MLN64 and MENTHO, two mediators of endosomal cholesterol transport

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Abstract

MLN64 (metastatic lymph node 64) and MENTHO (MLN64 N-terminal homologue) are two late-endosomal proteins that share a conserved region of four transmembrane helices with three short intervening loops called the MENTAL domain (MLN64 N-terminal domain). This domain mediates MLN64 and MENTHO and hetero-interactions, targets both proteins to late endosomes and binds cholesterol in vivo. In addition to the MENTAL domain, MLN64 contains a cholesterol-specific START domain [STAR (steroidogenic acute regulatory protein)-related lipid transfer domain]. The START domain is a protein module of approx. 210 residues that binds lipids, including sterols, and is present in 15 distinct proteins in mammals. Thus MLN64 and MENTHO define discrete cholesterol-containing subdomains within the membrane of late endosomes where they may function in cholesterol transport. The MENTAL domain might serve to maintain cholesterol at the membrane of late endosomes prior to its shuttle to cytoplasmic acceptor(s) through the START domain.

Introduction

Cholesterol is a major biological component; it is an essential constituent of biological membranes and a precursor for steroid hormones and bile acids. At the membrane, cholesterol is involved in the formation of lipid rafts and caveolae implicated in a variety of processes including lipid sorting, protein trafficking and signal transduction [1]. Cholesterol also covalently modifies proteins involved in developmental processes such as the morphogen proteins from the hedgehog family [2].

Homoeostasis of cellular cholesterol is an essential process as its imbalance may lead to different pathologies such as inherited lysosomal storage diseases and atherosclerosis [3]. Cellular cholesterol content is the result of an equilibrium between de novo synthesis using the acetyl-CoA pathway in the endoplasmic reticulum, salvage through circulating lipoproteins through the LDL (low-density lipoprotein) pathway and efflux to circulating HDL (high-density lipoprotein). Efflux via the HDL, or reverse cholesterol transport, is a physiological process that allows the transport of cholesterol from peripheral tissues to the liver for its subsequent elimination into bile acids [4]. Cellular cholesterol homoeostasis requires a variety of intracellular cholesterol movements through vesicular and non-vesicular mechanisms [5,6]. The endo/lysosomal system plays a central role in cholesterol uptake from LDL particles. Indeed, on binding to their receptors, circulating LDLs are endocytosed at the plasma membrane. LDLs are released from their receptors in endosomes and cholesteryl esters are hydrolysed into free cholesterol that is redistributed from late endosomes and lysosomes to different cellular compartments [7].

We have isolated two genes coding for related proteins from late endosomes potentially involved in cellular cholesterol homoeostasis: MLN64 (metastatic lymph node 64) and MENTHO (MLN64 N-terminal homologue) (Figure 1). MLN64 also known as STARD3 (StAR (steroidogenic acute regulatory protein)-domain-containing protein) was identified as a gene overexpressed in malignant breast tumours [8]. It is overexpressed in approx. 25% of breast cancers [9–15]. MLN64 contains a cytoplasmic C-terminal START domain (StAR-related lipid transfer domain) and a conserved N-terminal region (Figure 1). This conserved domain is also present in MENTHO (also called STARD3 N-terminal-like protein) [16].

MLN64 and MENTHO are two late endosome resident proteins

Subcellular localization studies have shown that MLN64 partially co-localizes with markers associated with early, recycling, sorting and late endosomes and with lysosomes [17]. A complete co-localization of MLN64 was found with LBPA (lysobisphosphatidic acid), a marker of late endosomes [18], showing that MLN64 is a resident protein of late endosomes. Similarly MENTHO co-localizes with MLN64 (Figure 1) and other late-endosomal proteins such as the Niemann–Pick type C1 protein [16].

MLN64 and MENTHO share a conserved domain named the MENTAL domain (MLN64 N-terminal domain) composed of four transmembrane helices with three short

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Abbreviations used: HDL, high-density lipoprotein; LDL, low-density lipoprotein; MLN64, metastatic lymph node 64; MENTAL domain, MLN64 N-terminal domain; MENTHO, MLN64 N-terminal homologue; STARD, StAR-related lipid transfer domain; START, StAR-related lipid transfer domain.

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Figure 1 | MLN64 and MENTHO: two late endosomal proteins

(A) Schematic representation of MLN64 and MENTHO proteins. The START and MENTAL domains are in light and dark grey respectively. Transmembrane helices within the MENTAL domain are in dark grey. Numbers indicate the amino acid positions. (B) MLN64 and MENTHO are two late-endosomal proteins. CHO cells (Chinese-hamster ovary cells) transfected with expression vectors encoding MENTHO and MLN64 were fixed, permeabilized and then incubated with anti-MLN64 (green) and anti-MENTHO (red) antibodies. Images were obtained using a confocal microscope. Overlay together with nuclei counterstained with Hoescht-33258 dye (blue) is shown in the right-hand panel. The yellow staining indicates co-localization of MENTHO and MLN64 in late-endosomal structures.

