Advances in the Structure, Function, and Mechanism of Mitofusin 2 in Liver Diseases (Postprint)

Authors: Yuan Xiwei, Nan Yuemin, Nan Yuemin

Date: 2023-05-05T00:00:00+00:00

Abstract

The liver is the largest metabolic organ in the human body, and impaired liver function can cause various acute and chronic liver diseases, which affect quality of life in mild cases and endanger life in severe cases; therefore, identifying precise and effective molecular diagnostic markers and therapeutic targets is crucial. Mitofusin 2 (Mfn2) is a transmembrane dynamin protein on the mitochondrial outer membrane that not only regulates mitochondrial fusion but also plays important roles in cellular energy metabolism, apoptosis, proliferation, mitochondria-endoplasmic reticulum tethering, endoplasmic reticulum stress, and mitophagy. Studies have found that abnormal expression or functional loss of Mfn2 can lead to mitochondrial dysfunction, thereby triggering various liver diseases. Through a systematic review of the structure, function, and mechanisms of action of Mfn2 in liver diseases, this article finds that Mfn2 can participate in the occurrence and development of chronic liver diseases through multiple pathways, and regulating Mfn2 overexpression can improve liver function and further slow or reverse disease progression. This article aims to provide a scientific reference for basic research and clinical applications of Mfn2 in liver diseases.

Full Text

Research Progress on the Structure, Function, and Mechanism of Action of Mitofusin 2 in Liver Diseases

YUAN Xiwei[1],[2], NAN Yuemin[1],[2]*

[1]Department of Traditional and Western Medical Hepatology, Third Hospital of Hebei Medical University, Shijiazhuang 050051, China
[2]Hebei Province Key Laboratory of Study on Mechanism of Hepatic Fibrosis in Chronic Liver Disease, Shijiazhuang 050051, China

*Corresponding author: NAN Yuemin, Professor, Chief physician; E-mail: nanyuemin@163.com

Abstract

The liver is the largest metabolic organ in the human body, and impaired liver function can lead to various acute and chronic liver diseases that affect quality of life in mild cases and become life-threatening in severe cases. Therefore, identifying accurate and effective molecular diagnostic markers and therapeutic targets is crucial. Mitofusin 2 (Mfn2) is a transmembrane motor protein on the mitochondrial outer membrane that not only regulates mitochondrial fusion but also plays important roles in cellular energy metabolism, apoptosis, proliferation, mitochondrial-endoplasmic reticulum (ER) tethering, ER stress, and mitophagy. Abnormal expression or functional loss of Mfn2 can cause mitochondrial dysfunction, leading to various liver diseases.

This paper systematically reviews the structure and function of Mfn2 and its mechanisms of action in liver diseases. We found that Mfn2 participates in the development of chronic liver diseases through multiple pathways, and modulating Mfn2 overexpression can improve liver function and slow or even reverse disease progression. This review aims to provide a scientific reference for basic research and clinical applications of Mfn2 in liver diseases.

Keywords: Mitochondrial dynamics; Mitofusin 2; Structure; Function; Liver disease; Review

Funding: National Natural Science Foundation of China (81970504)

1. Introduction

The liver is the largest metabolic organ in the human body, participating not only in the synthesis and decomposition of proteins, lipids, carbohydrates, and vitamins, but also in the transformation and metabolism of hormones and drugs. The liver also performs important functions such as bile secretion, phagocytosis, defense, and hematopoiesis during embryonic development. Various hepatotoxic factors can damage liver cells, leading to metabolic, synthetic, detoxification, secretory, biotransformation, and immune dysfunction, which often results in fatty liver disease, viral hepatitis, hepatic fibrosis, and in severe cases, progression to hepatocellular carcinoma (HCC) and liver failure [1]. However, precise and effective molecular diagnostic markers and therapeutic targets remain lacking, making the search for new molecular identifiers essential.

Mitofusin 2 (Mfn2) is a transmembrane motor protein localized on the mitochondrial outer membrane and a key factor regulating mitochondrial fusion and maintaining mitochondrial structure [2]. In addition to mediating mitochondrial

fusion, Mfn2 plays important roles in cellular energy metabolism, apoptosis, proliferation, mitochondrial-ER tethering, ER stress, and mitophagy. Recent studies have shown that abnormal expression or functional loss of Mfn2 is closely associated with the development of various liver diseases, including metabolic-associated fatty liver disease (MAFLD), viral hepatitis, hepatic fibrosis, HCC, and acute-on-chronic liver failure (ACLF). Therefore, understanding the structure, function, and mechanisms of Mfn2 in liver diseases has important scientific and clinical value and may provide a scientific basis for potential therapeutic targets and drug development to delay or reverse various liver diseases.

