LETM1 is required for mitochondrial homeostasis and cellular viability (Review)

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Abstract. Leucine zipper/EF-hand-containing transmembrane protein 1 (LETM1) has been identified as the gene responsible for Wolf-Hirschhorn syndrome (WHS), which is characterized by intellectual disability, epilepsy, growth delay and craniofacial dysgenesis. LETM1 is a mitochondrial inner membrane protein that encodes a homolog of the yeast protein Mdm38, which is involved in mitochondrial morphology. In the present review, the importance of LETM1 in WHS and its role within the mitochondrion was explored. LETM1 governs the mitochondrion ion channel and is involved in mitochondrial respiration. Recent studies have reported that LETM1 acts as a mitochondrial Ca^{2+}/H^{+} antiporter. LETM1 has also been identified as a K^{+}/H^{+} exchanger, and serves a role in Mg^{2+} homeostasis. The function of LETM1 in mitochondria regulation is regulated by its binding partners, carboxyl-terminal modulator protein and mitochondrial ribosomal protein L36. Therefore, we describe the remarkable role of LETM1 in mitochondrial network physiology and its function in mitochondrion-mediated cell death. In the context of these findings, we suggest that the participation of LETM1 in tumorigenesis through the alteration of cancer metabolism should be investigated. This review provides a comprehensive description of LETM1 function, which is required for mitochondrial homeostasis and cellular viability.

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Contents

1. Introduction

Wolf-Hirschhorn syndrome (WHS) patients display pleiotropic phenotypes including growth delay, intellectual disability, congenital hypotonia, distinct facial appearance, congenital heart defects, midline defects and seizures (1,2). The deletion of either of two critical regions (WHSCR and WHSCR-2) within chromosome band 4p16.3 has been proposed to be necessary for minimal clinical manifestation of WHS (3,4). A gene within WHSCR-2, leucine zipper/EF-hand-containing transmembrane protein 1 (LETM1), which is deleted in almost all patients with the full phenotype, and was therefore suggested as an excellent candidate gene for the seizure development (2,3,5). LETM1 was proposed to contribute to the neuromuscular features of WHS patients (6). Multiple genes are responsible for the characteristics of WHS disorders; thus, mouse models have been developed to complement ongoing patient-based studies (7). Phenotypes of a genetically modified mouse model for FGFR33, MAEA, Sax2/Nkx1-1 and CTBP1 showed skeletal malformations, hematopoietic dysgenesis, post-natal growth defects, and later growth defects, respectively (7). The most severe pathogenic phenotype of WHS is epilepsy, which has been shown in mouse lines carrying TACC3 and FAM53A mutantions (7,8). LETM1 is also accepted to be tightly linked to the epilepsy pathogenesis in WHS. In *Drosophila melanogaster* model, neuronal-specific knockdown of LETM1 resulted in the impairment of locomotor behavior in the fly and reduced synaptic neurotransmitter release. These results revealed the function of LETM1 in epilepsy: One of the severe pathogenic phenotypes of WHS (2,9). Decreased LETM1 levels in the neo-cortices of temporal lobe epilepsy patients and in the hippocampi and adjacent cortices of rats

following the onset of seizures has suggested that reduction of LETM1 contributes to the seizure phenotype. Knockdown of LETM1 leads to reduced MT-CYB expression and induction of susceptibility to seizures, which suggested that dysfunctional LETM1 together with mitochondrial swelling and disturbed MT-CYB expression is important for the deteriorated behavioral phenotype of epilepsy (10). However, Nigericin, a K^+/H^+ ionophore, fails to prevent epileptic seizures, and does not reverse lentivirus-shLETM1-mediated facilitation of epilepsy, indicating that further studies are required to determine the detailed mechanism of LETM1 function and delineate the involvement of LETM1 in seizure pathogenesis (10). Therefore a detailed understanding of LETM1 function will provide great advantages for WHS patients.

2. LETM1 functions in mitochondria

LETM1 structure and localization. LETM1 is a protein with a molecular mass of 70 kDa; it has also been identified as a 83-kDa endogenous protein in HeLa cells, suggesting that it is synthesized as a cytosolic precursor with a presequence (11). LETM1, like its yeast counterpart Mdm38 (12), is a mitochondrial inner membrane protein (1,9,13); it comprises a C-terminal domain bearing two EF-hand domains (578-590 and 676-698) and an N-terminal domain bearing a protein kinase C phosphorylation site (Fig. 1A) (6,14). LETM1 is an integral mitochondrial inner membrane protein facing toward the matrix (Fig. 1B, right). LETM1 is co-localized with HSP60, a mitochondria matrix protein (11). Partial digestion of LETM1 under hypotonic conditions has suggested that LETM1 contains a small and/or protease-resistant region exposed to the inner membrane space (11). The N-terminus of LETM1 is located in the inter-membrane space, connected to the inner membrane by a transmembrane domain containing three conserved proline residues (206-228), and the C-terminus extends to the mitochondrial matrix (6,15) [Fig. 1B, left (1)]. However, a recent study (16) revealed that LETM1 contains another transmembrane domain, from residues 413 to 421. This discovery has led to controversy regarding LETM1 N-terminal localization, which was strongly suggested to face the matrix [Fig. 1B, left (2)] (15).

