STARTing to understand MLN64 function in cholesterol transport[1]

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In most cell types, intracellular transport mechanisms that mediate cholesterol movement into the mitochondrial outer and inner membranes are not well understood. By contrast in steroidogenic tissues, the steroidogenic acute regulatory (StAR) protein in conjunction with the benzodiazepine receptor have been defined as key mediators of cholesterol flux toward the mitochondrial inner membrane (1, 2). Furthermore, StAR-mediated cholesterol transfer is critical for steroid synthesis, and deficiencies in this protein are responsible for the rare but severe disorder of human steroidogenesis known as congenital lipoid adrenal hyperplasia (1, 2).

A family of genes has now been identified based upon predicted structural homology to the StAR protein (3). Each protein contains a ~245 amino acid StAR-related lipid transfer (START) domain. The mammalian genome contains 15 START domain proteins (StARD1–StARD15) with StARD1 being a synonym for StAR (4). Considering that five START domain proteins have been demonstrated to bind cholesterol (1, 2), this opens the possibility that some members of this protein family may be involved in cholesterol transport to the mitochondria in nonsteroidogenic cells in which they are expressed. If true, START domain proteins could mediate the synthesis of additional cholesterol-derived products such as oxysterols, neurosteroids, and bile acids.

MLN64 (a.k.a. StARD3) exhibits some features suggesting its role in mitochondrial cholesterol delivery. It contains an N-terminal transmembrane domain called MENTAL (MLN64 N-terminal) in addition to its C-terminal START domain, which binds cholesterol at a 1:1 ratio (5). MLN64 was proposed to participate in steroidogenesis in tissues that do not express StAR, such as the placenta (6). However, the full-length protein has negligible prosteroidogenic activity, and only a mutant form containing the isolated START domain significantly promotes steroid hormone synthesis via mitochondrial P450scc (6). Moreover, mice with targeted mutation of the MLN64 START domain appear normal and show no defect in steroidogenesis (7). Interestingly, MLN64 is tethered to the membrane of late endosomes through its MENTAL domain, leaving the C-terminal START domain oriented toward the cytoplasm (8). Considering that the MENTAL domain also binds cholesterol (9), it has been suggested that MLN64 may capture cholesterol that is released from lipoproteins within the endolysosomal compartment and transfer this cholesterol to cytosolic acceptors via its cytoplasmic START domain (2). The localization and topology of MLN64 in late endosomes strongly suggests that this protein participates in intracellular cholesterol traffic delivered by the endocytic pathway for further metabolism in mitochondria. Niemann Pick Type C (NPC) 1, another late endosome transmembrane protein, is also involved in intracellular cholesterol transport, and its deficiency leads to cholesterol accumulation in lysosomes and to the neurodegenerative Niemann Pick type C disease (10). However, a functional relationship between MLN64 and NPC1 protein has yet to be demonstrated.

The paper by Charman et al. (11), "MLN64 mediates egress of cholesterol from endosomes to mitochondria in the absence of functional Niemann-Pick Type C1 protein" in the May, 2010 issue of the *Journal of Lipid Research* nicely demonstrates that MLN64 acts independently of NPC1 in mediating cholesterol transport to the mitochondria. In this study, the authors exploited a classic assay for analyzing cholesterol import into mitochondria that is based on pregnenolone production in Chinese hamster ovary (CHO) cells transfected with a fusion protein containing P450scc. In this manner, they showed that cholesterol transport to the mitochondrial inner membrane was not affected by NPC1 deficiency either using cells harboring NPC1 mutations or cells in which NPC1 was knocked down using siRNA technology. Moreover, Charman et al. (11) demonstrated that cholesterol transport to the mitochondrial inner membrane increased upon exposure of

Supported by FONDECYT grants 1070634 (to A.R.) and 1030415 and 1070622 (to S.Z.), and by the National Institutes of Health grants DK48873 and DK56626 (to D.E.C.). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or other granting agencies.

Manuscript received 28 May 2010.