Literature search strategy: We searched the CNKI, Wanfang Data, and PubMed databases from inception to February 2023. Chinese search terms included "mitofusin 2," "liver disease," "fatty liver disease," "viral hepatitis," "hepatic fibrosis," "hepatocellular carcinoma," and "liver failure." English search terms included "mitofusin 2," "liver disease," "fatty liver disease," "viral hepatitis," "hepatic fibrosis," "hepatocellular carcinoma," and "liver failure." Inclusion criteria: literature addressing Mfn2 structure and function or the relationship between Mfn2 and liver diseases. Exclusion criteria: poor relevance, low-quality literature, retracted articles, and unavailable full text. A total of 46 articles were included.

1.1 Structure of Mfn2

Mfn2 is a highly conserved transmembrane guanosine triphosphate (GTP) enzyme located on the mitochondrial outer membrane, sharing 77% homology with Mfn1 and possessing identical functional domains [3]. The structure includes an amino-terminal GTPase domain, a heptad-repeat domain (HR1), and a carboxy-terminal second heptad-repeat domain (HR2), with two transmembrane domains between HR1 and HR2 [2-3]. Both the GTPase and HR domains are exposed to the cytoplasm and are crucial for the fusion process [4]. The GTPase domain contains five functional motifs: G1 binds the phosphate of GTP molecules; G3 coordinates Mg^{2+} required for hydrolysis; G1, G2, and G3 together form the catalytic center; and G4 and G5 provide the specific conformation required for GTP binding. The HR2 domain forms homodimeric (Mfn1-Mfn1 or Mfn2-Mfn2) or heterodimeric (Mfn1-Mfn2) complexes through antiparallel coiled-coil structures between juxtaposed mitochondria, participating in the connection of two adjacent mitochondria. Subsequently, GTP hydrolysis by the GTPase domain provides energy to mediate membrane conformational changes, thereby promoting mitochondrial outer membrane fusion [5].

1.2 Regulation of Mfn2 Expression

The steady-state level of Mfn2 depends on ubiquitin-proteasome system regulation. During mitochondrial depolarization or cellular stress, Mfn2 is phos-

phorylated by PTEN-induced putative kinase 1 (PINK1), ubiquitinated by Parkin, and subsequently targeted for proteasomal degradation [3,6]. Additionally, stress-induced activation of c-Jun N-terminal kinase (JNK) can promote E3 ligase HUWE1-mediated phosphorylation of Mfn2, leading to ubiquitination and proteasomal degradation that affects mitochondrial fusion [6].

Mfn2 activity is also regulated by recombinant mothers against decapentaplegic homolog 2 (Smad2). Smad2 serves as a scaffold to recruit Rab-Ras interacting factor 1 (RIN1) and forms a Smad2-RIN1-Mfn2 complex. This complex allows RIN1 to act as a guanine nucleotide exchange factor that activates Mfn2-GTPase, thereby promoting mitochondrial adenosine triphosphate (ATP) synthesis and mitochondrial fusion [7]. The transcriptional regulator peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1) also plays an important regulatory role in Mfn2. PGC-1α can stimulate transcriptional activity of the Mfn2 promoter region 2kb fragment in muscle and brown adipose tissue. This specific region of the Mfn2 promoter binds to and is activated by estrogen-related receptor alpha (ERRα), which is further co-activated by PGC-1α [6,8]. PGC-1β, a PGC-1α homolog, is also essential for maintaining Mfn2 expression [6,8]. The Mfn2 promoter region also contains Krüppel-like factor 4 (KLF4) binding sites, and KLF4 overexpression can increase Mfn2 and glucose transporter 4 (GLUT4) expression, improving insulin resistance (IR) in skeletal muscle cells [9]. Additionally, glucocorticoids and pro-inflammatory factor tumor necrosis factor-α (TNF-α) also participate in regulating hepatic Mfn2 expression [4].

2.1 Regulation of Cellular Energy Metabolism

Mfn2 is essential for maintaining metabolic homeostasis [10]. Inhibition of Mfn2 expression reduces mitochondrial membrane potential, decreases glucose oxidation and oxygen consumption in L6E9 rat skeletal muscle and fibroblasts, manifested by reduced palmitate oxidation rate, increased glucose transport and lactate production, and decreased glucose conversion to glycogen [6]. Ablation of Mfn2 in mouse hepatocytes alters mitochondrial morphology, reduces mitochondrial respiratory complexes I and II, and promotes gluconeogenesis [11]. Conversely, Mfn2 overexpression in HeLa cells causes perinuclear clustering of mitochondria, enhances mitochondrial membrane potential, increases glucose oxidation, and upregulates expression of oxidative phosphorylation complexes I, IV, and V subunits [12]. Therefore, promoting Mfn2 expression can enhance mitochondrial function, facilitate cellular energy metabolism, and maintain organismal homeostasis.

