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Review

Functional biology of the neuronal ceroid lipofuscinoses (NCL) proteins

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Abstract

Neuronal ceroid lipofucinoses (NCLs) are a group of severe neurodegenerative disorders characterized by accumulation of autofluorescent ceroid lipopigment in patients' cells. The different forms of NCL share many similar pathological features but result from mutations in different genes. The genes affected in NCLs encode both soluble and transmembrane proteins and are localized to ER or to the endosomes/lysosomes. Due to selective vulnerability of the central nervous system in the NCL disorders, the corresponding proteins are proposed to have important, tissue specific roles in the brain. The pathological similarities of the different NCLs have led not only to the grouping of these disorders but also to suggestion that the NCL proteins function in the same biological pathway. Despite extensive research, including the development of several model organisms for NCLs and establishment of high-throughput techniques, the precise biological function of many of the NCL proteins has remained elusive. The aim of this review is to summarize the current knowledge of the functions, or proposed functions, of the different NCL proteins.

Keywords: Batten disease; Lysosomal degradation; NCL; Neurodegeneration; Storage disease

1. Introduction

Neuronal ceroid lipofucinoses (NCLs) are a group of severe neurodegenerative disorders affecting up to 1: 12,500 live births worldwide. Although certain forms of NCL are rare and concentrated regionally, as a whole the NCLs are considered to be among the most common hereditary dementing illnesses of childhood. NCL disorders are characterized by massive accumulation of autofluorescent lipopigment in patients' tissues. Lysosomal inclusions are seen as granular osmiophilic deposits (GRODs), curvilinear, fingerprint, or rectilinear profiles upon electron microscopic analysis. The central nervous system is the most dramatically affected organ; however, the accumulation of the autofluorescent material is ubiquitous, and in most forms of NCL subunit c of mitochondrial ATP synthase constitutes a large part of the proteinaceous component of storage bodies. NCL brains show massive neuronal cell atrophy and neuronal cell loss (reviewed in [1–3]). Clinical symptoms of NCLs include loss of vision, epilepsy, progressive mental retardation and a reduced lifespan.

NCL disorders result from mutations in different CLN genes (CLN1–CLN9). Six of these genes have been cloned (CLN1–3, 5, 6, and 8) and these encode both soluble and transmembrane proteins located in either endosomes/lysosomes or the ER (reviewed in [4]). (CLN4, which encodes the autosomal dominant form of adult NCL, CLN7, a Turkish variant late infantile NCL, and CLN9, found in Serbian and German patients, have yet to be identified). More recently, the group of NCL disorders has been expanded to encompass disorders or protein deficiencies with highly similar pathological findings to "classical" human NCLs. These include deficiencies of the lysosomal aspartyl proteinase cathepsin D [5–9] and other cathepsins [10–12]. Defects in members of the ClC chloride channel family have also been shown to share pathological findings characteristic of NCLs [13,14].

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The precise function of the all NCL proteins is currently unknown. The heterogeneity of the NCL proteins and diversity in their subcellular localizations suggests that they may play roles in a common functional pathway especially important in neuronal cells. Cell biological studies, characterization of model organisms, and development of high throughput screening methods have shed light on potentially important functional pathways. The results of these studies are discussed in this review.

1.1. CLN1 (Palmitoyl-protein thioesterase)

The infantile form of NCL (CLN1 disease) is caused by severe deficiency in the lysosomal enzyme, palmitoyl protein thioesterase (PPT1) [15]. PPT1 is an enzyme that removes fatty acids in covalent linkage to cysteine residues in proteins [16,17], ensuring the eventual metabolic disposal of S-acylated cysteine residues during lysosomal degradation [18,19]. A role for PPT1 in the fatty acylation cycle of cytosolic signaling molecules has not been convincingly demonstrated; this function appears to be performed by another enzyme, acyl-protein thioesterase (APT) [20]. A homolog of PPT1, PPT2, is a lysosomal fatty acyl thioesterase that hydrolyzes palmitoyl-CoA but not palmitoyl-cysteine [21,22]. No case of human PPT2 deficiency has been described, although deficiency in PPT2 causes an NCL-like disorder with unusual visceral features in mice [23].

