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Regulation of plasmalogen biosynthesis in mammalian cells and tissues

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ABSTRACT

Plasmalogens are a unique family of cellular glycerophospholipids that contain a vinyl-ether bond. Synthesis of plasmalogens is initiated in peroxisomes and completed in the endoplasmic reticulum. The absence of plasmalogens in several organs of patients with deficiency in peroxisome biogenesis suggests that *de novo* synthesis of plasmalogens contributes significantly to plasmalogen homeostasis in humans. Plasmalogen biosynthesis is spatiotemporally regulated by a feedback mechanism that senses the amount of plasmalogens in the inner leaflet of the plasma membrane and regulates the stability of fatty acyl-CoA reductase 1 (FAR1), the rate-limiting enzyme for plasmalogen biosynthesis. Dysregulation of plasmalogen synthesis impairs cholesterol synthesis in cells and brain, resulting in the reduced expression of genes such as mRNA encoding myelin basic protein, a phenotype found in the cerebellum of plasmalogen-deficient mice. In this review, we summarize the current knowledge of molecular mechanisms underlying the regulation of plasmalogen biosynthesis and the link between plasmalogen homeostasis and cholesterol biosynthesis, and address the pathogenesis of impaired plasmalogen homeostasis in rodent and humans.

1. Introduction

Plasmalogens, a subclass of glycerophospholipids harboring a vinyl-ether bond, are one of the major components of mammalian cellular membranes. Plasmalogens are found in anaerobic bacteria, invertebrates and vertebrate animal species (Braverman and Moser, 2012), whereas plasmalogens are not found in fungi or plants (Goldfine, 2010). Mutations in genes essential for plasmalogen biosynthesis lead to a drastic decrease in plasmalogen levels and cause the human genetic disease, rhizomelic chondrodysplasia punctata (RCDP) (Barøy et al., 2015; Braverman et al., 1997; Buchert et al., 2014; Motley et al., 1997; Purdue et al., 1997; Wanders et al., 1994, 1992). Patients with RCDP show severe growth retardation, proximal shortening of the upper limbs, and congenital cataracts, and diagnosed from a marked decrease in erythrocyte plasmalogens (Braverman and Moser, 2012). Since the level of plasmalogens in vegan's erythrocytes does not differ from that of human erythrocytes on a normal diet (Moser et al., 2011), it can be inferred that plasmalogens are supplied by biosynthesis rather than by dietary means. Furthermore, a recent finding of mutations in fatty acyl-CoA reductase 1 (FAR1), the rate-limiting enzyme in plasmalogen biosynthesis (Honsho et al., 2010), causes upregulation of plasmalogen biosynthesis, hence implying the physiological importance of the regulation of plasmalogen biosynthesis (Ferdinandusse et al., 2021). In this review, we summarized the regulation of plasmalogen biosynthesis in cells and tissues and discussed the pathology of dysregulation of plasmalogen homeostasis.

2. Regulation of plasmalogen biosynthesis in cells

Plasmalogen biosynthesis is initiated in the peroxisomal matrix where glyceronephosphate O-acyltransferase (GNPAT; also known as dihydroxyacetonephosphate acyltransferase) catalyzing the synthesis of acyl-dihydroxyacetone phosphate (acyl-DHAP) which is further metabolized to alkyl-DHAP by alkylglycerone phosphate synthase (AGPS) by replacing fatty acids of acyl-DHAP with long-chain fatty alcohols (Fig. 1). Activity of GNPAT and AGPS is not elevated in AGPS-deficient

Abbreviations: 24OHC, 24-hydroxycholesterol; AG, alkylglycerol; AGPS, alkylglycerone phosphate synthase; DHAP, dihydroxyacetone phosphate; FAR, fatty acyl-CoA reductase; GNPAT, glyceronephosphate O-acyltransferase; LXR, liver X receptor; MBP, myelin basic protein; RCDP, rhizomelic chondrodysplasia punctata; SM, squalene monooxygenase.