2.2 Apoptosis

Mitochondria are the primary target of intrinsic apoptosis in its early stages. The B-cell lymphoma/leukemia-2 (Bcl-2) protein family plays an important role

in this process. Anti-apoptotic protein Bcl-2 can maintain mitochondrial integrity by inhibiting cytochrome C (Cyt C) release, whereas high Mfn2 expression can inhibit Bcl-2 expression, disrupt mitochondrial integrity, and promote apoptosis [6]. Additionally, apoptosis regulators Bcl-2-associated X (Bax) and Bak co-localize with Mfn2 on the mitochondrial outer membrane [6]. High Mfn2 expression can also promote increased Bax and Bak expression, leading to increased mitochondrial permeability and Cyt C release, which activates Caspase-9 through enzymatic cascade reactions to form the apoptosome and activate Caspase-3, ultimately triggering apoptosis through proteolysis. If the Mfn2 gene is knocked out, the binding sites of Bax and Bak to mitochondria disappear, and cellular resistance to apoptosis increases [13]. Furthermore, Mfn2 can inhibit proto-oncogene Ras expression and activate mitochondrial apoptosis pathways by suppressing Ras-PI3K-Akt pathway phosphorylation, thereby promoting apoptosis [14]. This suggests that enhancing Mfn2 expression to promote apoptosis in cancer cells, inflammatory cells, and abnormal cells could serve as a therapeutic target for related diseases.

2.3 Cell Proliferation

Mfn2 is also a proliferation-suppressing gene. Mfn2 overexpression can inhibit cell proliferation by binding to Ras and blocking its activation, thereby preventing extracellular signal-regulated kinase 1/2 (ERK1/2) activation. In other words, Mfn2 inhibits Ras-Raf-MAPK-ERK1/2 signal transduction phosphorylation, suppresses DNA synthesis, and causes mitotic cells to enter a quiescent phase, thereby inhibiting proliferation of various cell types [15]. Additionally, ZHANG et al. [16] demonstrated that Mfn2 can inhibit cell proliferation by upregulating cyclin-dependent kinase inhibitor p21 to block ERK1/2 activation. Studies have shown that the amino-terminal fragment (aa1-264) and carboxy-terminal fragment (aa265-757) of Mfn2 can block cell proliferation through different mechanisms: the amino-terminal fragment inhibits proliferation by interacting with Raf-1, while the carboxy-terminal fragment inhibits proliferation by interacting with Ras [17]. Moreover, unphosphorylated retinoblastoma protein (Rb) combined with transcription factors can cause cell cycle arrest in the G0/G1 phase, and Mfn2 can regulate cell proliferation by reducing Rb phosphorylation levels [18]. These findings suggest that modulating Mfn2 expression to inhibit cancer cell proliferation and promote normal cell growth could help maintain liver function homeostasis and reverse liver damage.

2.5 ER Stress

Mfn2 also participates in regulating the ER stress response. This reaction depends on a complex signal transduction mechanism known as the unfolded protein response (UPR), which aims to clear unfolded proteins and restore ER

homeostasis [11]. Mfn2 can regulate ER stress response signaling through protein kinase R-like ER kinase (PERK) located on the ER membrane. PERK monitors the accumulation of unfolded proteins and activates specific signaling pathways to induce ER stress. Mfn2 is an upstream regulator of PERK and can directly interact with PERK to maintain its inactivation under basal conditions [23]. Studies have shown that Mfn2 ablation can induce activation of UPR proteins in the ER membrane, causing ER stress [24]. Conversely, promoting Mfn2 expression can ameliorate ER stress, thereby slowing or preventing ER stress-related liver injury.

2.6 Mitophagy

Mitophagy is a selective autophagy phenomenon that specifically clears damaged mitochondria to maintain mitochondrial network homeostasis. When mitochondria are damaged in cells, the PINK1/Parkin signaling pathway is activated. PINK1 translocates and accumulates on the mitochondrial outer membrane, recruits the E3 ligase Parkin and activates it. Activated Parkin can ubiquitinate Mfn2 to form ubiquitin chains and recruit lipidated autophagy receptor LC3 to the mitochondrial outer membrane to further form autophagosomes. The damaged mitochondria are then surrounded and engulfed by autophagosomes, which fuse with lysosomes to degrade autophagosome contents, thereby inducing mitophagy [3]. Studies have shown that Mfn2 deficiency can increase the accumulation of dysfunctional mitochondria and cause defects in autophagosome formation and autophagosome-lysosome fusion, leading to autophagy impairment [25-26]. Mfn2 loss in muscle is associated with autophagy inhibition and abnormal mitochondrial accumulation, potentially contributing to sarcopenia [27].