LETM2, a homolog of LETM1, has already been identified (11,17). The LETM2 gene is located at human chromosome 8 and was also found in rat through a homolog search using human LETM1 cDNA (17). LETM2 is a mitochondrial inner membrane protein that shares some features with LETM1 (e.g., they are both mitochondrial inner membrane proteins with a leucine-zipper motif); however, it has a function distinct from that of LETM1, as demonstrated by its failure to suppress mitochondrial swelling caused by LETM1 knockdown (11). LETM1 has been detected in nearly all rat tissues, whereas LETM2 is expressed specifically in the testis, with a molecular size of 45 kDa, during the developmental stages from spermatocyte to spermatozoon. These expression environments indicate its probable function in inner and crista membrane reorganization in the mitochondria during spermatogenesis (11).

LETM1 regulates mitochondrial ion-channels. As indicated in Fig. 2, LETM1 was recently reported to function as a mitochondrial ion channel regulator. The presence of two putative Ca^{2+} binding sites on LETM1 suggestes that impaired mitochondrial Ca^{2+} homeostasis due to lack of LETM1 could explain the seizures observed in some WHS patients (1). An RNAi screen was conducted to identify the genes that control mitochondrial Ca^{2+} transport in *Drosophila* S2 cells which stably express mitochondria-targeted ratiometric pericam. CG4589, the *Drosophila* homolog of the human gene LETM1, was identified as a gene strongly affecting $(Ca^{2+})_{mito}$ and $(H^+)_{mito}$ responses (18). LETM1 catalyzes the 1:1 electrogenic exchange of Ca^{2+} for H^+ which is driven by the negative potential of mitochondria and by protons leaving the mitochondrial matrix (18,19). The mitochondrial calcium unipoter (mCU) drives rapid and massive Ca^{2+}entry, but only at the high cytosolic Ca^{2+} concentrations (>10 μM) that are reached in microdomains near Ca^{2+} release channels on the endoplasmic reticulum (ER) (20). The new Ca^{2+}/H^+ antiporter operates at low cytosolic Ca^{2+} concentrations (<100 nM) and is limited by the pH gradient generated by the mitochondrial electron transport chain (21). LETM1 shares a role with mCU in catalyzing Ca^{2+} uptake into mitochondria; this role can be inhibited by ruthenium red. Thus, unlike mCU, which is critical only for Ca^{2+} uptake, LETM1 catalyzes Ca^{2+} uptake into mitochondria in exchange for H^+, implying that proton efflux from mitochondria can drive LETM1-dependent Ca^{2+} entry into mitochondria. mCU conveys rapid Ca^{2+} transient from the cytosol to the matrix but exposes mitochondria to Ca^{2+} overload and alterations in ER Ca^{2+} handling. LETM1 is bidirectional and can extrude Ca^{2+} along with the Na^+/Ca^{2+} exchanger during large Ca^{2+} loads, allowing mitochondria to bear small cytosolic Ca^{2+} elevations without risking Ca^{2+} overload (21). Knockdown and overexpression of LETM1 in cells has been shown to alter $[Ca^{2+}]_{mito}$ and $[pH]_{mito}$ responses in a pattern consistent with Ca^{2+}/H^+ exchange (18). Reconstitution of the purified protein in liposomes confirmed that LETM1 mediates Ca^{2+}/H^+ exchange. This transport is electrogenic and therefore blocked by ruthenium red. Functional data in LETM1-depleted HeLa cells have indicated that the new antiporter can mediate both Ca^{2+} uptake and Ca^{2+} extrusion from mitochondria; however, these observations remain to be confirmed by simultaneous Ca^{2+} and pH measurements during physiological stimulation (18). LETM1 and UCP2/3 independently contribute two molecularly distinct pathways for mitochondrial Ca^{2+} uptake in endothelial cells. Knockdown of LETM1 resulted in highly depleted sequestration of entering Ca^{2+}, but had no effect on Ca^{2+} uptake at ER-mitochondrial junctions, suggesting that LETM1 and UCP2/3 contribute Ca^{2+} uptake from different sources and at different Ca^{2+} concentrations (22). However, it has been proposed that LETM1 works as a Ca^{2+}/H^+ antiporter in mitochondria, but also that LETM1 functions as a mitochondrial K^+/H^+ exchanger (12). Measurements in isolated mitochondria labeled with K^+ and H^+ fluorescent dyes confirmed the presence of a K^+/H^+ exchanger in the inner mitochondrial membrane of yeast (12). Mutants in the yeast LETM1 homolog Mdm38, which lacks the two Ca^{2+} binding sites present in LETM1, cause loss of mitochondrial K^+/H^+ exchange activity, resulting in highly abundant K^+ within the mitochondrial matrix and low membrane potential ($\Delta\Psi m$), followed by water influx and organelle swelling (23,24). LETM1 expression restores Mdm38 and potassium acetate indicating