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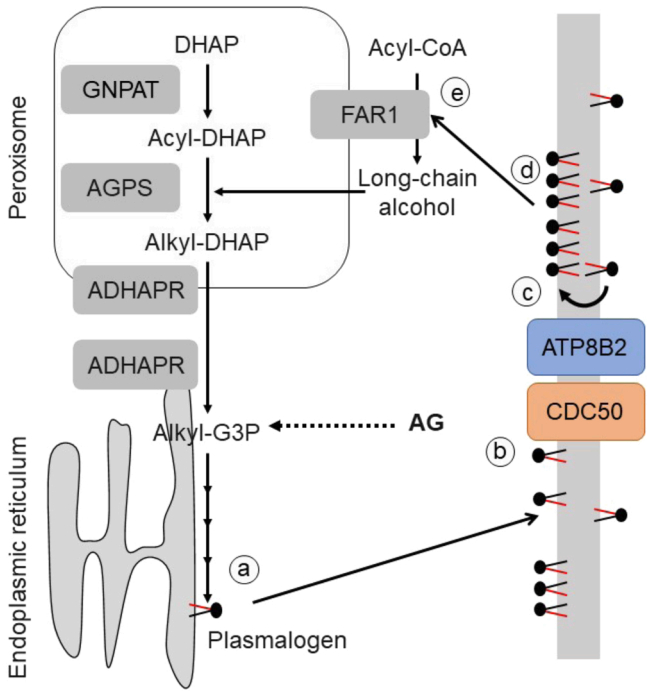

Fig. 1. Schematic view of regulation of plasmalogen biosynthesis. The initial two steps of plasmalogen biosynthesis are catalyzed by peroxisomal matrix enzymes, GNPAT and AGPS, where AGPS catalyzes the formation of alkyl-DHAP by replacing the acyl chain of acyl-DHAP with a long-chain alcohol. FAR1, a peroxisomal C-tail anchored protein, catalyzes the synthesis of long-chain alcohols. Alkyl-DHAP is further reduced to alkyl-glycerol-3-phosphate (Alkyl-G3P) by acyl/alkyl DHAP reductase (ADHAPR) localized to both peroxisome and endoplasmic reticulum (Honsho et al., 2020). Plasmalogens are synthesized *via* the remaining four steps in the ER (a). Plasmalogens are transported to the post-Golgi compartment, including endosomes and plasma membranes in a manner dependent on ATP but not vesicular transport (Honsho et al., 2008) (b). Plasmalogens are preferentially localized in the inner leaflet of plasma membrane in a manner dependent on P4-type ATPase ATP8B2 that associates with CDC50 subunit (c). Plasmalogens localized in the inner leaflet of plasma membrane are sensed (d) and the signal monitoring the cellular level of plasmalogens is conveyed to peroxisomes, where the stability of FAR1 is regulated, thereby controlling the synthesis of plasmalogens (e). Alkylglycerol (AG) is taken up into cells and incorporated to the plasmalogen biosynthetic pathway.

and GNPAT-deficient cells, respectively (de Vet et al., 1999; Nagan et al., 1998), whereas earlier studies by Rizzo and co-workers showed an increase in FAR activity and accumulation of fatty alcohol, the product of FAR-catalysis, in plasmalogen-deficient cells (James et al., 1990; Rizzo et al., 1993). These studies suggested that FAR is a rate-limiting enzyme in plasmalogen synthesis. This scenario was supported by the finding that accumulation of fatty alcohol in plasmalogen-deficient cells was reduced by restoration of the plasmalogen level (Honsho et al., 2010). By successful isolation of genes encoding FAR proteins, FAR1 and FAR2 (Cheng and Russell, 2004), the protein level of FAR1 but not FAR2 is shown to be correlated with the level of fatty alcohol required for the synthesis of plasmalogens (Honsho et al., 2013). Importantly, restoration of plasmalogens by incubating plasmalogen-deficient cells with purified plasmalogens reduces the elevated protein level of FAR1 to that in control cells without altering the transcription level of *FAR1*. Similarly, plasmalogen synthesis in wild-type cells is downregulated by enhancing degradation of FAR1 when the cellular plasmalogen level is elevated by incubating cells with purified plasmalogens (Honsho et al., 2010, 2013). These studies imply that FAR1 is the rate-limiting enzyme in plasmalogen biosynthesis, by which plasmalogen synthesis is regulated in response to intracellular plasmalogen levels (Honsho et al., 2010; Honsho and Fujiki, 2017). The physiological importance of plasmalogen biosynthesis is further appreciated by the discovery of a missense mutation in FAR1 that inhibits FAR1 degradation and increases plasmalogens (Ferdinandusse et al., 2021).