In summary, Mfn2 exerts numerous functional roles through its unique molecular structure and expression regulation, participating in various cell death pathways. Upregulating or downregulating Mfn2 expression can be involved in the development of various liver diseases through different signaling pathways.

3.1 MAFLD

The diagnosis of MAFLD is based on metabolic dysfunction such as hyperlipidemia, type 2 diabetes, and hypertension, and can coexist with other liver diseases [28]. Studies have confirmed that Mfn2 expression is reduced in obese and type 2 diabetic patients, while exercise and weight loss can increase Mfn2 expression. The underlying mechanism may be that physical exercise promotes activation of PGC-1α and ERRα, Mfn2 transcription, mitochondrial fusion, and GLUT4 activation, thereby increasing insulin sensitivity and reducing lipid deposition [29-30]. Conversely, inhibiting mitochondrial Mfn2 expression can

cause mitochondria to lose their normal reticular structure and become scattered isolated aggregates, reduce mitochondrial membrane potential, inhibit aerobic glucose metabolism, increase mitochondrial membrane pore size causing proton leak, stimulate the JNK pathway, promote formation of lipid intermediates, and cause insulin resistance in muscle and liver, thereby inducing MAFLD and related metabolic diseases [14,31]. Insulin administration can upregulate Mfn2 expression by blocking the mitogen extracellular signal regulated kinase (MEK)-dependent cascade reaction, promote Mfn2 binding to Ras to activate the PI3K-Akt signaling pathway, reverse mitochondrial structural changes, induce mitochondrial fusion, and improve IR [32]. Therefore, IR induced by metabolic abnormalities can be reversed by targeting the insulin signaling pathway to promote Mfn2 overexpression. Additionally, specific knockout of Mfn2 in hypothalamic pro-opiomelanocortin neurons can also cause ER stress and leptin resistance, leading to increased appetite, reduced energy expenditure, and obesity [33].

Non-alcoholic steatohepatitis (NASH) is a severe form of non-alcoholic fatty liver disease (NAFLD)/MAFLD that is also associated with Mfn2. HERNÁNDEZ-ALVAREZ et al. [34] found that liver-specific Mfn2 ablation in mice can cause hepatic inflammation, triglyceride accumulation, and promote NASH development. Promoting Mfn2 overexpression in NASH mouse models enables Mfn2 to bind phosphatidylserine (PS) and specifically transfer PS to membrane domains, further transferring it to mitochondria and promoting mitochondrial phosphatidylethanolamine synthesis, inhibiting ER stress, reducing inflammatory responses and triglyceride accumulation, thereby ameliorating the NASH phenotype. In summary, promoting Mfn2 expression may become a new approach for improving MAFLD.

3.2 Viral Hepatitis

Mitochondrial damage and oxidative stress are prominent features of chronic hepatitis B and C, and mitochondrial liver injury has long been considered a consequence of hepatitis B virus (HBV) infection in chronic hepatitis [35]. HBV infection is associated with downregulated cellular Ca^{2+} signaling, mitochondrial depolarization and dysfunction, and reactive oxygen species production. Studies have shown that many DNA and RNA viruses participate in regulating mitophagy, which can suppress host immune responses, prevent clearance by phagocytosis, and facilitate viral replication and maturation [35]. HBV and its encoded hepatitis B virus X protein can induce mitochondrial cascade reactions, stimulate expression of Parkin, PINK1, and autophagy microtubule-associated protein light chain $ $3 (LC3B), and induce Parkin recruitment and translocation to mitochondria, further promoting ubiquitination and degradation of its substrate Mfn2. This leads to altered mitochondrial dynamics (such as mitochondrial swelling, cristae loss, and mitochondrial fission) and ultimately clears damaged mitochondria through mitophagy, promoting viability of infected cells

and inhibiting apoptosis of HBV-infected cells, thereby facilitating persistent infection and chronic hepatitis. Similarly, hepatitis C virus, a positive-sense single-stranded RNA virus, can also induce Parkin-mediated selective autophagy that benefits viral replication. Therefore, designing novel therapeutic approaches against chronic viral infection by targeting Mfn2 overexpression to promote apoptosis of infected cells may be feasible.

