might reflect the regional differences in glycolipid metabolism of CNS. According to a previous study, DNA damage with mitomycin C down-regulates *UGCG*, thereby increasing ceramides and up-regulating *UGT8*. These findings are consistent with our current results of *ACOX1* and *GNPAT* silencing. Although our findings were obtained from a limited number of samples, we suggest that peroxisomal dysfunction may affect glycolipid metabolism via ceramide metabolism in ZS.

I investigated whether accumulated glycolipids in peroxisome-deficient Z65 relates to the pathomechanism of PBDs. Treatment of D-PDMP reduced the amount of GlcCer and gangliosides in Z65 and suppressed cellular proliferation. Our group had previously reported that Z65 are more fragile to H_2O_2 than CHO-K1. D-PDMP aggravated fragility of Z65 to H_2O_2 exposure, suggesting protective effect of glycolipids against oxidative stress. Cell adhesion assay revealed that Z65 were more tightly bound to laminin or type IV collagen precoated plates than CHO-K1. Z65 expressed weak binding to extracellular matrix molecules after D-PDMP treatment, suggesting that the accumulation of GlcCer and gangliosides affects cell adhesion. I showed that the amount of β1-integrin in Z65 was smaller than in CHO-K1. Integrins mediate the formation of focal adhesions where integrins link to intracellular cytoskeletal complexes. These complexes play important roles in modulating cell adhesion and inducing cell shape

changes involved in cell spreading and locomotion. Integrins also regulate the trafficking of lipid raft (membrane microdomain), which involves gangliosides on the cell membrane Altered interaction of gangliosides and β1-integrin may affect cell adhesion, leading to impaired cell movements in the CNS with ZS. Further investigatition of altered interaction between gangliosides and β1-integrin in peroxisome deficient cells sould provide better understanding of neuropathology of ZS.

Conclusion

In conclusion, the results of the present study provide evidence that accumulation of glycolipids in ZS patients'tissues and their impact on cellular functions, suggesting the pathogenetic role of accumulated glycolipids in the neuropathology of ZS.