The role of FAR2 in the synthesis of plasmalogens remains unexplored. Analyses of substrate specificity between FAR1 and FAR2 in culture cells and the role of Far2 in the synthesis of fatty alcohol in meibomian glands and formation of tear film lipid layer indicate that Far2 is involved in the synthesis of fatty alcohol with carbon chains longer than C20 (Otsuka et al., 2022). These results suggest that FAR2 is less likely involved in the synthesis of plasmalogens under physiological conditions because the *sn*-1 position of plasmalogens is normally occupied by either C16 or C18 carbon attached *via* vinyl-ether moieties (Braverman and Moser, 2012; Nagan and Zoeller, 2001).

Sensing of plasmalogens is an important step in the regulation of plasmalogen synthesis. The precise mechanism of sensing plasmalogens remains unknown. However, plasmalogens in the plasma membranes appear to be important for sensing plasmalogens from the studies modulating plasmalogens in the plasma membranes (Honsho et al., 2017, 2008; Pike et al., 2002). Protein level of FAR1 is increased in HeLa cells treated with nystatin, a membrane-impermeable cholesterol-chelating compound (Guyader et al., 2002), which is likely due to the distortion of the structure and function of cholesterol-rich membrane domain (Ros-Baró et al., 2001) without lowering cellular plasmalogen level (Honsho et al., 2017). In the plasma membranes, plasmalogens are thought to be concentrated in the inner leaflet by the flippase activity of P4-type-ATPases (Honsho et al., 2017) as in the case of other glycerophospholipids such as phosphatidylserine (Shin and Takatsu, 2019). Indeed, plasmalogens are mostly found in the inner leaflet of plasma membranes (Fellmann et al., 1993; Kirschner and Ganser, 1982), whereas the localization of plasmalogens in the outer leaflet of plasma membranes is augmented by lowering the expression of *CDC50A*, a gene encoding β-subunit of P4-ATPases that is essential for exiting of most of P4-ATPases from the endoplasmic reticulum (ER) by forming a hetero-oligomer (Bryde et al., 2010; van der Velden et al., 2010). Under this *CDC50A*-knockdown condition, protein level of FAR1 is elevated without changing total cellular level of plasmalogens (Honsho et al., 2017). By expanding these findings, we recently identified that ATP8B2 acts as a flippase for plasmalogens in HeLa cells (Honsho et al., 2022). Given these findings, we proposed that plasmalogens in the inner leaflet of plasma membranes are sensed. However, several steps in the regulation of plasmalogen biosynthesis remain unexplored. To fully uncover the regulatory mechanism of plasmalogen biosynthesis, understanding of the molecular mechanisms involving asymmetric distribution of plasmalogens, plasmalogen sensing, signaling of intracellular plasmalogen levels, and regulation of FAR1 degradation is essential. In this regard, the observation that an ectopic expression of ER-localizing TMEM189, a plasmalogen desaturase, promotes degradation of FAR1 is interesting (Cui et al., 2021). The precise mechanism how TMEM189 lowers FAR1 protein level has not been addressed. However, the enhanced degradation of FAR1 is less likely caused by the TMEM189-mdediated elevation of plasmalogen synthesis because TMEM189 is not thought to be involved in the regulation of the level of plasmalogens (Werner et al., 2020). It is tempting to speculate that the interactions between peroxisomes and ER or plasma membrane play a role in the degradation of FAR1.

3. Regulation of plasmalogen biosynthesis in peripheral tissues and brain

Regulation of plasmalogen biosynthesis in tissues has not been directly addressed. Feeding alkylglycerol (AG) to *Pex7*- and *Gnpat*-knockout (KO) mice or wild-type rats provided insight into how plasmalogen synthesis is regulated in animals. Administration of AG (1-O-octadecyl-*sn*-glycerol) to *Pex7*- or *Gnpat*-KO mice successfully increased the level of plasmalogen containing C18:0 fatty alcohol, in peripheral

