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DDHD domain-containing lipases: Targets for the treatment of rare diseases

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ABSTRACT

The DDHD domain-containing lipases belong to the intracellular phospholipase A_1 ($iPLA_1$) family. Phospholipases have been implicated in the regulation of lipid metabolism, intracellular membrane trafficking, and signaling. In addition, phospholipases have been linked to the development of rare and neurodegenerative diseases. The rare and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease have been focused on phospholipase A_2. But there is a scarcity of literature on the role of PLA_1 in rare and neurodegenerative diseases. Recently, in humans, mutation in DDHD1 and DDHD2 ($iPLA_1$ members) has been identified as a cause of specific types of hereditary spastic paraplegia (HSP) termed as SPG28 and SPG54, respectively. Ddl1 (DDHD domain-containing lipase 1), a yeast homolog of human DDHD1/2, hydrolyzes cardiolipin (CL), phosphatidylethanolamine, and phosphatidylglycerol. Ddl1 has an important role in the mitochondrial phospholipids remodeling. Defects in phospholipids remodeling and mitochondrial functions have been implicated in the development of the Barth syndrome, HSPs, and other neurodegenerative disorders. Mutations in DDHD1 and DDHD2 produce *DDL1*-defective yeast strain like phenotypes (mitochondrial dysfunction and defects in lipid metabolism). Therefore, the *DDL1*-defective yeast could be a good model system to understand hereditary spastic paraplegia.

DDHD domain

The DDHD domain was first identified as a long stretch of amino acids in the central part of the Nir/rdgB (N-terminal domain-interacting receptor/*Drosophila* retinal degeneration B) proteins[1]. This domain possesses four conserved amino acid residues (DDHD), which may play an important role in forming a metal-binding site. The name DDHD domain is based on these four conserved amino acid residues. This domain is also found in the C-terminal region of the phosphatidic acid (PA)-preferring phospholipase A_1 ($PA\text{-}PLA_1$)[1]. The recently identified lipid-metabolizing enzyme family, named as intracellular phospholipase A_1 ($iPLA_1$) family also possesses the DDHD domain-containing proteins. It has been predicted that the DDHD domain may play a role in the phospholipid metabolism, organelle biogenesis, membrane trafficking, and signaling[1]. In humans, there are three $iPLA_1$ family members namely $PA\text{-}PLA_1$/DDHD1, KIAA0725p/DDHD2 and p125/Sec23IP. The $iPLA_1$ family members have also been reported in yeasts, nematodes, and plants[2]. The first $iPLA_1$ member, $PA\text{-}PLA_1$, was identified by Higgs and Glomset[3]. The DDHD1[4] and DDHD2[5] have hydrolase activities, while the lipase activity of p125 is yet to be demonstrated. A study of the substrate specificity of the purified rat DDHD2 provides new insights into the enzymatic nature of the human DDHD2[6]. The human DDHD1 and DDHD2 are the PA preferring lipases and play an important role in the intracellular membrane trafficking. PA has diverse functions in biological systems. It is a precursor for the biosynthesis of polar and non-polar lipids. It has structural functions

in the biological membranes[7]. It has also been reported that PA has an important role in the lipid signaling[8]. Like iPLA$_1$ family members, the DDHD domain is also conserved in some phosphatidylinositol transfer proteins[9,10]. Inoue et al. have shown that the DDHD domain is crucial for the PLA$_1$ activity as well as in phosphatidylinositol phosphate (PIP) binding and oligomerization[4]. PIPs play crucial parts in the membrane trafficking[11,12].