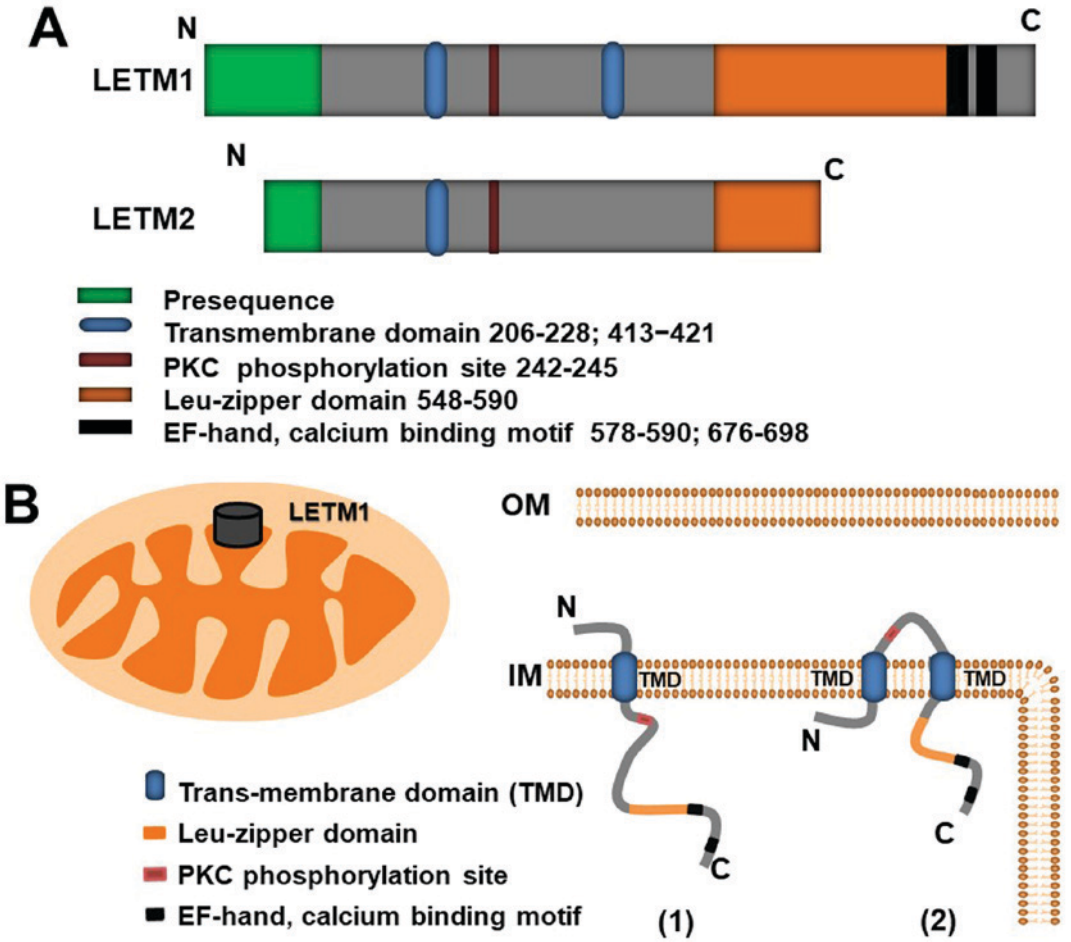

Figure 1. LETM1 structure and localization. (A) Schematic representation of LETM1 and LETM2. Specific domains and sites are symbolized by respective colors as indicated. (B) Sub-mitochondrial localization of LETM1. OM, Outer Membrane; IM, inner membrane; LETM1, leucine zipper/EF-hand-containing transmembrane protein 1.