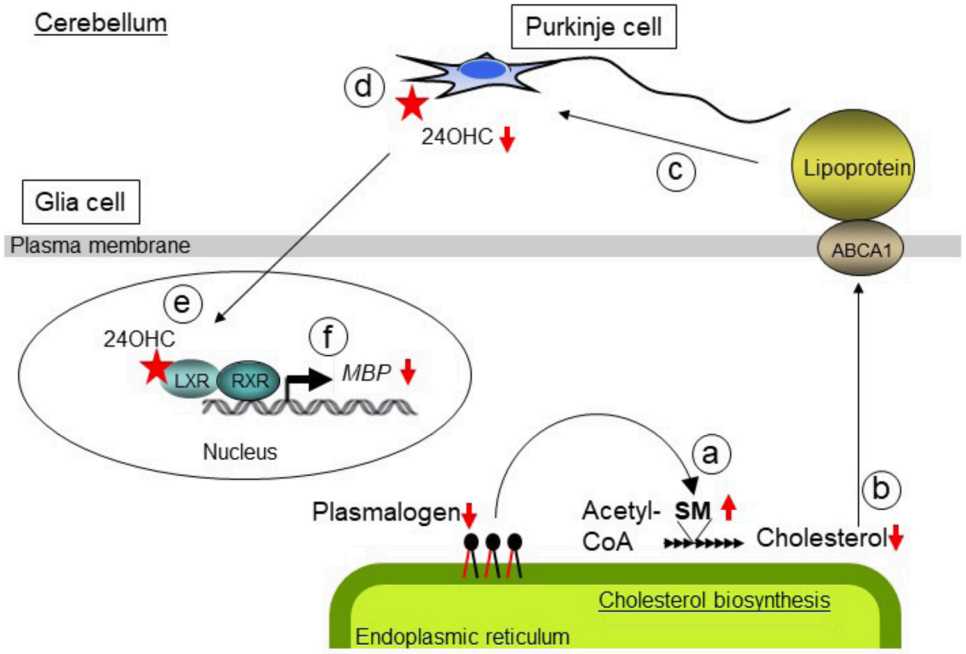

Fig. 2. Plasmalogen-mediated regulation of cholesterol biosynthesis and its functions in cerebellum. Homeostasis of plasmalogens regulates cholesterol synthesis by modulating stability of squalene monooxygenase (SM) (a). In *Pex14*$^{\Delta C/\Delta C}$ and GNPAT-deficient mice, the reduction of plasmalogens in cerebellum stabilizes SM by suppressing the degradation of SM. The elevated protein level of SM lowers the synthesis of cholesterol (b). Cholesterols are transferred from glia cells to neurons by ATP-binding cassette transporter A1 (ABCA1) and lipoprotein particles (c) and converted to the most abundant oxysterol 24(S)-hydroxycholesterol (24OHC, a red star) in Purkinje cells (d). The reduced level of 24OHC due to the less efficient synthesis of cholesterol lowers the transcriptional activity of liver X-receptor (LXR) (e), resulting in the reduced expression of LXR target genes such as *MBP* encoding myelin basic protein (f). RXR, retinoid X receptor. Upward and downward arrows indicate up- and down-regulations, respectively.

tissues but not brain (Brites et al., 2011; Dorninger et al., 2022; Todt et al., 2020). Consistent with this study, feeding of 1-O-heptadecyl-*sn*-glycerol to young wild-type rat results in the detection of plasmalogens containing C17:0 fatty alcohol in all tissues except for the brain. However, the total plasmalogen amount is not increased in peripheral tissues (Das and Hajra, 1988). These studies addressed two important issues regarding to the plasmalogen homeostasis in animals. Firstly, there is some mechanism regulating the synthesis of plasmalogens by sensing cellular plasmalogens in peripheral tissues. Secondly, plasmalogens in the brain are most likely synthesized by brain cells, rather than taking up peripheral tissue-derived plasmalogens from the bloodstream. Based on the studies on the regulation of plasmalogen biosynthesis in cells, FAR1-mediated regulation of plasmalogen biosynthesis is expected as a control mechanism of plasmalogen synthesis in tissues, which is partly supported by the elevated FAR1 protein level in kidney of plasmalogen-deficient *Pex7*-KO mice (Wiese et al., 2012). Similarly, increased FAR1 protein is reported in the cerebellum of *Gnpat*-KO mice and the mouse model with deletion of gene encoding C-terminus part of Pex14, termed the *Pex14*$^{\Delta C/\Delta C}$, in which import of peroxisomal matrix proteins such as Agps and catalase is partially defective and plasmalogens are about a half of that in control mice (Abe et al., 2018; Honsho et al., 2019). Clearly, it is important to analyze the fate of FAR1 in tissues of these KO mice upon restoring the plasmalogen levels.

