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Research Highlight

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TMEM41B, a novel ER phospholipid scramblase mediating systemic lipid metabolism

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The phospholipid molecules forming the phospholipid bilayer membrane have difficulties to complete spontaneous trans-bilayer shuttling due to the physicochemical properties and biological functions of the membrane bilayer [1]. However, this transbilayer phospholipid transport is essential in biology. Phospholipid synthesis is concentrated on the membrane of the endoplasmic reticulum (ER) of cells, which occurs only in the monolipid layer on the cytoplasmic side. Therefore, the formation of a complete bilayer membrane requires the phospholipids to be flipped to the other side. Transmembrane translocation of lipids controls transbilayer lipid asymmetry in membranes. ATP-independent scramblases and energy-driven flippases are key factors in maintaining asymmetric phospholipid distribution by promoting transbilayer movement of specific lipids across the cytoplasmic leaflet [2]. ER scramblases promote rapid turnover of lipids and allow them to equilibrate between two membrane leaflets in an ATP-independent manner. Intriguingly, ER transmembrane protein 41B (TMEM41B) was recently identified as a lipid scramblase which mediates transbilayer phospholipid movement and plays a prominent role in maintaining lipoprotein biogenesis and lipid metabolism [3–5]. The ER is considered to initiate protein and lipid synthesis/transport in eukaryotic cells. Many neutral lipids, including phospholipids and cholesterol utilized for lipoprotein assembly, are synthesized in the ER lumen lipoproteins, indicating that there are tissue-specific phospholipid biosynthetic pathways that vigorously produce phospholipids on the rough ER membrane of liver or intestinal cells. One recent study revealed that the transmembrane cargo receptor SURF4 selectively transports hepatic lipid-carrying lipoproteins from the ER to the Golgi through coat protein complex II (COPII)-coated transport vesicles [6]. Synergistic pairing between SURF4 and the molecular switch SAR1B operates a sensitive transport program for hepatic lipoprotein secretion and maintains circulating lipid homeostasis (Figure 1). Furthermore, TMEM41B is detected in a hepatic protein complex of SURF4 and lipoproteins, which is present in the SURF4-mediated ER transport of lipid cargos and implicated in lipid equilibration between the membrane leaflets [3].

The maturation of lipoproteins requires continuous supplementation of neutral lipids and phospholipids across bilayer membranes in the ER or Golgi lumen. Increasing amounts of phospholipids are actively shuttled into the ER or Golgi luminal leaflet to be accessible to the growing lipoproteins and cover its expanding surface. TMEM41B's functionally related protein VMP1 is important for the homeostasis of neutral lipids and lipoproteins in a whole organism [7]. Recently, the ER transmembrane protein TMEM41B was identified as a crucial phospholipid scramblase for regulating the cellular distribution of cholesterol and phosphatidylserine [8]. In addition, a recent study suggested that TMEM41B connects to the COPII cargo receptor SURF4 and apolipoprotein B (APOB)-containing lipoproteins during VLDL lipid transport and acts as the scramblase equilibrating phospholipids between the cytosolic and lumenal ER leaflets (Figure 1) [3]. In other words, TMEM41B catalyzes the flipping of phospholipids into the cytosolic leaflet in an ATP-independent manner. TMEM41B is responsible for the biogenesis of lipoproteins and controls systemic lipid metabolism. Hepatic scramblase deficiency leads to the elimination of plasma lipids because of the complete diminishment of circulating lipoproteins within the ER. The paradoxical activation of lipid production has also been confirmed with hepatic TMEM41B loss despite stalled lipid secretion, leading to rapid nonalcoholic steatohepatitis (NASH) development with the characteristics of liver fibrosis and immune cell infiltration [3]. Downregulation of TMEM41B is prevalent in fatty liver patients, obese mice and rhesus monkeys, while phospholipid scramblase supplementation can alleviate the symptoms of fatty liver [3]. Massive accumulation of neutral lipids (triglycerides and cholesterol esters) in TMEM41B-deficient hepatocytes is accompanied by decreased amounts of major phospholipids, including phosphatidylcholine and phospha-