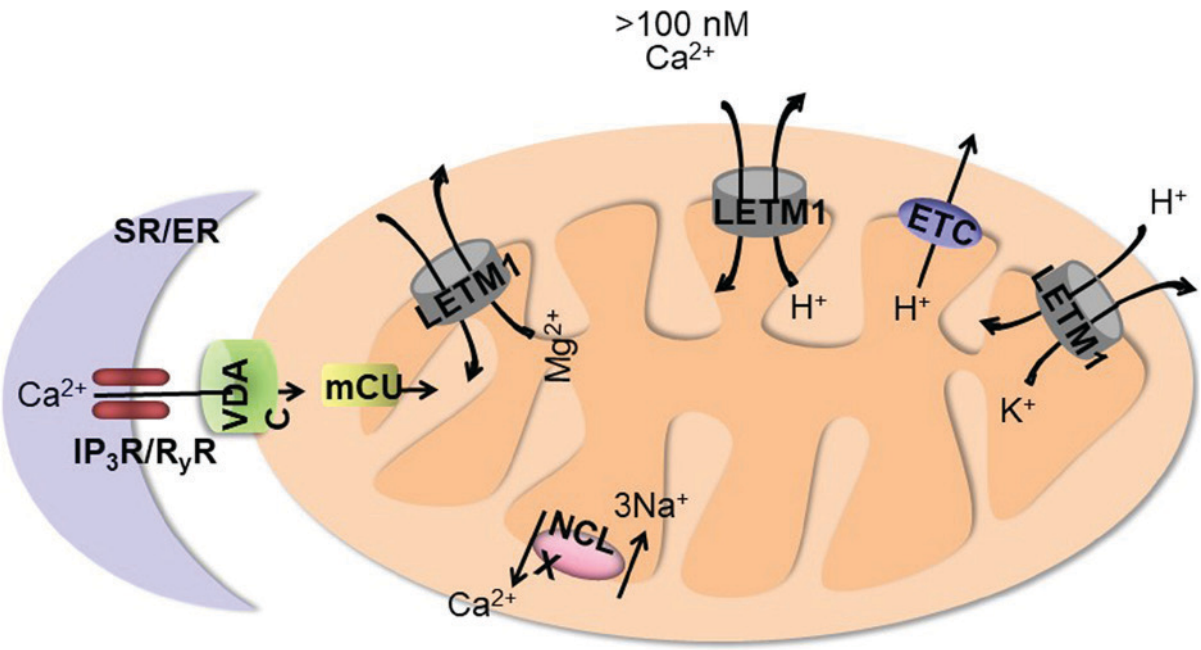

Figure 2. Regulation of ion-channels in mitochondria by LETM1. MCU drives massive calcium entry with high cytosolic Ca^{2+} concentrations (>10 μM) in microdomains near Ca^{+2} release channels on ER but LETM1 functions as Ca^{2+}/H^{+} antiporter, at low cytosolic Ca^{2+} concentrations (>100 nM). The pH gradient generated by the ETC limits its action. It can also extrude Ca^{2+} along with the NCLX during Ca^{2+} overload. Furthermore, it also functions as the K^{+}/H^{+} exchanger as well as a Mg^{2+} transporter. ETC, electron transport chain; MCU, Mitochondrial Ca^{2+} uniporter; NCLX, Na^{+}-Ca^{+} exchanger; VDAC, voltage dependent anion channel; SR, sarcoplasmic Reticulum; ER, endoplasmic reticulum; LETM1, leucine zipper/EF-hand-containing transmembrane protein 1.

a defect in K^+/H^+ exchange activity-induced swelling (23). Furthermore, Mdm38 depletion resulted in an early loss of K^+/H^+ exchange-mediated swelling capacity of mitochondria, loss of ΔΨm and mitophagy during the extensive interaction of mitochondria with vacuoles (24,25). These effects strongly support the notion that K^+/H^+ exchange activity is the primary cause of morphological changes in mitochondria, which in turn trigger the process of mitophagy (24). This evidence of mitophagy induction via Mdm38 depletion feasibly suggests that LETM1 regulates mitophagy and cell viability.

Mitochondrial Mg^{2+} transport processes may play an important role in cellular Mg^{2+} homeostasis and the regulation of cell and mitochondrial functions (26). MRS2 encodes a mitochondrial membrane protein, that is an essential component of the major electrophoretic Mg^{2+} influx system in mitochondria (26). Mutant alleles of MRS2 and its overexpression increase intra-mitochondrial Mg^{2+} concentrations. Two yeast homolog genes of LETM1, MRS7 and YOL027, are multicopy suppressors of MRS2D (disruptiing the open reading frames of MRS2 and defective in mitochondrial Mg^{2+} influx, respectively), whereas Yo1027p overexpression may improve Mg^{2+} influx in MRS2D cells (23,26). Furthermore, Mdm38 mutation suppresses the growth deficiency caused by mutation in the MRS2 gene (27,28). Together, these findings suggest LETM1 acts as an Mg^{2+} transporter in the mitochondrion.