Role of phospholipases in rare diseases

Phospholipids are an important constituent of biological membranes. Several studies[13-19] have shown that phospholipases play an important role in rare and neurodegenerative diseases. Phospholipases are enzymes that hydrolyze the phospholipids. Phospholipases have been implicated in the processes like regulation of lipid transport and metabolism, intracellular membrane trafficking, and vesicular transport[13]. Phospholipases are categorized in A, B, C and D groups according to their site of action. Most of the studies related to disease conditions have been focused on phospholipase A$_2$ (PLA$_2$)[13-16]. As different types of PLA$_2$ have been identified in the central nervous system (CNS); therefore, the role of PLA$_2$s in rare and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease have been widely studied[14-19]. But the knowledge regarding the roles of different types of PLA$_2$ is sketchy because of the complexity of the brain which possesses different types of cells. On the other hand, there is a scarcity of reports on the role of PLA$_1$ in rare and neurodegenerative diseases. Recently, in humans, mutations in DDHD1 and DDHD2 (iPLA$_1$ members) have been identified as a cause of specific types of hereditary spastic paraplegia termed as SPG28[20] and SPG54[21,22], respectively. Hereditary spastic paraplegia (HSP, also known as Strumpell-Lorrain disease) is a heterogeneous group of genetic neurodegenerative disorders, and these disorders are mainly characterized by slowly progressive spasticity (contraction) and weakness of the lower limbs. Many types of HSP have been discovered and are numbered according to the order of their discovery [spastic paraplegia (SPG) 1-72]. Affected individuals are clinically grouped according to the absence (uncomplicated or pure HSP) or presence (complicated or complex HSP) of additional phenotypes[23,24]. By modes of inheritance, HSPs are grouped as autosomal dominant, autosomal recessive, and X-linked recessive HSPs[23,24].

DDHD domain-containing lipase in Saccharomyces cerevisiae

We[25] have characterized an unannotated *Saccharomyces cerevisiae* open reading frame, YOR022C as a Ddl1 (DDHD domain-containing lipase 1). Pairwise sequence alignment showed that yeast Ddl1 has ~33% and ~34% similarities with human DDHD1 and DDHD2, respectively. The Ddl1 is a novel mitochondrial phospholipase of the iPLA$_1$ family (Figure 1)

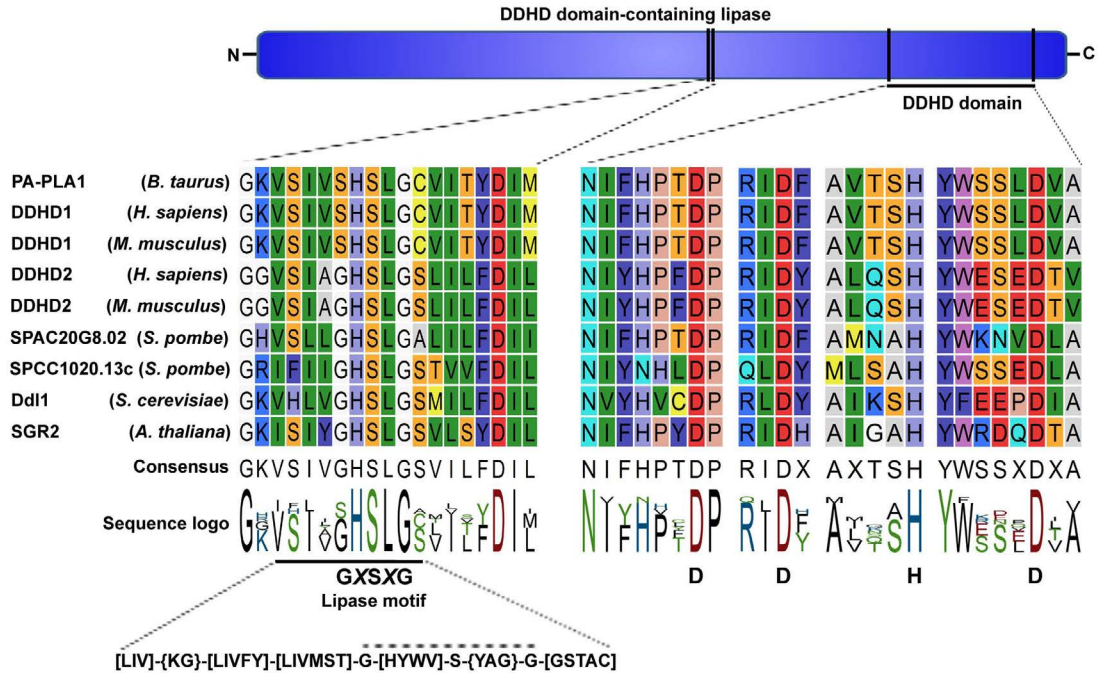

Figure 1. Ddl1 and its homologs

A schematic diagram of the DDHD domain-containing lipase and multiple sequence alignment of two conserved regions: the lipase motif (GXSXG) and DDHD domain. A list of homologs of the iPLA1 family is available on the UniProtKB database. An analysis of the sequences in the PROSITE database showed that the lipase motif is present inside the PROSITE domain PS00120 consensus sequence [LIV]-{KG}-[LIVFY]-[LIVMST]-G-[HYWV]-S-{YAG}-G-[GSTAC]. In some family members, the lipase consensus sequence (GXSXG) was replaced by the SXSXG sequence. In Ddl1, the lipase motif spanned from the 499th to 503rd amino acids, while the DDHD domain spanned from the 519th to 700th amino acids.