3.3 Hepatic Fibrosis

Activation and proliferation of hepatic stellate cells (HSCs) are central to hepatic fibrosis formation, and chronic inflammatory response is the prerequisite and driving force for hepatic fibrosis formation. Therefore, promoting apoptosis of activated HSCs and inhibiting hepatic inflammation are important means to prevent and treat hepatic fibrosis [36]. Mfn2 can inhibit hepatic fibrosis by suppressing production of fibrosis-related factors through multiple signaling pathways. ZHU et al. [37] found that Mfn2 overexpression can inhibit the TGF-$ $1/Smad signaling pathway, downregulate α-smooth muscle actin (α-SMA) and types I, III, and IV collagen, significantly reduce immune cell infiltration, inhibit hepatic inflammatory responses, promote HSC apoptosis, and ameliorate hepatic fibrosis. Additionally, Mfn2 can inhibit HSC proliferation and promote apoptosis through PI3K/Akt and mTOR signaling pathways, thereby inhibiting hepatic fibrosis development [39-40]. Since Mfn2 has been confirmed to be a negative regulator of MAPK signaling, the mechanism by which Mfn2 inhibits hepatic fibrosis-related factor generation in HSCs may be related to MAPK signaling pathway transmission [38]. In summary, Mfn2 is crucial in regulating HSC apoptosis and hepatic fibrosis and may be a potential therapeutic target for alleviating hepatic fibrosis.

3.4 HCC

The Mfn2 gene is located at 1p36.22 on human chromosome 1, a region with high mutation frequency in malignant tumors, suggesting that abnormal expression or functional loss of Mfn2 may be an important factor in tumor development [18]. Studies have shown that Mfn2 is an independent prognostic factor for HCC patients. Mfn2 expression is significantly lower in HCC tissues than in surrounding normal liver tissues, and patients with high Mfn2 expression have longer overall survival than those with low expression, making it a new reference indicator for determining tumor differentiation and pathological staging [41].

In tumors, Mfn2 has dual functions of promoting apoptosis and inhibiting proliferation. High Mfn2 expression promotes tumor cell apoptosis and inhibits tumor cell proliferation through the PI3K/Akt apoptosis pathway and ERK1/2 proliferation pathway, blocking the cell cycle at G0/G1 or G2/M phases to inhibit mitosis and suppress tumor growth [14]. Additionally, high Mfn2 expression can

mediate apoptosis in HepG2 cells, downregulate mitochondrial membrane potential, and cause ER Ca^{2+} to enter mitochondria, leading to decreased ER Ca^{2+} concentration, increased mitochondrial Ca^{2+} concentration, elevated intracellular reactive oxygen species, and induced tumor cell apoptosis [41]. Studies have shown that 17β-hydroxysteroid dehydrogenase 13, an enzyme that catalyzes steroid and lipid metabolism and is closely related to hepatic lipid metabolism and hepatocarcinogenesis, is significantly positively correlated with Mfn2 expression [42], though its direct regulatory relationship requires further investigation. Additionally, microRNA (miRNA) is an important factor inhibiting HCC cell proliferation and promoting apoptosis. Studies have shown that miR-150 and miR-761 may inhibit HCC cell proliferation, migration, and invasion while promoting apoptosis by upregulating Mfn2 levels [43-45]. In summary, Mfn2 is an independent prognostic predictor for HCC patients and a potential therapeutic target for HCC.

3.5 ACLF

Despite remarkable advances in clinical treatment, ACLF still maintains high incidence and mortality rates. XUE et al. [45] showed that Mfn2 overexpression can reduce serum transaminase levels in SD rats and improve massive hepatocyte necrosis and sinusoidal dilation with congestion symptoms caused by ACLF, reducing eosinophil and neutrophil infiltration in hepatocytes. Mfn2 may inhibit lipid accumulation, protein aggregation, chronic cell death, oxidative stress, and inflammation by suppressing the PI3K/AKT/mTOR signaling pathway to induce autophagy, thereby maintaining cellular homeostasis and delaying disease progression to alleviate liver injury in ACLF [46]. Additionally, Mfn2 exerts anti-apoptotic functions in ACLF [45-46]. First, autophagy may be the trigger for Mfn2's anti-apoptotic function in ACLF. Second, Bcl-2/adenovirus E1B interacting protein 3 (BNIP3) is a pro-apoptotic protein that competes with Beclin-1 for binding to Bcl-2 through its BH3 domain, inhibiting Bcl-2 gene expression and promoting apoptosis. In ACLF models, Mfn2 can reduce BNIP3 expression in hepatocyte autophagy injury models and inhibit apoptosis. Therefore, Mfn2 plays a protective role in ACLF and may provide a promising therapeutic target for ACLF patients.