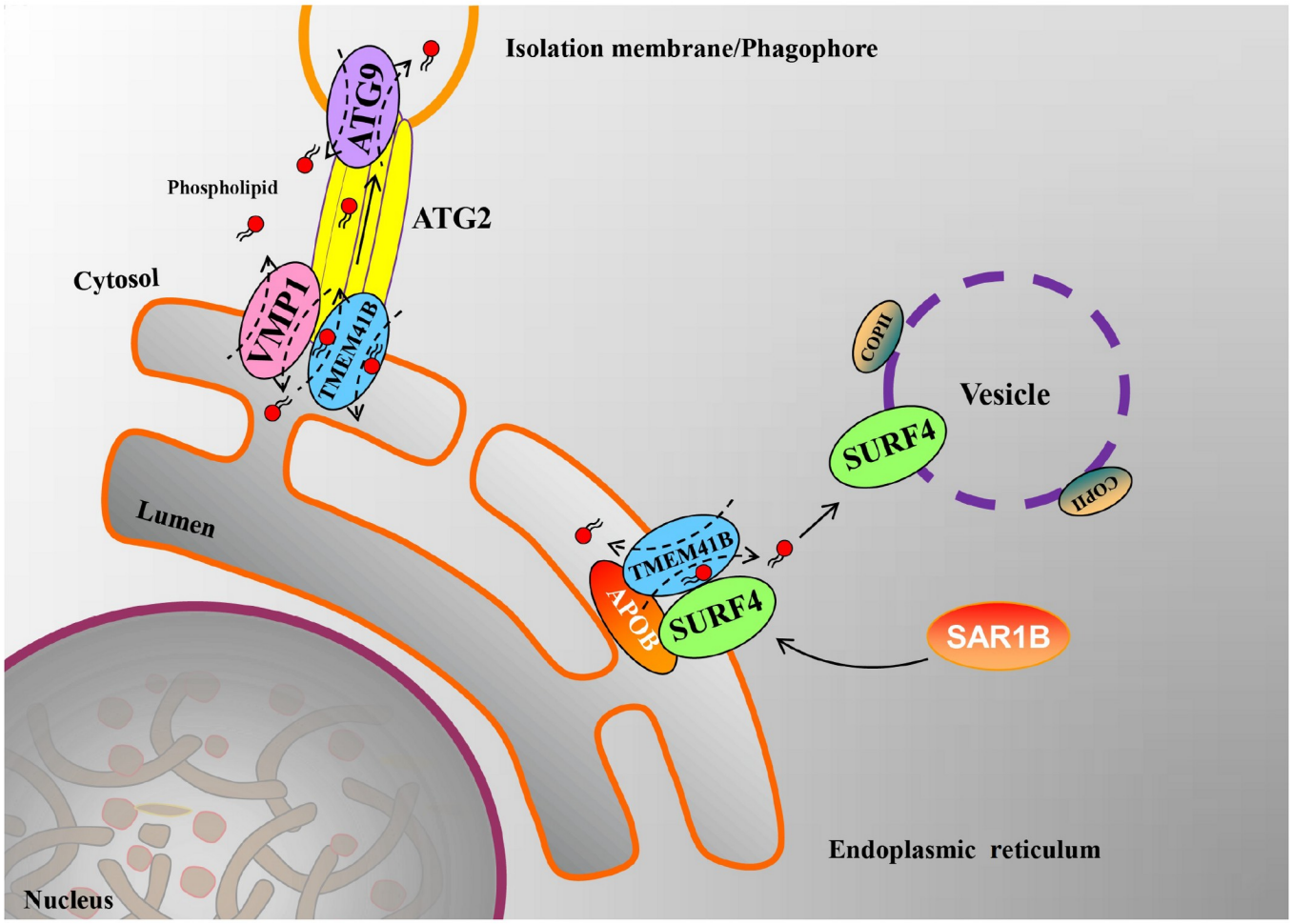

Figure 1. A schematic diagram depicting the action of phospholipid transfer complexes involving TMEM41B in lipid transfer and lipoprotein biogenesis The cargo receptor SURF4 partnered with the molecular switch SAR1B selectively transports lipoproteins from the ER to the Golgi apparatus via COPII-coated transport vesicles. And the lipid scramblase TMEM41B colocalized with SURF4 and lipoproteins mediates the shuttling process of bulk phospholipids and controls lipoprotein biogenesis and lipid homeostasis. The phospholipid scramblases TMEM41B, VMP1 and ATG9 form a phospholipid transfer complex with the intermembrane and interleaflet lipid transporter ATG2 to ensure lipid transport and organelle contact from the ER to the isolation membrane (also called phagophores) to regulate autophagosome membrane expansion and autophagy activity.

tidylethanolamine. The ER membrane in TMEM41B-deficient hepatocytes displays drastic morphological changes (curved, sparse and dilated) with a severe deficiency of lipoproteins within [3]. TMEM41B scramblase deficiency triggers ER membrane transformation characterized by leaflet imbalance, which is thought to be a novel stress response distinct from the typical unfolded protein response and DNA damage response. TMEM41B deficiency also induces the upregulation of factors involved in lipid peroxidation and leads to a large induction of phospholipid-modifying enzymes involved in the Land's cycle and enzymes in cholesterol biosynthetic pathways and fatty acid synthesis and esterification. In general, TMEM41B scramblase deficiency promotes cells to convert excessive phospholipids into storage lipids such as triglycerides and cholesterol esters, driving hepatic lipid deposition and NASH progression. The above results reveal a safe fundamental mechanism by which TMEM41B-mediated lipid scrambling protects the function and integrity of the ER in cells, while its dysfunction disrupts lipid homeostasis.