The fatty acyl thioesterase activity of PPT1 was first described in relation to its ability to cleave palmitate from a model palmitoylated substrate, H-Ras. The enzyme was purified by classical techniques from bovine brain (33,000-fold) using [^{3}H]palmitoyl H-Ras as a substrate [16,24]. [^{3}H]palmitate was shown to be a product of the cleavage reaction. The enzyme is effectively inhibited by the histidine-modifying reagent, diethyl pyrocarbonate, but not by phenylmethanesulfonyl fluoride (PMSF), which modifies serine. Resistance to PMSF was later shown to be due to steric hindrance around the active site serine, as PPT1 is a serine hydrolase [25]. The enzyme is stabilized by EDTA and inactivated by heavy metal contamination of buffers, presumably due to the presence of a vicinal disulfide bridge critical for the structural integrity of the enzyme. In addition to palmitoylated Ras, the enzyme was shown to remove palmitate from the subunits of modified Gα proteins, a number of other proteins, and from palmitoyl-CoA. No phospholipase C, phospholipase A2, triglyceride or diglyceride lipase activity was detected [16].

PPT1 is encoded by a 25-kb gene containing nine exons on human chromosome 1p32, which produces a single 2.5 kb messenger RNA [26]. The encoded protein contains a signal peptide, which is cleaved co-translationally. The mature protein migrates as a 37/35-kDa doublet and is reduced to 31-kDa upon deglycosylation [27]. The amino terminal sequence of the purified native protein, Asp–Pro–Pro–Ala–Pro… differs from the amino terminus of the recombinant protein produced in Sf9 cells (His–Leu–Asp–Pro–Pro–Ala–Pro…), indicating the action of dipeptidyl aminopeptidase during processing in the lysosome [16,24]. PPT1 contains three asparagine-linked glycosylation sites, all of which are utilized and contribute to enzyme activity and/or stability [28]. Lysosomal targeting of the enzyme through the classical mannose 6-phosphate receptor pathway has been demonstrated in peripheral tissue [27,29,30] and the enzyme has been identified in a proteomic study of mannose 6-phosphate binding proteins in brain [31]. Proteomic studies have also identified PPT1 as a prominent component of placental lysosomes [32] and macrophage phagosomes [33].

The X-ray crystallographic structure of PPT1 has been determined [28]. The enzyme is a classical α/β serine hydrolase consisting of two major domains with a fatty acid binding groove down the center of the enzyme. The catalytic triad consists of Ser115, Asp233, and His289. The structure of the covalent fatty acyl-enzyme intermediate shows the palmitate bound in an extended conformation along a hydrophobic groove in the second domain of PPT1. There is no evidence for a movable lid that regulates the interfacial behavior of many lipases [34].

The role of PPT1 in the metabolism of lipid-modified proteins has been demonstrated through metabolic labeling studies [18,19]. Cell lines from patients deficient in PPT1 were labeled with [^{35}S]cysteine and small lipophilic compounds migrating as small peptides were demonstrated by thin-layer chromatography. The formation of these compounds was blocked by protein synthesis inhibitors and by inhibitors of lysosomal metabolism, demonstrating that they were derived from lipid-modified proteins in the process of lysosomal degradation.

How PPT1 deficiency leads to selective central nervous system degeneration is unclear. The storage material is widespread, and virtually every organ displays storage material. This suggests a selective vulnerability of neurons, presumably enhanced by the post-mitotic nature of neurons and their limited turnover. Transcript expression profiling (microarray) studies on whole brain indicate up-regulation of genes associated with the inflammatory response in INCL [35] and in other lysosomal storage disorders affecting the brain [36–39]. It is conceivable that signals produced by distressed post-mitotic cells (neurons) provoke this inflammatory response, but the nature of such signals remains to be determined. Of note, not all neurons in the central nervous system may be equally vulnerable. Detailed studies of neuronal loss in PPT1 knockout mice have shown selective drop-out of GABAergic interneurons of the hippocampus early in the course of the disorder [40].

Saposins A and D have been identified as major protein components of the granular osmiophilic bodies [41], but neither protein is a substrate for PPT, as they are not S-acylated. In addition, saposin D accumulates to a high degree (10–20-fold) when normal lymphoblasts are cultured in the presence of lysosomotropic aminothiols [42], suggesting that saposin accumulation may be an interesting but secondary marker for lysosomal dysfunction.

Selective apoptosis of neurons has been observed pathologically in humans and in animal models of INCL, prompting studies of the effect of PPT on apoptosis in cell culture models [43–47]. These studies have shown a tendency for decreased PPT activity to correlate with increased sensitivity to apoptosis induced by selected agents, particularly C2-ceramide, whereas overexpression of PPT was associated with decreased sensitivity. The conclusions that can be drawn from these studies are limited by the use of highly overexpressed, tagged versions of PPT (with concerns about correct localization), use of