4. Conclusion and Outlook

In summary, with the deepening of Mfn2 research, the functions of Mfn2 have received increasing attention. In addition to mediating mitochondrial fusion and maintaining normal mitochondrial structure and function, Mfn2 plays important roles in regulating cellular energy metabolism, apoptosis, proliferation, mitochondrial-ER tethering, ER stress, and mitophagy, and regulates the development of various liver pathological states. With advances in molecular biology,

the structure, mechanism, and regulatory mechanisms of Mfn2 will be further clarified. Investigating its regulatory roles in different liver diseases is expected to provide crucial new targets and ideas for disease treatment. However, clinical studies on Mfn2 and liver diseases remain limited. Given the important regulatory role of Mfn2 in liver diseases, future research should further investigate the sensitivity and specificity of Mfn2 as a diagnostic marker for various liver diseases in clinical settings, as well as its effectiveness as a therapeutic target, to achieve translation of Mfn2 from basic research to clinical application, ultimately benefiting patients and reducing the incidence and mortality of liver diseases.

Author Contributions: YUAN Xiwei was responsible for data collection and manuscript writing; NAN Yuemin was responsible for quality control and revision, and is accountable for the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

References

[1] WANG Jianzhi, QIAN Ruizhe. *Pathophysiology* [M]. Beijing: People's Medical Publishing House, 2015.
[2] ZACHARIOUDAKIS E, AGIANIAN B, KUMAR MV V, et al. Modulating mitofusins to control mitochondrial function and signaling [J]. *Nat Commun*, 2022, 13(1): 3775. DOI: 10.1038/s41467-022-31324-1.
[3] JOAQUIM M, ESCOBAR-HENRIQUES M. Role of mitofusins and mitophagy in life or death decisions [J]. *Front Cell Dev Biol*, 2020, 8: 572182. DOI: 10.3389/fcell.2020.572182.
[4] HERNÁNDEZ-ALVAREZ M I, ZORZANO A. Mitochondrial dynamics and liver cancer [J]. *Cancers*, 2021, 13(11): 2571. DOI: 10.3390/cancers13112571.
[5] DI NOTTIA M, VERRIGNI D, TORRACO A, et al. Mitochondrial dynamics: molecular mechanisms, related primary mitochondrial disorders and therapeutic approaches [J]. *Genes (Basel)*, 2021, 12(2): 247. DOI: 10.3390/genes12020247.
[6] ZORZANO A, HERNÁNDEZ-ALVAREZ M I, SEBASTIÁN D, et al. Mitofusin 2 as a driver that controls energy metabolism and insulin signaling [J]. *Antioxid Redox Signal*, 2015, 22(12): 1020-1031. DOI: 10.1089/ars.2014.6208.
[7] KUMAR S, PAN C C, SHAH N, et al. Activation of Mitofusin2 by Smad2-RIN1 complex during mitochondrial fusion [J]. *Mol Cell*, 2016, 62(4): 520-531. DOI: 10.1016/j.molcel.2016.04.010.
[8] BASSOT A, PRIP-BUUS C, ALVES A, et al. Loss and gain of function of Grp75 or mitofusin 2 distinctly alter cholesterol metabolism, but all promote triglyceride accumulation in hepatocytes [J]. *Biochim Biophys Acta Mol Cell Biol Lipids*, 2021, 1866(12): 159030. DOI: 10.1016/j.bbalip.2021.159030.
[9] ZHANG Zhe, ZHAO Zhiying, WANG Chao. KLF4 alleviates palmitate-induced insulin resistance in L6 skeletal muscle cells by upregulating Mfn2