that hydrolyzes important mitochondrial phospholipids, and it has roles in cardiolipin (CL), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG) remodeling. The deletion of the *DDL1* gene caused an increase in molecular species with saturated fatty acids while *DDL1* overexpression caused an increase in the molecular species with unsaturated fatty acids. In addition, we have shown that Ddl1 has roles in CL degradation. Our findings showed that Ddl1 is a CL-preferring lipase; therefore deletion of the DDL1 gene caused an increase in the CL content while overexpression of the *DDL1* gene caused a decrease in the cellular CL content. Also, the CL degradation was affected in the *DDL1* deleted cells (monolysocardiolipin to dilysocardiolipin conversion was affected by the deletion of the *DDL1* gene), leading to the CL accumulation. On the other hand, the *DDL1* overexpression increased the CL degradation leading to reduced CL content. Therefore, optimal regulation and activity of Ddl1 are required for the proper CL metabolism. As CL is the signature phospholipid species of the mitochondria; misregulation of Ddl1 causes mitochondrial dysfunction in yeast[25].

Role of mitochondria in rare diseases

The mitochondria are vital organelles of the cell. Important metabolic reactions and the regulation of some signaling cascades occur in the mitochondria[26]. The mitochondria are sites of synthesis of major non-bilayer-forming phospholipids PE and CL[27,28]. CL and PE play an important role in the mitochondrial fusion[29]. CL is required for the proper mitochondrial inner membrane potential $\Delta\psi$ and in protein import into the mitochondria[30]. CL and mitochondrial PE have overlapping functions, and they can compensate for the loss of the other[31]. Defective mitochondrial phospholipids remodeling has been identified as a major cause of Barth syndrome[32,33]. We[25] have proposed that CL is remodeled through a deacylation-transacylation cycle, in which the acyl specificity of CL is achieved by extensive remodeling through PLA_1 (Ddl1), PLA_2 (Cld1), and transacylase (Taz1) activities. A deacylation (by Ddl1)-reacylation (by CoA-dependent acyltransferase, Ale1) cycle was proposed for the PE and PG remodeling in yeast (Figure 2).

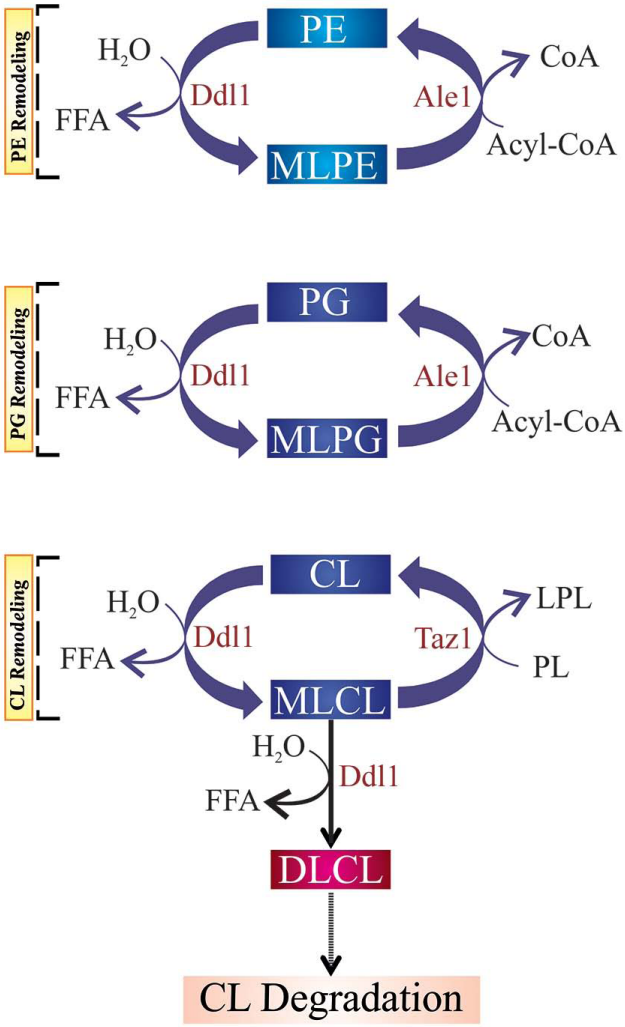

Figure 2. A schematic model of the proposed roles of the DDL1 gene in yeast

We propose that Ddl1 plays an important role in phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL) remodeling; as well as CL degradation. Ddl1 overexpression enhances the degradation of the mitochondrial membrane lipids, causing mitochondrial dysfunction in yeast. MLPE, monolysophosphatidylethanolamine; MLPG, monolysophosphatidylglycerol; MLCL, monolysocardiolipin; DLCL, dilysocardiolipin; and LPL, lysophospholipid.