In addition to TMEM41B, Vacuole Membrane Protein 1 (VMP1) and autophagy-related 9 (ATG9) were recently identified as phospholipid scramblases responsible for phospholipid flipping and lipid equilibration in such cellular processes [8–10]. VMP1, as an ER-resident transmembrane protein, shares an identical conserved VTT domain with TMEM41B, which is essential for autophagosome formation, lipoprotein metabolism and cellular phospholipid and cholesterol homeostasis in mammals [7–9]. Ablation of VMP1 in organisms causes accumulation of neutral lipids within lipid bilayers of the ER membrane, thus affecting lipoprotein secretion and homeostasis [7]. The normal cellular distribution of cholesterol and phosphatidylserine is impaired in VMP1-deficient cells [8]. ATG9 on the preautophagosome ATG9 vesicles is another lipid scramblase that colocalizes with the lipid transfer protein ATG2 to translocate phospholipids from the cytoplasm to the luminal leaflet, thereby regulating autophagosome membrane expansion and autophagy activity [11]. New discoveries show that TMEM41B and VMP1 (which act locally in autophagosome biogenesis) as well as ATG9 form a phospholipid transfer complex with ATG2 to ensure that lipids flow from the ER to the

expanding autophagosome without disrupting the autophagosome or ER ultrastructure (Figure 1) [9,10,12]. Intriguingly, depletion of TMEM41B/VMP1 but not ATG9 causes lipoprotein secretion defects, implying that the locations of different scramblases may account for their different roles. Moreover, some members of the TMEM16 protein family, including TMEM16F and TMEM16K, exhibit calcium-dependent lipid scramblase activity required for phosphatidylserine exposure on the cell surface [13,14].

Nevertheless, TMEM41B was first identified as a major target of spinal muscular atrophy (SMA)-dependent U12 splicing and is essential for normal synaptic transmission of cholinergic motor circuit neurons, but its molecular function remains uncertain [15]. In addition, TMEM41B, as a key autophagy-related ER protein, functions together with a structurally related protein, VMP1, in autophagosome formation [16,17]. Loss of TMEM41B protein causes inhibition of autophagosome formation and recruitment of ATG proteins and small vesicles. TMEM41B-deficient cells display accumulation of cytosolic lipid droplets and lower endogenous fatty acid (FA) utilization rates, supporting a potential role of lipid mobilization and mitochondrial β-oxidation of FA. TMEM41B is also identified as a novel ER-localized regulator of autophagosome biogenesis and lipid mobilization [18]. Likewise, VMP1 and ATG9 are essential for early autophagosome biogenesis events. VMP1 modulates ER isolation membrane contact disassembly during autophagosome formation by increasing ER calcium transporter SERCA activity [19]. ATG9A is present on postGolgi vesicles required to initiate autophagosome formation [10].

Genome-wide CRISPR-Cas9 screening identified the VTT domain protein TMEM41B as an essential host-dependent factor for flavivirus infection, including SARS-CoV-2 infection [20]. The cellular localization of TMEM41B changes from a diffuse reticular-like pattern to a large cytosolic aggregate and colocalizes with flavivirus nonstructural proteins NS4A/NS4B upon virus infection [20]. TMEM41B is recruited to sites of viral RNA replication through direct interaction with NS4A/NS4B or through mobilizing lipids and facilitating NS4A/NS4B-inducing membrane remodelling [21]. Flavivirus NS4A/NS4B proteins assemble and viral RNA replication initiates in abnormal replication complexes exposed to the cytoplasm in TMEM41B-deficient cells. Intriguingly, naturally occurring TMEM41B single nucleotide polymorphisms present at a striking ∼20% frequency in East and Southeast Asia reduce flavivirus infection and have the ability to maintain normal lipid distribution in cells. Loss of TMEM41B confers decreased viral RNA replication and exaggerated innate immune response to flavivirus infection [21]. Therefore, TMEM41B is also considered a candidate drug target for antiviral therapeutics.

In general, efficient phospholipid scrambling is crucial to life activities. Although the existence of phospholipid scramblases in biological membrane formation has been proposed by researchers for half a century, this factor has not been discovered until recently, and its molecular mechanisms and pathophysiology are still unknown. Recent studies identified a few phospholipid scramblases, especially TMEM41B, required for lipoprotein biogenesis and lipid homeostasis in organisms [3,8,10]. Deficiency of phospholipid scrambling molecules often causes drastic changes in organelle morphology, severe disorder of cellular lipid metabolism and full-blown hepatic steatosis. Further studies should focus on the catalytic and regulatory mechanisms of lipid scramblases and the distinct functions and phenotypes resulted from different scramblases. In addition, the biophysical or biochemical properties of the phospholipid imbalance response and their relevance to fatty liver diseases still await further exploration.

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Conflict of Interest

The authors declare that they have no conflict of interest

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