LETM1 and its partners cooperate to influence mitochondria morphology, respiration and biogenesis. Yeast Mdm38, a homolog of LETM1 was identified in a comprehensive genome-wide screen for mutants with mitochondrial morphology defects (29). Within LETM1-knockdown cells, mitochondrial swelling and the disorganization of cristae structures were observed using electron microscopy (11). LETM1 knockdown caused mitochondria to become dot-like structures and lose their tubular networks to a significantly greater extent than fragmented mitochondria observed in OPA1-knockdown cells (11,30). Images of mitochondria lacking LETM1 were reminiscent of observations following overexpression of pro-fission proteins such as Fis1 (31) or knockdown of pro-fusion proteins sfiguch as OPA1 (32). In both cases, inhibition of the dynamin-related protein Drp1-dependent fission machinery, by silencing or using a dominant negative, caused the Drp1K38A mutant to recover its mitochondrial morphology (33,34). Nevertheless, Drp1 inhibition in LETM1 knockdown cells, did not recover the fragmented phenotype, indicating that blockage of the fission machinery is not induced by reduction of LETM1 and that lack of LETM1 causes Drp1-independent mitochondrial fragmentation (30). Moreover, following the silencing of both Drp1 and LETM1, mitochondria remained partly swollen, suggesting that mitochondrial membrane fusion is unaffected by downregulation of LETM1 or co-silencing of Drp1 (11). These results suggest that LETM1 is not directly implicated in mitochondrial membrane fission and fusion. Thus, neither lack nor excess of LETM1 is beneficial to cells, and fragmented mitochondria and swollen cristae were observed in LETM1-overexpressed HeLa cells (14).

LETM1 downregulation caused reduced numbers, and morphological changes in cristae, leading to a substantially lower membrane potential (ΔΨm), which is consistent with reports that isolated mitochondria from yeast Mdm38 mutants exhibited low membrane potential (23,35); however, no significant changes in ΔΨm were observed between controls and LETM1-transduced cells (14). LETM1 knockdown caused the disassembly of three different proton pumps complex I (NADH dehydrogenase), III (cytochrome *b* complex), and IV (cytochrome *c* oxidase) (36), suggesting that LETM1 regulates the biogenesis of respiratory chains. Low membrane potential appears to be a consequence of the failure to form super-complexes: Complexes I, III and IV of the respiratory chains (11).

Therefore, LETM1 is critical for respiratory chain biogenesis, being physically associated with mitochondrial ribosomes to mediate membrane insertion of several proteins of nuclear and mitochondrial origin, and facilitating their transport across the inner membrane (24,35). A ribosome-associated site has been identified on the LETM1 ribosome binding domain (RBD); it displays a matrix-exposed 14-3-3-like fold, which is critical for respiratory chain assembly through the regulation of Cox1 and Cytb translation (37). Mitochondrial ribosomal protein L36 (MRPL36) has been reported to be associated with the inner-membrane (38). The addition of puromycin was used to access possible interactions between LETM1 and MRPL36; LETM1 associated with MRPL36 both *in vivo* and *in vitro*. MRPL36 siRNA significantly recovered LETM1-mediated ATP reduction, suggesting that LETM1 may regulate the mitochondrial translation system and reduce mitochondrial biogenesis through association with MRPL36 (14). The inhibition of mitochondrial biogenesis by LETM1 offers a possible explanation of WHS phenotypes, especially neuromuscular defects and seizures, which likely reflect oxidative phosphorylation defects and thus resembles classical mitochondrial encephalomyopathies such as mitochondrial encephalomyopathy, lactic acidosis, and stroke like episodes (35).

Cells lacking mitochondrial DNA lose active respiratory chains, but maintain mitochondrial tubular networks, indicating that mitochondrial swelling caused by LETM1 knockdown is not caused by the disassembly of respiratory chains (11). Human AAA-ATPase BCS1L, which is a mitochondria inner membrane protein, is responsible for human disorders and the assembly of respiratory chains (39-41). BCS1L interacts specifically with LETM1, stimulating the formation of the LETM1 major complex; thus, BCS1L and LETM1 function in different process to maintain mitochondria and form tubular network structures (11).