MLN64 and MENTHO are cholesterol-binding proteins

The C-terminal half of MLN64 contains a START domain exposed to the cytoplasm [17]. This domain is a protein module of approx. 210 residues that binds lipids, including sterols, which is evolutionarily conserved in plants and animals [22–24]. In humans, 15 proteins have been found to contain a START domain, either alone or in association with other domains. The ligands of StAR/MLN64/STARD5, STARD5, PCTP (phosphatidylcholine-transfer protein)/STARD10, STARD10 and CERT (ceramide-transport protein) are cholesterol, 25-hydroxycholesterol, phosphatidylcholine, phosphatidylethanolamine and ceramides respectively [23]. StAR, MLN64, STARD4, STARD5 and STARD6 are closely related. StAR (also known as STARD1) and MLN64 belong to the STARD1 subfamily, while STARD4, STARD5 and STARD6 belong to the STARD4 subfamily. StAR is a well-characterized START protein that regulates the limiting step in steroid hormone production by mobilizing cholesterol to mitochondrial membranes [25]. Patients with StAR deficiency have an autosomal recessive disease named lipid congenital adrenal hyperplasia, which is characterized by impaired steroidogenesis and subsequent accumulation of cholesterol in the gonads and adrenals [26]. In cultured cells, forced expression of a mutant MLN64 containing only the START domain induces steroid synthesis, while the wild-type protein has negligible activity [27]. Similarly, forced expression of STARD4 and STARD5 promotes steroidogenesis in vitro [28].

The crystal structure of the MLN64 START domain has been solved [29]. The three-dimensional organization of this domain forms a hydrophobic inner tunnel wide enough to accommodate one molecule of cholesterol. Indeed, titration experiments have shown that the START domain of MLN64 binds cholesterol at a 1:1 ratio but not cholesteryl ester [29]. Moreover, two residues lining the tunnel wall of this domain (Met27 and Asn11) are required for in vitro interaction with photocholesterol, a photoactivatable and radiolabelled cholesterol [19,30].

To evaluate whether the MENTAL domain could be implicated in the handling of cholesterol by MENTHO and MLN64, the cholesterol binding properties of the MENTAL domain of MLN64 were studied in vivo using photocholesterol [20]. We showed that the MENTAL domain of MLN64 binds cholesterol.

The START domains within the STARD1 and STARD4 subfamilies are closely related, suggesting that StAR, MLN64, STARD4, STARD5 and STARD6 are probably cholesterol-specific START proteins and may share a common function in cholesterol transport and steroidogenesis. However, a mouse model in which the START domain of MLN64 was selectively removed showed no obvious phenotype, indicating that MLN64 is not critical for steroidogenesis [31]. START-containing proteins are less represented in flies than in mammals [24]. Among the STARD1 and STARD4 subfamilies protein members, only MLN64 has a counterpart in Drosophila.
melanogaster named DmStart1 that contains both a MENTAL and a START domain [32]. DmStart1 is highly expressed in prothoracic gland cells where ecdysobioids are synthesized from cholesterol. Its expression pattern correlates with the larval stage humoral ecdysone titre wave, suggesting that Start1 functions in cholesterol transport during ecdysiosis [32]. Intriguingly, the silkworm Bombyx mori possesses a Start1 counterpart that is highly similar to MLN64 [33]. By alternative splicing, the BmStart1 gene generates two differently expressed transcripts encoding BmStart1 and a shorter isoform lacking the MENTAL domain named CBP (carotinoid-binding protein) highly similar to the StAR protein [33]. These findings illustrate the central and conserved role for the START domain whose function might be further modulated by the presence of additional protein motifs and by its expression pattern.

**Potential mode of action of MLN64 and MENTHO**

Given their position in discrete cholesterol-rich subregions within the membrane of late endosomes, MLN64 and MENTHO are potentially involved in cholesterol exit from this organelle. MLN64 and MENTHO may capture cholesterol via their MENTAL domain in the late-endosomal membranes, and then cholesterol could be transferred to the cytoplasmic START domain of MLN64 to a cytosolic acceptor protein or membrane. Accordingly, the START domain in MLN64 has been shown to transfer cholesterol from donor to acceptor vesicles or from liposomes to acceptor mitochondrial membranes [21,34]. This mechanism may occur at intracellular contact sites between different membranes [5]. Indeed, it has been shown that MLN64-containing late-endosomal tubules align in a parallel way to StAR-labelled mitochondria and transiently come into contact with them [21]. Thus the START domain of MLN64 could transfer cholesterol from one membrane to the other, at the contact site, by a flippering mechanism to allow a rapid and efficient transfer of several cholesterol molecules.

MENTHO, MLN64 and other cholesterol START-specific proteins may act in concert during intracellular movement of cholesterol. The START domain acts as a shield to protect cholesterol from a hydrophilic environment during non-vesicular transfer. The exact nature of the acceptor site of cholesterol transfer mediated by these proteins, as well as the molecular mechanisms involved, should be further explored.

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**References**


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