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Review

Cell biology and function of neuronal ceroid lipofuscinosis-related proteins ☆

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ABSTRACT

Neuronal ceroid lipofuscinoses (NCL) comprise a group of inherited lysosomal disorders with variable age of onset, characterized by lysosomal accumulation of autofluorescent ceroid lipopigments, neuroinflammation, photoreceptor- and neurodegeneration. Most of the NCL-related genes encode soluble and transmembrane proteins which localize to the endoplasmic reticulum or to the endosomal/lysosomal compartment and directly or indirectly regulate lysosomal function. Recently, exome sequencing led to the identification of four novel gene defects in NCL patients and a new NCL nomenclature currently comprising CLN1 through CLN14. Although the precise function of most of the NCL proteins remains elusive, comprehensive analyses of model organisms, particularly mouse models, provided new insight into pathogenic mechanisms of NCL diseases and roles of mutant NCL proteins in cellular/subcellular protein and lipid homeostasis, as well as their adaptive/compensatorial regulation at the transcriptional level. This review summarizes the current knowledge on the expression, function and regulation of NCL proteins and their impact on lysosomal integrity. This article is part of a Special Issue entitled: The Neuronal Ceroid Lipofuscinoses or Batten Disease.

1. Introduction

The neuronal ceroid lipofuscinoses (NCLs) are inherited progressive degenerative diseases that primarily affect the brain and retina. They are considered lysosomal storage disorders (LSD), because of their characteristic accumulation of autofluorescent ceroid lipopigments, subunit c of mitochondrial ATP synthase or sphingolipid activator proteins A and D in lysosomes of most cells. In contrast to classical LSDs that are caused by dysfunctional lysosomal enzymes or transporters, resulting in the notable lysosomal accumulation of undegraded substrates or metabolites, respectively [1], the rather heterogenous storage material in NCLs is not clearly disease-specific. Therefore, the determination of the primary substrates/metabolites possibly accumulating in NCLs may first require the elucidation of the functions of NCL-causing gene products. The onset of clinical symptoms and subsequent classification range from prenatal/perinatal, infantile and juvenile to adult forms of the diseases [2]. However, the clinical features of dementia, physical deterioration, seizures and visual failure, that eventually lead to early death and blindness, are common to all NCLs [3]. Thirteen genetically distinct NCL variants, categorized by age of onset and pathological features, have been identified. NCL-causing mutated proteins (CLN1 to CLN14) represent soluble lysosomal enzymes, polytopic membrane proteins localized in lysosomes or in the ER, or synaptic vesicle associated proteins (Table 1). Since the function of most of the CLN proteins or their natural substrates are unknown, system biology approaches including genome-wide analyses, proteomics as well as bioinformatics have been applied in studies on cultured patient cells or various animal models of NCLs that revealed new and unpredicted interactions with CLN proteins and improved understanding of the molecular mechanisms of NCL diseases. The availability of mouse models for many NCL diseases (reviewed by C. Russell et al., this issue) also enabled research on experimental therapies such as gene or small molecule-based therapies.

This review summarizes our current knowledge on the expression, protein-chemical properties and post-translational modifications, the intracellular transport, subcellular localization, proposed function of the NCL-causing gene products, and their interactions with other proteins. The reader is also referred to recent reviews [4–9], and to other reviews in this issue (by Mink et al.; Williams et al., Kousi et al.) summarizing clinical courses, nomenclature and genetic variability in NCL diseases, experimental approaches to define the functions of NCL gene products, and the development of various model organisms to gain insight into pathogenic mechanisms of NCL diseases.

☆ This article is part of a Special Issue entitled: The Neuronal Ceroid Lipofuscinoses or Batten Disease.