Role of LETM1 in mitochondrial quality control. The term mitophagy was first introduced by Dr J.J. Lemasters for the selective autophagic degradation of damaged and dysfunctional mitochondria (42). An accumulation of studies suggest that mitochondrial dysfunction and morphological changes, which are interrelated factors, are responsible for the induction of mitophagy (24,43,44). Mitochondrial fission produces two subsets of daughter mitochondria with either increased or decreased membrane potential. The depolarized daughter mitochondrion, which is incapable of fusing into the polarized network, is removed through the process of mitophagy (44). Loss of K^+/H^+ activity and subsequent

decrease in membrane potential due to Mdm38 depletion leads to mitochondrial fragmentation into discrete spheres and their association with vacuoles, suggestive of mitophagy induction. Furthermore, the depletion of DNM1, a mitochondrial fission protein, and Mdm38 leads to the continued fusion of swollen mitochondria, indicating the blockage of selective removal by mitophagy (24). LETM1 overexpression has been reported to result in a mitochondrial ATP decrease, mitochondrial dysmorphology, swollen mitochondria cristae, and fragmentation in HeLa cells in an OPA1-dependent manner (45). The mitochondria within autophagosomes with reduced OPA1 levels (44) and fragmented discrete mitochondria targeted by autophagic vacuoles in an Mdm38-depleted yeast strain point toward a possible role for LETM1 in mitophagy induction. Mitochondrial depolarization also leads to the stabilization and accumulation of PINK1 in the outer membrane of the mitochondrion (46), which can recruit parkin; the depolarized, damaged mitochondria are subsequently removed by mitophagy (47). Interestingly, PINK1-phosphorylated LETM1 has been shown to modulate mitochondrial Ca^{2+} transport and protect neurons against mitochondrial stress (48). These studies paved the way for further studies to reveal the role of LETM1 in the quality control machinery of mitochondria.

3. New insights into tumorigenesis

Tumors have been reported in several WHS cases (49-52). In particular, the discovery of neuroblastoma in a child with WHS implicated the association of these two phenotypes (51). Because WHS is rare, the occurrence of these tumors raises concern that cancer may be a component feature of WHS. LETM1 is highly expressed in many human malignancies and is correlated with poor prognosis (14,53,54). LETM1 knockdown by siRNA repressed proliferation, migration, and invasion in bladder cancer cells (53). Several oncogenic proteins in the Wnt/β-catenin signaling pathway (β-catenin, cyclin D1 and c-Myc) were significantly decreased by LETM1 siRNA. These finding clearly indicate the roles of LETM1 in tumor progression. However, the potential molecular mechanism of LETM1-mediated tumorigenesis remains to be elucidated.

Because mitochondrial dysfunction has been implicated in a wide variety of human diseases, including cancers and age-related disorders, mitochondria have emerged as effective targets for anticancer therapy (55-57). Mitochondria function as central components of cell survival through ATP production and govern cell fate by mitochondrial membrane-dependent cell death signaling (58). Loss of Mdm38 results in a variety of phenotypic effects, including defects of respiratory chain, altered mitochondrial morphology, osmotic swelling, and mitophagy (24). Downregulation of LETM1 causes Drp1-independent fragmentation of the mitochondrial network, but is not associated with respiratory chain, whereas overexpression of LETM1 leads to mitochondrial fragmentation through OPA1 modulation (30). Although the functions and mechanisms of LETM1 with respect to cell viability and tumorigenesis remain controversial, accumulating data suggest that LETM1 will be a crucial candidate to clarify how mitochondria regulate the normal life of the cell.

Maintenance of mitochondrial morphology by LETM1 is required for cell viability. The regulation of mitochondrial volume and morphology has been associated with a wide range of important biological functions and pathologies. Any disruption in the osmotic balance between mitochondria and the cytosol, mainly as a result of intracellular ion fluxes (particularly K^+, due to its higher concentration), induces water movement between these two compartments, leading to loss of mitochondrial volume and homeostasis, which then cause morphological changes (59). Fission-fusion events can also result in mitochondrial morphological changes, which are modulated by a complex network of cytosolic and mitochondrial proteins and are coordinated to respond to specific cellular demands (60,61). Osmotic swelling, which indicates pathological states in mitochondria, has been found to activate downstream cascades, notably determining the viability of the cell as a whole (61).

Mitochondrial swelling also plays an important role in the release of cytochrome *c*, which is associated with apoptotic cell death (59). Adenovirus-meditated LETM1 overexpression leads to mitochondrial dysmorphology, swollen mitochondria cristae, and an increase in mitochondrial fragmentation, as shown by electron microscopy, and sensitizes the cell to apoptosis in an OPA-1-dependent manner (45).