As described above, AG has been used to restore the plasmalogen levels in plasmalogen-deficient cells and mice (Bozelli et al., 2020; Brites et al., 2011; Das and Hajra, 1988; Honsho et al., 2008; Nagan et al., 1997) (Dorninger et al., 2022; Todt et al., 2020) (Fig. 1). AG is incorporated and phosphorylated, resulting in a product, 1-O-alkyl-2-lyso-glycerophosphate, which enters to the plasmalogen biosynthetic pathway (Nagan and Zoeller, 2001). Consistent with these observations, AG is converted to plasmalogens in several peripheral tissues and ameliorate the pathological impairments of plasmalogen-deficient mice (Brites et al., 2011; Todt et al., 2020). Similarly, PPI-1040, a plasmalogen analogue, that is hydrolyzed at its cyclic phosphoethanolamine group upon administration and is converted to plasmalogen, elevates plasmalogens in several peripheral tissues of *Pex7* hypomorphic mice, although administration of PPI-1011, an another plasmalogens analogue containing lipoic acid at *sn*-3 failed to increase plasmalogens (Fallatah et al., 2020). These findings indicate that plasmalogens in peripheral tissues can be replaced by its precursor AG, its analogue, or even intact plasmalogens (Bozelli and Epand, 2021; Mawatari et al., 2020), thereby assessing the fate of FAR1 is the one of the key issue to understand how plasmalogen homeostasis is regulated in tissues.

4. Involvement of plasmalogen homeostasis in cerebellum myelination

Contrary to the elevation of plasmalogens in peripheral tissues, an increased level of plasmalogens in brain has not been reported by the administration of alkylglycerol (Brites et al., 2011; Dorninger et al., 2022), its analogue, or intact plasmalogens, although phenotypes such as impaired myelination in dorsal column track of *Gnpat*-KO mouse and hyperactive behavior observed in a hypomorphic *Pex7* mouse model (*Pex7*$^{hypo/null}$) were restored by the administration of glyceryl 1-myristyl ether and PPI-1040, respectively (Fallatah et al., 2020; Malheiro et al., 2019).

Since plasmalogens are abundant in myelin sheath, homeostasis of plasmalogens is likely important for myelination in different regions of central nervous system such as cerebellum and spinal cord (Malheiro et al., 2019; Teigler et al., 2009). Indeed, study using *Pex7*-deficient mouse model showed direct correlations between the severity of the disease, plasmalogen levels and brain phenotype such as myelination in cerebellum and corpus callosum (Fallatah et al., 2022). The role of plasmalogens homeostasis in myelination is addressed by several groups (da Silva et al., 2014; Honsho et al., 2019; Malheiro et al., 2019). Plasmalogens are required for the correct and timely differentiation of Schwann cells and for the process of myelination involving protein kinase B (AKT) signaling (da Silva et al., 2014). Subsequently, by establishing *in vitro* myelination assay and the analysis of myelination in central nervous system in *Gnpat*-KO mice, Malheiro et al. proposed that the complete absence of plasmalogens, together with the decreased levels of myelin basic protein (MBP), were the major causes of inadequate formation of the myelin sheath, and consequently impaired myelination in *Gnpat*-KO mouse (Malheiro et al., 2019). The authors further showed interesting results that glyceryl 1–myristyl ether rescues impaired myelination *in vitro* and *in vivo*, although how the chemical rescues a myelination was not addressed and mechanism underlying the reduced expression of MBP by the deficit of plasmalogens remains unknown.