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2. CLN1 (PPT1)

2.1. CLN1/PPT1 gene and protein expression

CLN1 encodes palmitoyl protein thioesterase 1, PPT1 [10], an enzyme that removes palmitate groups from *S*-acylated proteins [11]. The *CLN1* transcript is ubiquitously expressed [12–15]. The abundance of *CLN1* mRNA in several regions of human and rodent brain is developmentally regulated starting early in embryonic life [13,16,17]. The nascent PPT1 polypeptide contains 306 amino acids, including a 25 amino acid signal sequence which is co-translationally cleaved [12]. Mature PPT1 migrates as a ~37/35 kDa doublet, and is *N*-glycosylated at N197, N212, N232 in a manner essential for the activity, stability, and trafficking of the protein [12,18–20]. The crystal structure of bovine PPT1, which is 94% identical to the human homologue, shows the globular monomeric structure with classical α/β serine hydrolase fold and a catalytic triad composed of S115, H289, and D233 [18]. Recently, a structural basis for the effect of *CLN1* mutants was constructed showing that mutations associated with a total loss of enzymatic activity affect the core region of the enzyme, whereas less severe mutations are localized at the surface of PPT1 [21]. Whereas native substrates of PPT1-mediated depalmitoylation activity are unknown, several *in vitro* targets have been reported, including H-Ras, palmitoyl-CoA, α subunits of heterotrimeric G proteins, neuron-specific GAP43 and rhodopsin [11,22,23]. The expression of PPT1 in mouse brain preparations and cultured neurons parallels with the abundance of presynaptic marker protein and indicates a role for PPT1 in synaptogenesis [13,24]. Studies on the intracellular targeting of PPT1 and mannose 6-phosphate proteome analyses suggested that PPT1 is targeted to lysosomal compartments *via* a mannose 6-phosphate receptor-mediated pathway [23,25–27]. However, several findings indicate that the localization and functional activity of PPT1 is not limited to lysosomes, especially in neurons. First, enzymatic activity of PPT1 shows a substrate-dependent neutral pH optimum unusual for lysosomal enzymes which may suggest functionality in extralysosomal compartments [22]. Second, PPT1 has been demonstrated to partly associate with lipid raft domains [28] and third, both overexpressed and endogenous PPT1 have been observed in vesicular structures in the soma, dendrites, and axonal varicosities [13,24,29–32]. The localization of PPT1 in synaptic vesicles, however, is a matter of debate [24,29,31,33]. The difference in processing and trafficking of PPT1 between non-neuronal and neuronal cell types further highlights putative distinct functions of PPT1 in neurons [20]. Interestingly, a substantial amount of overexpressed PPT1 is secreted in neural and extraneural cells, and therefore, it has been speculated that the protein may also have substrates/functions in extracellular space [12,29].

2.2. CLN1/PPT1 protein function and interactome

Early changes in the pathogenesis in Ppt1$^{-/-}$ mice involve breakdown of axons and synapses [34], and PPT1 has been implicated in the recycling of synaptic vesicles. This was based on the observations of a reduced total and readily releasable synaptic vesicle pool size, and persistent membrane anchorage of the palmitoylated presynaptic proteins SNAP-25, VAMP-2, and syntaxin 1 in cultured cortical neurons and brain specimens of Ppt1$^{-/-}$ mice, and post-mortem brain samples of CLN1 disease patients [30,33]. The expression and co-localization of PPT1 with a glutamate receptor subunit, NMDAR2B, was enhanced after kainic acid-induced excitotoxicity suggesting a role for PPT1 in synaptic plasticity and protection from excitotoxicity [32]. Furthermore, altered sensitivity of cultured Ppt1$^{-/-}$ neurons to excitotoxicity caused by AMPA- and NMDA-treatments have been reported, and link PPT1 to glutamate receptor functions [35]. However, no fundamental alterations in the electrophysiological properties of Ppt1$^{-/-}$ and Ppt1$^{\Delta ex4}$-derived neurons have been observed, except a progressive decrease in the frequency of miniature synaptic currents in Ppt1$^{-/-}$ mice [33,36]. Additionally, studies with *Drosophila melanogaster* have further contributed to defining the role of PPT1 in axonal and synaptic compartments. Ppt1$^{-/-}$-fly shows reduced lifespan but no apparent neurodegenerative phenotype in adulthood [37]. However, analyses on Ppt1-deficient fly embryos suggest that *Drosophila* Ppt1 is required for proper neuronal development, including processes of neuronal cell fates and organization, and axon guidance [38]. Furthermore, loss-of-function and gain-of-function modifier screens for genes interacting with *Ppt1* have implied a connection between Ppt1 and endosomal/synaptosomal trafficking, and synaptic growth, as well as in lipid metabolism and intracellular signaling [39–41]. In addition, a genetic interaction between Ppt1 and Psd, a protein involved in autophagosomal clearance of accumulated rhodopsin, an *in vitro* substrate of PPT1, has been observed in *D. melanogaster* [42].