However, long-term LETM1 overexpression leads to necrotic cell death via decreases in intracellular ATP levels, in a time-dependent manner, because it is independent of AIF nuclear translocation and caspase activation. A previous study found that the addition of extra glucose led to a reduction in total mitochondrial mass and the amount of ATP per cell, and partial recovery, supporting the notion that LETM1-mediated cell death is influenced by energy deprivation (14). However, downregulation of LETM1 has also been shown to result in caspase-independent and Bcl-2-insensitive necrotic cell death. To date, it remains unknown how both the gain and loss of LETM1 causes similar phenotypes in cells.

Mitochondrial morphology is also strongly associated with energy metabolism, as mitochondria with increased respiration levels appear in morphologically interconnected networks with enlarged cristae compartments, whereas those with low respiration and therefore high glycolysis levels are fragmented, with smaller inter-cristae space (62). LETM1 has been demonstrated to cause such fragmented morphology (24,45) along with altered energy metabolism leading to tumorigenesis (14). Taken together, these findings demonstrate that the role of LETM1 in mitochondrial volume and morphological homeostasis is critical to determining cell viability and correlation with tumorigenesis.

LETM1 contributes to cancerous metabolic alteration. In 1931, Otto Warburg was awarded the Nobel Prize for oncology for his breakthrough hypothesis and research on cancerous metabolism (63). He observed that cancer cells are predominantly dependent on energy produced by glycolysis, rather than by pyruvate oxidation in mitochondria (57). Although the exact mechanisms responsible for this metabolic alteration remain to be elucidated, mitochondrial respiratory defects, due in part to mitochondrial DNA mutations/deletions and hypoxia, are thought to be important contributing factors. Mitochondrial respiration deficiency and an increase in ATP

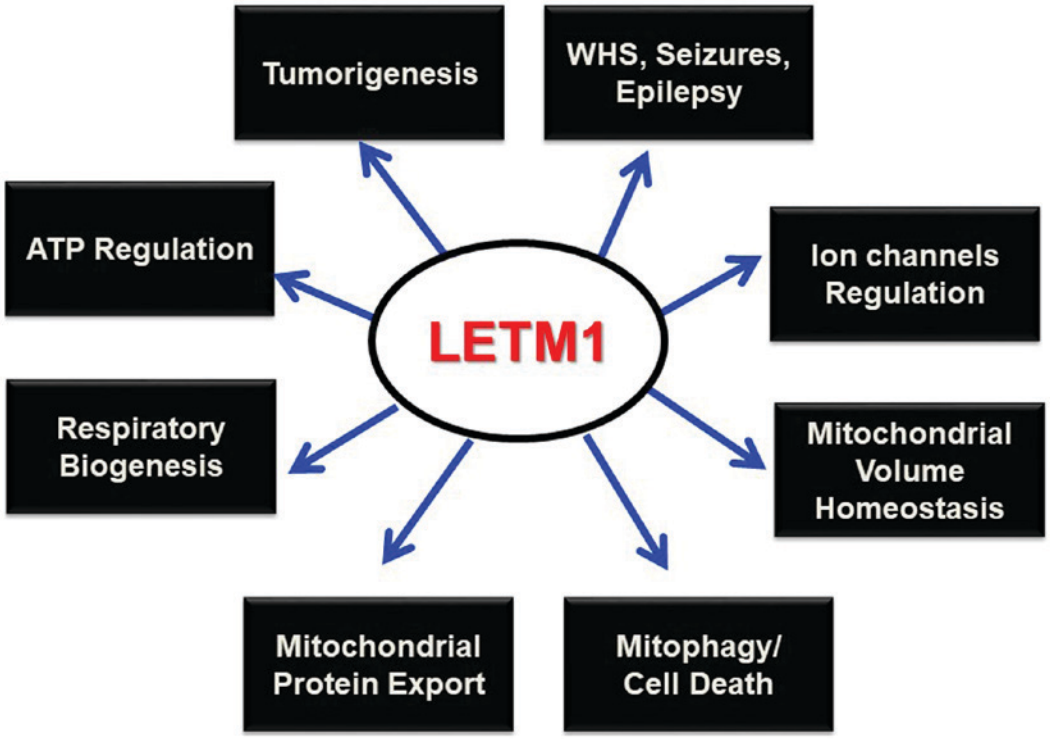

Figure 3. Schematic representation of proposed LETM1 function. Schematic diagram depicting the proposed physiological as well as pathophysiological roles of LETM1. It shows the functions of LETM1 in the mitochondria and the consequences of loss in these functions. LETM1, leucine zipper/EF-hand-containing transmembrane protein 1.