MBP is known to play essential roles in initiating and driving the

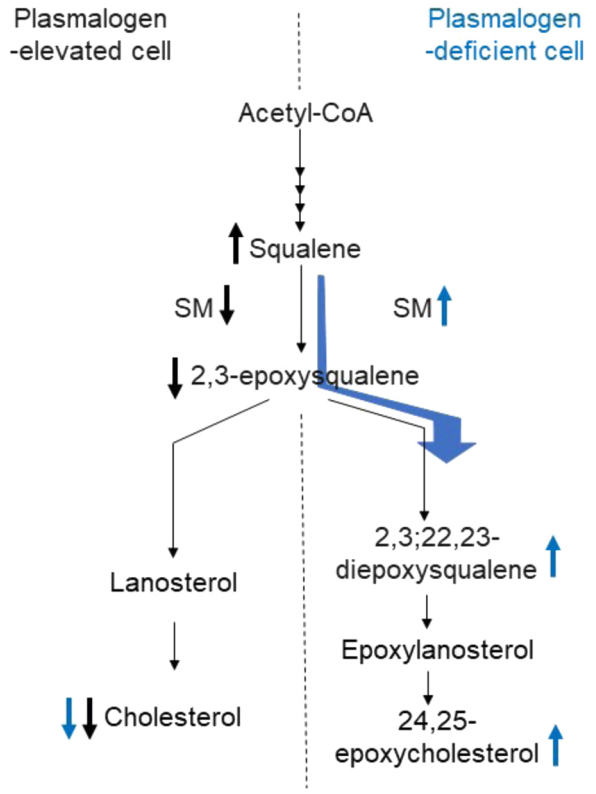

Fig. 3. Synthesis of cholesterol in plasmalogen-elevated and -deficient cells. Cholesterol is synthesized from acetyl-CoA by the isoprenoid biosynthetic pathway, followed by the conversion of two farnesyl pyrophosphates to squalene. Squalene is oxidized by SM to 2,3-epoxysqualene, which is further cyclized to the first sterol intermediate, lanosterol. Lanosterol is then converted to cholesterol through a series additional 19-step reactions. SM oxidizes 2,3-epoxysqualene to 2,3;22,23-diepoxysqualene (diepoxysqualene), followed by generating epoxylanosterol and its conversion to epoxycholesterol. Blue and black arrows indicate the level of metabolites or expression level of SM. (left) In plasmalogen-elevated cells, protein level of SM is decreased through enhanced degradation of SM, thereby lowering the synthesis of cholesterol and epoxycholesterol. (right) In plasmalogen-deficient cells, the protein level of SM is elevated due to the suppressed degradation of SM. Under such condition, SM preferentially synthesizes diepoxysqualene and its metabolite, epoxycholesterol, concomitantly decreasing the synthesis of cholesterol (Honsho et al., 2015). Note that cholesterol synthesis is decreased in both plasmalogen-elevated and -deficient cells, suggesting that homeostasis of plasmalogen is tightly linked to cholesterol synthesis.

axon wrapping process, myelin compaction, and maintaining the physical stability of the sheath (Snaidero et al., 2017; Weil et al., 2016). Therefore, clarifying how a reduced level of plasmalogens decreases MBP expression is an important issue for understanding the pathogenesis of impaired myelination in brain. This is partly explored from the viewpoint of plasmalogen homeostasis (Honsho et al., 2019) (Fig. 2). Expression of MBP in the cerebellum of *Gnpat*-KO, *Pex7*-KO, and *Pex7*-$^{hypo/null}$ mice is reduced as compared to control mice (Fallatah et al., 2022; Teigler et al., 2009). *MBP* is one of target genes of liver X receptor (LXR) which is a transcription factor activated by oxysterols such as 24-hydroxycholesterol (24OHC), the most abundant oxysterols in brain (Meljon et al., 2014). The amount of 24OHC in cerebellum of plasmalogen-deficient *Gnpat*-KO mouse and plasmalogen-depleted *Pex14*$^{\Delta C/\Delta C}$ mouse is reduced as compared to control mice, resulting in the reduced transcription of *MBP* (Honsho et al., 2019). The decrease in 24OHC of the cerebellum in plasmalogen-depleted mice is due to the less efficient biosynthesis of cholesterol, which is likely caused by the preferential synthesis of 2,3;22,23-diepoxysqualene from squalene, similar to the mechanism of the reduced cholesterol synthesis in the absence of plasmalogens as explored with plasmalogen-deficient cells (Honsho et al., 2015, 2019; Honsho and Fujiki, 2017) (Fig. 3). Given the fact that brain cholesterol separated from the circulating cholesterol with the blood-brain barrier is supplied primarily by glial cells in brain (Hayashi, 2011), these findings confer physiological consequence of plasmalogen homeostasis in cerebellum.