It has been shown that human DDHD1 is partially localized to the mitochondria[20,34] and mutations in DDHD1 gene produce mitochondrial dysfunction and defects in lipid metabolism. The DDHD2 protein has been reported to locate in cis-Golgi and endoplasmic reticulum (ER)-Golgi intermediate compartment, besides role of DDHD2 in the membrane trafficking and vesicle fusion has been proposed[4]. Defects in the intracellular membrane trafficking, mitochondrial morphology and functions, and lipid metabolism are key pathophysiological features of HSPs[20,35]. The neurodegenerative disorders, such as Alzheimer's disease, and Huntington's disease have also been associated with the defects in the intracellular membrane trafficking, mitochondrial morphology, and functions[36]. Alteration in the mitochondrial-membrane lipid composition has already been shown to cause mitochondrial dysfunction which in turn triggers secondary cellular dysfunctions[37-39]. The increased reactive oxygen species (ROS) production was observed in SPG28 and SPG49 cells, and it was hypothesized that the increased ROS production could cause neurodegeneration[40].

A Recent study has linked alterations in CL profile to an early development of the age-related neurodegenerative disorders such as Alzheimer's disease[41]. Human HSD10

is a multifunctional enzyme which is found in the brain and cerebral spinal fluid and has been associated with the mitochondrial disease. In Alzheimer's disease patients, the HSD10 expression was found elevated[42]. Recently, a study showed that the HSD10 protein has CL-specific phospholipase C-like enzyme activity[43].

Cures and future perspectives

In humans, many rare genetic diseases are associated with mutations in poorly characterized genes. Determination of biochemical functions of these genes is critical for understanding and formulating potential cures for these rare genetic diseases. The probable implication of cardiolipin and mitochondria in the pathophysiology of neurodegenerative disorders could help in the development of therapeutic strategies focused on the mitochondrial morphology and functions.

Polyunsaturated fatty acids (PUFAs) are enriched in the phospholipids of CNS membranes[44]. Metabolism of PUFA is strictly controlled by PLA_2 and acyltransferases through "deacylation-reacylation cycle" which is an energy-dependent process involving coenzyme A (CoA) and ATP[45-47]. We[25] have shown that Ddl1 (PLA_1), the yeast homolog of human DDHD1 and DDHD2 proteins, plays an important role in the mitochondrial phospholipids remodeling. Therefore, it will be interesting to study the role of DDHD1 and DDHD2 proteins in phospholipids remodeling.

There are several synthetic and phytochemical based inhibitors of PLA_2 available and which could be used for the treatment of neurological disorders[48]. *Ginkgo biloba* and *Centella asiatica* extracts have been used for the treatment of neurological disorders in the cell culture and animal model systems[49,50]. The neurological disorders wherein the PLA_2 expression is elevated, PLA_2 inhibitors could be used for the treatment. Likewise, identification of PLA_1 inhibitors would provide a base for the innovative development of therapeutic strategies focused on the PLA_1 activities.

Cardiolipin is always at the risk of oxidation as it is localized to the inner mitochondrial membrane. When cardiolipin becomes oxidized, it induces apoptosis and could trigger diseases such as Alzheimer's and Parkinson's. Researchers have suggested that HSD10 protein has CL-specific phospholipase C-like enzyme activity, and it prevents neurodegeneration by removing oxidized cardiolipin[43]. In humans, mutations in the *DDHD1* and *DDHD2* genes cause specific types of hereditary spastic paraplegia, and the yeast *DDL1*-defective strain exhibits the similar phenotypes of hereditary spastic paraplegia (mitochondrial dysfunction and defects in lipid metabolism). Yeast Ddl1 prefers CL as substrates. It has also been shown that DDHD1 is partially localized to the mitochondria[20,34]. Therefore, it will be interesting to explore the role of DDHD1 in CL metabolism. In addition, the *DDL1*-defective yeast strain could be a suitable model system to understand hereditary spastic paraplegia.

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