production via glycolysis, leading to activation of the PKB/Akt survival pathway through NADH-mediated inactivation of PTEN, is a novel mechanism contributing to altered cancerous metabolism (64). Consistent with these results, Piao and colleagues demonstrated that LETM1 overexpression (45); led to increased ATP production via glycolysis and increased lactate production, also causing PTEN inactivation and PKB activation, suggesting that LETM1-induced mitochondrial dysfunction resulted in PKB activation which increased the ratio of NADH/NADPH to inactivate PTEN enzyme activity. -Similar levels of LETM1 expression in cancerous tissue and in overexpressed HeLa cells, along with the detection of LETM1 in two bands from six patients who had undergone surgery for malignant cancer, strongly suggest that LETM1 overexpression is a feature of altered cancerous metabolism accompanied by metastasis (14). In contrast, AMPK activation and consequent inhibition of G1/S cell cycle progression, as well as decreased PKB and mTOR phosphorylation by LETM1 overexpression can alter lung cancer cell growth (65). Using liquid chromatography tandem mass spectrometry and a power law global error model for reliable bio-signature mapping of hepatocellular carcinoma,, LETM1 was found to have increased expression level, and its potential translocation to the tumor nuclear fraction was inferred, especially to the peripheral nuclear region and outer nucleus as confirmed by western blot, immunohistochemical, and immunofluorescent analyses (66). Collectively, these finding underscore the putative role of LETM1 in altered cancerous metabolism and indicate the need for further study to explore the roles of LETM1 in different tumors, and establish it as an effective therapeutic target in cancer-treatment.

Association of LETM1 with mitochondrial ribosomes and mitochondrial ATP regulation. Mutations in mitochondrial DNA have been shown to play a key role in tumorigenesis within various organs, as these mutations lead to the malformation or/and malfunctioning of the mitochondrial respiratory chain, compelling cells to depend on glycolysis to fulfill their ATP demand (67,68). The biogenesis of respiratory chain complexes requires the synthesis of proteins encoded by the mitochondrial genome and their subsequent insertion into the inner membrane. Mdm38 has been found to be associated with newly synthesized mitochondrial proteins via the ribosome, and is specifically required for efficient membrane insertion of cytochrome *b* and Atp6, and polytopic membrane proteins of complexes III and V, respectively (35). The LETM1 ortholog Mdm38 plays a role in respiratory chain function at the cellular level, as demonstrated by growth defects and reduced $\Delta\psi$ observed in Mdm38Δ mitochondria. LETM1 partially rescues growth defects in Mdm38Δ cells, suggesting that both proteins fulfill similar cellular functions (23).

An LETM1 RBD has been shown to be important to respiratory chain assembly through regulation of Cox1 and Cytb translation; this matrix-exposed domain displays a 14-3-3-like fold (37). LETM1 overexpression has been reported to decrease mitochondrial ATP production, oxygen consumption, and MRPL36 depletion through the use of siRNA-MRPL36 to significantly revert the LETM1-mediated ATP decrease, suggesting a functional association between LETM1 and mitochondrial ribosomes (14). Thus, the association of the LETM1 family with mitochondrial ribosomes and its consequent role in the mitochondrial translation system and biogenesis highlights the putative role of LETM1 in tumorigenesis.

4. Conclusion

LETM1 has been cloned in an attempt to identify the genes deleted in WHS (6), and was found to be responsible for seizure development (4,10). Since this discovery, LETM1 has been shown to play prominent roles in mitochondrial K^+ and Ca^{2+} homeostasis, volume, and morphology maintenance, and respiratory chain biogenesis, and to be indispensable in maintaining organelles and cellular physiological integrity. Its potential interaction with mitochondrial ribosomes and biogenesis, and regulation of mitochondrial ATP and morphological homeostasis underscore its putative candidacy in altered metabolism and subsequent tumorigenesis. Mitophagy has been reported as the selective degradation of depolarized mitochondria, as a result of lost K^+/H^+ exchange activity due to Mdm38 depletion (24); however, this process must be further explored to determine the contributing roles of the PINK/PARKIN pathway and LETM1. Studies of the possible interactions between LETM1 and mitochondrial ribosomes and its functional relevance as a translocase in mitochondrial protein synthesis and export machinery may help to establish this protein as a potential therapeutic target for various diseases such as WHS, epilepsy, cancer, and other pathophysiological conditions (Fig. 3).

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Availability of data and materials

All data used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YL conceived the present study. QT analyzed the LETM1 literature and made the substantial contribution in the finalization of this manuscript. LP assessed the experimental data. RS and SP contributed to data interpretation and writing the discussion. JiP contributed to data interpretation and discussion, particularly regarding the mitochondria function of LETM1. JoP contributed to designing the study and was involved in data interpretation and writing the discussion. JoP also approved the final manuscript for publication. All authors reviewed the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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