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Review

TRPML and lysosomal function

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Abstract

Mucolipin 1 (MLN1), also known as TRPML1, is a member of the mucolipin family. The mucolipins are the only lysosomal proteins within the TRP superfamily. Mutations in the gene coding for TRPML1 result in a lysosomal storage disorder (LSD). This review summarizes the current knowledge related to this protein and the rest of the mucolipin family.

Keywords: Lysosomal storage disorder; Mucolipin protein family; Endocytosis and lysosomal function; Mucolipidosis

1. Introduction

TRPMLs are encoded by the MCOLN genes. TRPML1 is encoded by MCOLN1 localized in humans at chromosome 19p13.2–13.3 while the 2 other genes—MCOLN2 and MCOLN3 are localized at chromosome 1p22.3 encoding TRPML2 and TRPML3 respectively. MCOLN1 is mutated in humans in a lysosomal storage disorder—Mucolipidosis type IV (MLIV) (MIM #252650), an autosomal recessive, neurodegenerative disease characterized by severe psychomotor retardation and vision impairment due to retinal degeneration and cornea opacity (Reviewed by [1–3]). Achlorhydria and iron deficiency are also part of the clinical symptoms [4,5]. Unlike most other lysosomal disorders MLIV progresses extremely slowly, thus patient ages range from 1 year to the late thirties and forties and life expectancy is not clear yet. Milder patients, particularly in the psychomotor impairment, were also reported [6–8].

MLIV is found in increased frequency among Ashkenazi Jews (AJ) with a heterozygote frequency of 1:100 [9,10]. Over 15 mutations were identified in MLIV patients, Jews and non-Jews, including two MCOLN1 founder mutations in the AJ population comprising 95% of the mutated alleles among AJ MLIV patients [3]. These founder mutations date approximately 40 generation ago [11].

The identification of MCOLN1 mutations opened the door for accurate patient diagnosis as well as prenatal diagnosis and heterozygote identification. Lately MLIV is included in the population screening program operated in various countries with high concentrations of AJ in an attempt to ascertain high risk couples, in which both parents are heterozygotes and offer them options for family planning (prenatal diagnosis, marriage decisions), as a preventative program to reduce the number of newly born MLIV patients in the high risk population [12].

1.1. Lysosomal storage

MLIV is classified as a mucolipidosis due to the simultaneous lysosomal accumulation of lipids together with water soluble substances [13]. This characteristic heterogeneous storage is observed in cells of every tissue and organ of MLIV patients and principally best demonstrated by electron microscopy [14–18]. The storage material in MLIV is autofluorescent [19]. This typical finding is also present in cultured amniotic fluid cells obtained from MLIV affected fetuses and thus this technique was used for prenatal diagnosis purposes in the era preceding the gene discovery [20,21]. Chemical analysis led to the identification of the stored substances as a variety of lipids namely, gangliosides, phospholipids and neutral lipids while the water soluble materials included primarily mucopolysaccharides and glyco-

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proteins [2,3]. Histochemical analysis indicated that gangliosides were predominately stored in neural tissues while phospholipids were mostly accumulating in visceral organs [22]. It should be noted that despite the early onset of the disease in early infancy and the clear evidence of the characteristic lysosomal storage in these early ages, the fact that the disease remains in an apparent steady state or very slow deterioration for at least 2–3 decades may suggest that the storage is not as massive and progressive as is found in most other lysosomal storage disorders.

What leads to the storage in MLIV? Earlier studies indicated that the heterogeneous storage does not stem from the impairment or deficiency of a lysosomal hydrolase that could account for this phenomenon [23]. Studies in cultured fibroblasts with radiolabeled compounds such as phosphatidylcholine or gangliosides, which constitute the bulk of the storage in MLIV, or with fluorescent lipid analogs, indicated that the abnormal accumulation results from a defect in the late stages of the endocytosis process of membrane compounds leading to the impairment of lysosomal biogenesis or, alternatively, a defect in the normal maintenance of the mature lysosomes [23–26]. It should be pointed out that another LSD, namely, Niemann–Pick disease type C (NPC) (MIM #257220) [27] is also suggested to be involved in defective vesicular trafficking of late endosomes, although with different cellular and biochemical characteristics [28–30], thus, these two disorders (MLIV and NPC) constitute a distinct group within the LSD of endosomal/lysosomal trafficking impairment leading to defective lysosomal biogenesis or function.