expression [J]. *Chinese Journal of Gerontology*, 2014, 34(21): 6076-6079. DOI: 10.3969/j.issn.1005-9202.2014.21.064.
[10] ZHANG C Y, JIA Y Z, LIU B, et al. TLR4 knockout upregulates the expression of Mfn2 and PGC-1α in a high-fat diet and ischemia-reperfusion mice model of liver injury [J]. *Life Sci*, 2020, 254: 117762. DOI: 10.1016/j.lfs.2020.117762.
[11] SEBASTIÁN D, HERNÁNDEZ-ALVAREZ M I, SEGALÉS J, et al. Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis [J]. *Proc Natl Acad Sci U S A*, 2012, 109(14): 5523-5528. DOI: 10.1073/pnas.1108220109.
[12] PICH S, BACH D, BRIONES P, et al. The Charcot-Marie-Tooth type 2A gene product, Mfn2, up-regulates fuel oxidation through expression of OXPHOS system [J]. *Hum Mol Genet*, 2005, 14(11): 1405-1415. DOI: 10.1093/hmg/ddi149.
[13] ZHANG Yangyang, PEI Haifeng, DUAN Haixia, et al. Research status of mitofusin 2 in cardiovascular diseases [J]. *Heart Magazine*, 2015, 27(2): 224-228. DOI: 10.13191/j.chj.2015.0065.
[14] SHI Yulong, YI Chengla. Research progress of mitofusin 2 [J]. *Neural Injury and Functional Reconstruction*, 2017, 12(3): 234-237. DOI: 10.16780/j.cnki.sjssgncj.2017.03.015.
[15] CHEN K H, DASGUPTA A, DING J H, et al. Role of mitofusin 2 (Mfn2) in controlling cellular proliferation [J]. *FASEB J*, 2014, 28(1): 382-394. DOI: 10.1096/fj.13-230037.
[16] ZHANG G E, JIN H L, LIN X K, et al. Anti-tumor effects of Mfn2 in gastric cancer [J]. *Int J Mol Sci*, 2013, 14(7): 13005-13021. DOI: 10.3390/ijms140713005.
[17] ZHU Xuemin, MENG Qinghua. Functions of mitofusin-2 [J]. *Beijing Medicine*, 2015, 37(12): 1177-1179. DOI: 10.15932/j.0253-9713.2015.12.013.
[18] GUAN Fei, FANG Kewei. Research progress on biological functions of mitofusin-2 and its role in diseases [J]. *Shandong Medical Journal*, 2017, 57(38): 106-109. DOI: 10.3969/j.issn.1002-266X.2017.38.035.
[19] NAON D, ZANINELLO M, GIACOMELLO M, et al. Critical reappraisal confirms that Mitofusin 2 is an endoplasmic reticulum-mitochondria tether [J]. *Proc Natl Acad Sci U S A*, 2016, 113(40): 11249-11254. DOI: 10.1073/pnas.1606786113.
[20] CASELLAS-DÍAZ S, LARRAMONA-ARCAS R, RIQUÉ-PUJOL G, et al. Mfn2 localization in the ER is necessary for its bioenergetic function and neuritic development [J]. *EMBO Rep*, 2021, 22: e51954. DOI: 10.15252/embr.202051954.
[21] DECUYPERE J P, MONACO G, BULTYNCK G, et al. The IP3 receptor-mitochondria connection in apoptosis and autophagy [J]. *Biochim Biophys Acta BBA Mol Cell Res*, 2011, 1813(5): 1003-1013. DOI: 10.1016/j.bbamcr.2010.11.023.
[22] FILADI R, GREOTTI E, TURACCHIO G, et al. Mitofusin 2 ablation increases endoplasmic reticulum-mitochondria coupling [J]. *PNAS*, 2015, 112(17): E2174-2181. DOI: 10.1073/pnas.1504880112.
[23] VAN VLIET A R, AGOSTINIS P. Mitochondria-associated membranes

and ER stress [J]. *Curr Top Microbiol Immunol*, 2018, 414: 73-102. DOI: 10.1007/82_{2017}2.
[24] NGOH G A, PAPANICOLAOU K N, WALSH K. Loss of mitofusin 2 promotes endoplasmic reticulum stress [J]. *J Biol Chem*, 2012, 287(24): 20321-20332. DOI: 10.1074/jbc.M112.359174.
[25] PENG C, RAO W, ZHANG L, et al. Mitofusin 2 exerts a protective role in ischemia reperfusion injury through increasing autophagy [J]. *Cell Physiol Biochem*, 2018, 46(6): 2311-2324. DOI: 10.1159/000489621.
[26] SONG M S, MIHARA K, CHEN Y, et al. Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts [J]. *Cell Metab*, 2015, 21(2): 273-286. DOI: 10.1016/j.cmet.2014.12.011.
[27] SEBASTIÁN D, SORIANELLO E, SEGALÉS J, et al. Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway [J]. *EMBO J*, 2016, 35(15): 1677-1693. DOI: 10.15252/embj.201593084.
[28] ESLAM M, SANYAL A J, GEORGE J, et al. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease [J]. *Gastroenterology*, 2020, 158(7): 1999-2014.e1. DOI: 10.1053/j.gastro.2019.11.312.
[29] GONÇALVES I O, PASSOS E, DIOGO C V, et al. Exercise mitigates mitochondrial permeability transition pore and quality control mechanisms alterations in nonalcoholic steatohepatitis [J]. *Physiol Appliquee Nutr Metab*, 2016, 41(3): 298-306. DOI: 10.1139/apnm-2015-0470.
[30] DONG J H, LOOR J J, ZUO R K, et al. Low abundance of mitofusin 2 in dairy cows with moderate fatty liver is associated with alterations in hepatic lipid metabolism [J]. *J Dairy Sci*, 2019, 102(8): 7536-7547. DOI: 10.3168/jds.2019-16544.
[31] BACH D, PICH S, SORIANO F X, et al. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity [J]. *J Biol Chem*, 2003, 278(19): 17190-17197. DOI: 10.1074/jbc.M212754200.
[32] PAWLIKOWSKA P, GAJKOWSKA B, ORZECHOWSKI A. Mitofusin 2 (Mfn2): a key player in insulin-dependent myogenesis in vitro [J]. *Cell Tissue Res*, 2007, 327(3): 571-581. DOI: 10.1007/s00441-006-0320-3.
[33] SCHNEEBERGER M, DIETRICH M O, SEBASTIÁN D, et al. Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance [J]. *Cell*, 2013, 155(1): 172-187. DOI: 10.1016/j.cell.2013.09.003.
[34] HERNÁNDEZ-ALVAREZ M I, SEBASTIÁN D, VIVES S, et al. Deficient endoplasmic reticulum-mitochondrial phosphatidylserine transfer causes liver disease [J]. *Cell*, 2019, 177(4): 881-895.e17. DOI: 10.1016/j.cell.2019.04.010.
[35] KIM S J, KHAN M, QUAN J, et al. Hepatitis B virus disrupts mitochondrial dynamics: induces fission and mitophagy to attenuate apoptosis [J]. *PLoS Pathog*, 2013, 9(12): e1003722. DOI: 10.1371/journal.ppat.1003722.
[36] Chinese Society of Hepatology, Chinese Society of Gastroenterology, Chinese Society of Infectious Diseases. Consensus on diagnosis and treatment of hepatic fibrosis (2019) [J]. *Gastroenterology*, 2019, 24(9): 546-556. DOI:

10.3969/j.issn.1008-7125.2019.09.007.
[37] ZHU H Z, SHAN Y Q, GE K, et al. Specific overexpression of mitofusin-2 in hepatic stellate cells ameliorates liver fibrosis in mice model [J]. *Hum Gene Ther*, 2020, 31(1/2): 103-109. DOI: 10.1089/hum.2019.153.
[38] WANG R, ZHANG H, WANG Y Y, et al. Inhibitory effects of quercetin on the progression of liver fibrosis through the regulation of NF- B/I Bα, p38 MAPK, and Bcl-2/Bax signaling [J]. *Int Immunopharmacol*, 2017, 47: 126-133. DOI: 10.1016/j.intimp.2017.03.029.
[39] LUO Q Y, HUO P, WANG L L, et al. The influencing mechanism of mTOR signal pathway mediated by mitofusin-2 in development of follicle [J]. *Eur Rev Med Pharmacol Sci*, 2018, 22(8): 2212-2217. DOI: 10.26355/eurrev_{{{201804}}_{{14806}}}.
[40] CHEN Z P, LIN Z Y, YU J D, et al. Mitofusin-2 restrains hepatic stellate cells' proliferation via PI3K/Akt signaling pathway and inhibits liver fibrosis in rats [J]. *J Healthc Eng*, 2022, 2022: 6731335. DOI: 10.1155/2022/6731335.
[41] WANG X M, LIU Y D, SUN J, et al. Mitofusin-2 acts as biomarker for predicting poor prognosis in hepatitis B virus related hepatocellular carcinoma [J]. *Infect Agent Cancer*, 2018, 13: 36. DOI: 10.1186/s13027-018-0212-7.
[42] ZENG Chaiming, YAN Maolin, SHI Yihai, et al. Expression changes and bioinformatics analysis of 17β-hydroxysteroid dehydrogenase 13 and mitofusin 2 in hepatocellular carcinoma tissues [J]. *Journal of Clinical Hepatology*, 2019, 35(12): 2736-2740. DOI: 10.3969/j.issn.1001-5256.2019.12.019.
[43] WEN Feng, XIANG Yan. Study on the mechanism of MiR-150, CCNB1 and MFN2 in regulating apoptosis and inhibiting invasion and migration of Huh-7 hepatocellular carcinoma cells [J]. *Journal of Hainan Medical College*, 2019, 25(9): 650-653. DOI: 10.13210/j.cnki.jhmu.20190325.002.
[44] ZHOU X H, ZHANG L S, ZHENG B C, et al. MicroRNA-761 is upregulated in hepatocellular carcinoma and regulates tumorigenesis by targeting Mitofusin-2 [J]. *Cancer Sci*, 2016, 107(4): 424-432. DOI: 10.1111/cas.12904.
[45] XUE R, YANG J, JIA L, et al. Mitofusin2, as a protective target in the liver, controls the balance of apoptosis and autophagy in acute-on-chronic liver failure [J]. *Front Pharmacol*, 2019, 10: 601. DOI: 10.3389/fphar.2019.00601.
[46] XUE R, ZHU X M, JIA L, et al. Mitofusin2, a rising star in acute-on-chronic liver failure, triggers macroautophagy via the mTOR signalling pathway [J]. *J Cell Mol Med*, 2019, 23(11): 7810-7818. DOI: 10.1111/jcmm.14658.

(Received: March 1, 2023; Revised: April 7, 2023)

Note: Figure translations are in progress. See original paper for figures.