Cholesterol synthesis is downregulated in cells showing either plasmalogen-deficient or -elevated conditions (Honsho et al., 2015) (Fig. 3). In plasmalogen-deficient cells, squalene monooxygenase (SM), a key enzyme in cholesterol biosynthesis, is stabilized, thereby reducing the metabolic flow from squalene to cholesterol, rather inducing preferential synthesis of diepoxysqualene that is not utilized for the synthesis of cholesterol. In contrast, cholesterol synthesis in plasmalogen-elevated cells is lowered by enhancing degradation of SM. Based on these studies, it is tempting to speculate that the impaired plasmalogen-mediated downregulation of cholesterol synthesis in brain give rise to the shared phenotypes of patients with plasmalogen-deficient and patients with elevated plasmalogens (Ferdinandusse et al., 2021).

Recent studies showed a reduction of monoamine neurotransmitters such as dopamine, norepinephrine, and serotonin in *Gnpat*-KO mice manifesting a hyperactive phenotype with deficient social interaction (Dorninger et al., 2019), and Braverman and coworkers subsequently showed correlations between plasmalogens and brain neurotransmitter levels as well as hyperactivity phenotype in *Pex7* deficient mice (Fallatah et al., 2022). Exact mechanism underlying the reduction of neurotransmitters remains to be defined. However, impaired homeostasis of plasmalogens might contribute to a less expression level of monoamine transporter 2, a transporter of the monoamine neurotransmitters from the cytosol into synaptic vesicles, in striatal vesicular preparations obtained from *Gnpat*-KO mice (Dorninger et al., 2019). The report that PPI-1040 restores hyperactivity of *Pex7*$^{hypo/null}$ mouse is interesting, although the plasmalogen level in cortex and cerebellum is not augmented (Fallatah et al., 2020). Nevertheless, analysis of the protein level of monoamine transporter 2 and monoamine neurotransmitters in brain of *Pex7* deficient mice before and after the administration of PPI-1040 would provide novel insight into the physiological role of plasmalogen homeostasis in brain.

5. Conclusions

Plasmalogen homeostasis is maintained by a combination of regulation of plasmalogen biosynthesis and degradation. In cultured cells, plasmalogen synthesis is known to be regulated in a manner dependent on the amount of plasmalogens present in the plasma membrane. Plasmalogen synthesis in tissues is likely to be regulated by a feedback mechanism similar to that in cultured cells. Plasmalogens can be metabolized to lysoplasmalogens (Beckett et al., 2007; Brites et al., 2004; Farooqui, 2010; Hayashi et al., 2022) and N-acylplasmalogens (Tsuboi et al., 2011) or degraded by cytochrome *c* in cells (Jenkins et al., 2018). Regulation of plasmalogen degradation in tissue is largely unknown, although a reduced level of brain plasmalogens has been reported in Alzheimer's disease (Ginsberg et al., 1995; Guan et al., 1999; Huynh et al., 2020), Parkinson's disease (Dragonas et al., 2009; Fabelo et al., 2011), and schizophrenia (Huang et al., 2017; Tessier et al., 2016). Elevation of plasmalogens by the infection of virus such as human cytomegalovirus and West Nile virus and requirement of plasmalogens for the assembly of human cytomegalovirus has been shown (Beltran et al., 2018; Martín-Acebes et al., 2014). Just as the elucidation of the regulation of plasmalogen biosynthesis has conferred rapid identification of the cause of diseases with elevated plasmalogen levels (Ferdinandusse et al., 2021), we should await further studies to elucidate whether the dynamic changes of plasmalogens as exemplified here are the cause or merely the apparent phenotypes of disease. Replacement of plasmalogens in peripheral tissues is shown to be available at least in animals (Brites et al., 2011; Fallatah et al., 2020). In contrast, elevation

of plasmalogens in brain is still challenging, which may require any strategies for enhancing biosynthesis or inhibiting degradation of plasmalogens. Therefore, further studies on the regulation of plasmalogen homeostasis and development of small molecules that regulate the process of plasmalogen homeostasis are awaited.

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Conflict of Interest

None.

Data Availability

No data was used for the description of this article.

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