Published, JLR Papers in Press, May 28, 2010
DOI 10.1194/jlr.E008854

[1] See referenced article, *J. Lipid Res.* 2010, 51:1023–1034

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cholesterol-deprived cells to LDL, indicating preferential transport of endosomal cholesterol to mitochondria under these conditions. Knockdown of MLN64 by siRNA reduced cholesterol transport to the mitochondrial inner membrane in both wild-type and NPC1 deficient cells, demonstrating a significant role for MLN64 in the mitochondrial delivery of cholesterol. Interestingly, mitochondrial cholesterol contents were higher in CHO cells with loss of NPC1 compared with wild-type cells, a finding that is consistent with similar results previously reported in NPC1 neurons and hepatocytes (12–14) and suggests preferential shunting of cholesterol to mitochondria in the absence of a functional NPC1 pathway. Consistent with this possibility, depletion of MLN64 mitigated the increase in mitochondrial cholesterol in NPC1-deficient cells. Taken together, these observations indicate that MNL64-mediated cholesterol transfer is responsible for the accumulation of mitochondrial cholesterol that is observed in NPC disease. This is pathophysiologically relevant because the increase in cholesterol content has been linked to mitochondrial dysfunction characterized by reduced glutathione content, membrane potential, and ATP synthesis (12–14).

The findings by Charman et al. (11) complement our previous report that adenovirus-mediated overexpression of MLN64 induces an increase in hepatic free cholesterol that is associated with apoptosis and liver damage (15). Based on the findings of Charman et al. (11), we hypothesize that the liver damage observed in MLN64-overexpressing mice is mediated, at least in part, by an increase in mitochondrial cholesterol transport. Additional studies will be required to test this hypothesis.

MLN64 is expressed in all tissues (4, 6), suggesting the possibility that it broadly regulates sterol metabolism. In the liver, it may be critical for cholesterol movement into mitochondria in order to initiate the acidic pathway for bile acid synthesis (16). Indeed, two studies (17, 18) have demonstrated that overexpression of StAR, the closest MLN64 homolog, leads to an increase in bile acid synthesis in hepatocytes. By contrast, MLN64 overexpression in primary rat hepatocytes and in mouse liver caused a rather small increase in bile acid synthesis and no change in bile acid pool size and composition (18; Rigotti, Cohen, and Zanlungo, unpublished observations). Clearly, additional studies are required to understand the small effect observed in bile salt synthesis by MLN64 overexpression in hepatocytes. Nevertheless, it is noteworthy that Charman et al. (11) demonstrated that MLN64 depletion in CHO cells inhibited pregnenolone formation by only 30–40%, an indication that MLN64-dependent cholesterol transport is not the only pathway for mitochondrial cholesterol import in these cells. MLN64 may also be relevant for cholesterol transport into mitochondria in other tissues and important for the synthesis of 27-hydroxycholesterol, an oxysterol that regulates cholesterol metabolism and homeostasis in the mammalian brain (19). One potential alternative route for cholesterol transport into hepatic mitochondria is via StARD4, a START domain protein that binds cholesterol and is highly expressed in the liver (2). Overexpression of StARD4 in primary hepatocytes increases bile acid production (20). However, mice with targeted mutation for StARD4 exhibited only modest alterations in hepatic steroid metabolism (21).

The findings of Charman et al. (11) highlight the relevance of cholesterol transport to the mitochondria and suggest that abnormalities in this process may lead to mitochondrial dysfunction as a key pathogenic event underlying several neurodegenerative diseases. In addition to the association between increased mitochondrial cholesterol contents, reduced glutathione and ATP production in NPC1 cells, cholesterol chelation by cyclodextrin treatment of NPC1 mitochondria restores ATP synthesis and mitochondrial function (12). Mitochondrial dysfunction appears to be a key element not only in certain neurodegenerative diseases but also in diseases associated with damage to the liver and heart (22–25). For instance, hepatocytes from leptin-deficient *ob/ob* mice or rats with diet-induced mitochondrial cholesterol accumulation exhibited reduced mitochondrial membrane fluidity and glutathione levels that correlated with increased sensitivity to TNFα-dependent cell death (13). In this connection, we have previously shown increased MLN64 mRNA and protein levels in a murine model of liver damage induced by bile acids (15), suggesting that MLN64-mediated cholesterol transport to mitochondria may underlie bile acid-induced cytotoxicity. In addition, mitochondrial dysfunction is commonly associated with increased generation of reactive oxygen species, induction of apoptosis, inflammation, and fibrosis, depending upon the tissue (22–25). Future studies will be required to determine the pathophysiological relationships between MLN64 expression/activity to abnormal mitochondrial cholesterol transport in human diseases.

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