Phenotypic analysis of *Schizosaccharomyces pombe* deficient for Pdf1, a yeast orthologue of PPT1, suggests a function for PPT1 in modulation of lysosomal/vacuolar protein sorting and pH [43] which is supported by increased lysosomal pH values measured in fibroblasts of Finnish CLN1 patients [44]. Furthermore, trafficking of endocytosed material in Finnish CLN1 patient fibroblasts was observed to be defective at the level of late endosomes/lysosomes [45].

Several studies found that changes in the levels of PPT1 expression correlated with the activation of caspase-mediated apoptotic pathways in neuroblastoma cells and lymphoblasts [46–48], which appear to be caused by ER and oxidative stress [49–51]. More recently, PPT1 has been suggested to function in negative regulation of tumor necrosis factor (TNF)-induced pathway of apoptosis possibly *via* modulating the specific membrane domain association of TNF receptors *via* depalmitoylation [52].

Abnormalities in the number, intracellular localization pattern and morphology of mitochondria, as well as defects in the mitochondrial enzyme activities and adaptive energy metabolism have been observed in patient fibroblasts, sheep, Ppt1$^{-/-}$ mice, and *Caenorhabditis elegans* [53–56] which could not be confirmed, however, in Ppt1$^{\Delta ex4}$ mice [57].

PPT1 has been repeatedly associated with lipid metabolism. Post-mortem brain samples of CLN1 disease patients revealed changes in phospholipid content. Severe loss of phospholipids due to neuron loss and demyelination were shown to be accompanied by abnormalities in remaining phospholipid content [58]. Ceramide levels of lipid rafts have been reported to be decreased in PPT1 overexpressing CHO cells most likely due to the fact that PPT1 is involved in the processing of saposin D, a storage component in CLN1 disease, and involved in ceramide catabolism [28,45]. Additionally, an increase in cholesterol biosynthesis and a direct link to apolipoprotein A-I metabolism have been observed in Ppt1$^{\Delta ex4}$ mice [36,57]. PPT1 interacts with and modulates the cell surface levels of F_1 complex of ATP synthase, a protein originally found in the mitochondria but recently also shown to act as a receptor for apolipoprotein A-I/HDL at the plasma membrane [59]. Furthermore, Ppt1-deficient mouse cortical neurons showed increased amounts of F_1 complex at the plasma membrane and consistently, increased apolipoprotein A-I uptake accompanied by abnormalities in serum lipid/lipoprotein profiles of Ppt1$^{\Delta ex4}$ mice [57]. Further analyses are required to determine the localization of this PPT1 interaction.

3. CLN2

3.1. CLN2/TPP1 gene and protein expression

The *CLN2* gene encodes the tripeptidyl peptidase 1 (TPP1, CLN2), originally identified as an abundant 46 kDa mannose 6-phosphorylated protein that was absent in the brain specimens from late infantile NCL patients [60]. The *CLN2* gene is expressed ubiquitously in human, rat and mouse tissues and is developmentally regulated [61–64]. TPP1 is a lysosomal serine protease that removes tripeptides from the N-terminus of small polypeptides [65]. It is synthesized as an inactive 66/67 kDa precursor protein consisting of a 19 amino acid signal peptide, a 176 amino acid prosegment and a 368 amino acid catalytically active