Catabolism of the accumulated materials in MLIV still does occur in the storage vacuoles (early lysosomes? Multivesicular bodies?) of MLIV patients, though in an altered rate and pattern (for instance; degradation products are not exported normally from these organelles to the Golgi apparatus) [24,26]. This helps to explain the clinical manifestations of this disease, as outlined above; since despite the early onset of the disease and severe neurological involvement, MLIV patients remain in an apparent steady state for decades. This may indicate that indeed the slower catabolism rate prevents a massive storage as is the case in most other LSD, and hence, the extremely slow progression of the clinical picture in these patients.

1.2. Genetics

The gene MCOLN1 was identified by linkage studies [31–33] following its mapping to human chromosome 19p13.2–13.3 [34]. The identification of this gene triggered the identification of two other homologous genes, namely, MCOLN2 and MCOLN3, which mapped to chromosome 1p22.3. The MCOLN genes code for TRPML proteins that show about 60% amino acid homology to each other. The identification of the human genes lead to the identification of similar genes in mice, *C. elegans* and *Drosophila*. Over 15 different MCOLN1 mutations have been identified in MLIV patients, including, partial gene deletions, insertions, splicing, missense and nonsense mutations [3]. It should be noted that most missense mutations are located in the putative transmembrane domains. The clinical severity among the various patients is very similar regardless the nature of the mutations; but one in-frame deletion mutation of amino-acid No. 408—phenylalanine, is found in a mild patient in compound heterozygosity with a splicing mutation [3]. Except for AJ, MLIV is considered to be rare in other populations.

Unlike MCOLN1 there are no reports associating MCOLN2 or MCOLN3 with human diseases. Nevertheless, spontaneous mutations in MCOLN3 in Varitint–Waddler mice were shown to be associated with early deafness, vestibula defects, pigmentation abnormality and perinatal lethality [35]. At present no human counterpart was reported. Mutations in a mucolipin-like gene in *C. elegans* — CUP5 was reported to be associated with general lysosomal storage phenomena of a similar nature as is present in cells of MLIV patients, as well as increased cell death leading to embryonic lethality [36,37]. Recent reports have linked the embryonic lethality in CUP5-null *C. elegans* to starvation of embryonic cells and general developmental defects [38]. These observations were suggested to stem from defective endocytosis of nutrients and the general accumulation of a genetic suppressor, the ATP-binding cassette (ABC) transporter MRP-4, in CUP5-null cells [38,39].

Abnormal mitochondrial function due to increased mitochondrial fragmentation is observed in MLIV cultured fibroblasts, as well as in cell lines from other lysosomal storage disorders, as a result of inefficient autophagolysosomal recycling of mitochondria [40]. This leads to decreased Ca^{2+} buffering capacity in MLIV cells which increases susceptibility to a caspase-8-dependent apoptotic pathway. This phenomenon might also explain the increased sensitivity to apoptosis in *C. elegans* as described above. The functional link between mutations in the MCOLN1 gene, mitochondrial aberrations and the clinical manifestations in MLIV still remains to be seen.

1.3. Mucolipins and subcellular localization

Three proteins are encoded by the MCOLN genes, namely, TRPML1, TRPML2, and TRPML3 also referred as MLN1–3 or Mucolipin 1–3, respectively. Following the identification of the relevant genes the putative structure of the protein products was deduced by computerized analysis [31]. This indicated that TRPML1 is 580 amino acids long consisting of 6 transmembrane domains which attribute this protein to the TRP superfamily [41,42], although it does not contain ankirin domains. The two other TRPML proteins have similar structure.

The physiological function of the mucolipins has not yet been fully elucidated, but electrophysiological analyses indicate that they function as cation channels. This was particularly demonstrated in TRPML1 channels. Based on previous findings (see above) it is expected that TRPML1 function would be related to the biogenesis of lysosomes or their normal maintenance. Indeed, studies with expression vectors and immunohistochemistry demonstrated that TRPML1 is located to lysosomal vacuoles [26,43–47].

Site directed mutagenesis indicated the role of two dileucine motifs, one at the N-terminal and the second at the C-terminal tail of TRPML1, as subcellular targeting motives. Experiments