

REVIEW

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Extracellular vesicles: opening up a new perspective for the diagnosis and treatment of mitochondrial dysfunction

Jiali Li^{1,2†}, Tangrong Wang^{1,2†}, Xiaomei Hou³, Yu Li², Jiabin Zhang², Wenhuan Bai², Hui Qian² and Zixuan Sun^{1,2*}

Abstract

Mitochondria are crucial organelles responsible for energy generation in eukaryotic cells. Oxidative stress, calcium disorders, and mitochondrial DNA abnormalities can all cause mitochondrial dysfunction. It is now well documented that mitochondrial dysfunction significantly contributes to the pathogenesis of numerous illnesses. Hence, it is vital to investigate innovative treatment methods targeting mitochondrial dysfunction. Extracellular vesicles (EVs) are cell-derived nanovesicles that serve as intercellular messengers and are classified into small EVs (sEVs, < 200 nm) and large EVs (lEVs, > 200 nm) based on their sizes. It is worth noting that certain subtypes of EVs are rich in mitochondrial components (even structurally intact mitochondria) and possess the ability to transfer them or other contents including proteins and nucleic acids to recipient cells to modulate their mitochondrial function. Specifically, EVs can modulate target cell mitochondrial homeostasis as well as mitochondria-controlled apoptosis and ROS generation by delivering relevant substances. In addition, the artificial modification of EVs as delivery carriers for therapeutic goods targeting mitochondria is also a current research hotspot. In this article, we will focus on the ability of EVs to modulate the mitochondrial function of target cells, aiming to offer novel perspectives on therapeutic approaches for diverse conditions linked to mitochondrial dysfunction.

Keywords Mitochondrial dysfunction, Pathogenesis, Extracellular vesicles, Biomarkers, Targeted therapies

Introduction

Mitochondria are ubiquitous in nearly all eukaryotic cells and serve as indispensable organelles for maintaining cellular energy metabolism. They fulfill a vital role in cellular processes by serving as the primary source of ATP synthesis, controlling the creation of reactive oxygen species (ROS), facilitating intracellular Ca²⁺ cycling, and participating in apoptosis [1]. The occurrence of even a

minor disruption in any of the mitochondrial membrane, respiratory chain, enzyme activity, or mitochondrial DNA (mtDNA) can result in the disruption of cellular processes and subsequent mitochondrial dysfunction, which can have direct or indirect implications on the overall normal function of the cell [2]. Therefore, therapeutic modulation by targeting mitochondria could lead to better management of diseases involving mitochondrial dysfunction, such as neurodegenerative diseases, cardiovascular diseases (CVD), and cancer [3]. Currently, mitochondrially targeted antioxidants [4], mitochondrial transplantation [5], and mitochondrial fission inhibitor Mdivi-1 [6] have made progress in experimental and clinical studies. Nevertheless, considering the drawbacks of these medications, such as poor efficacy and weak

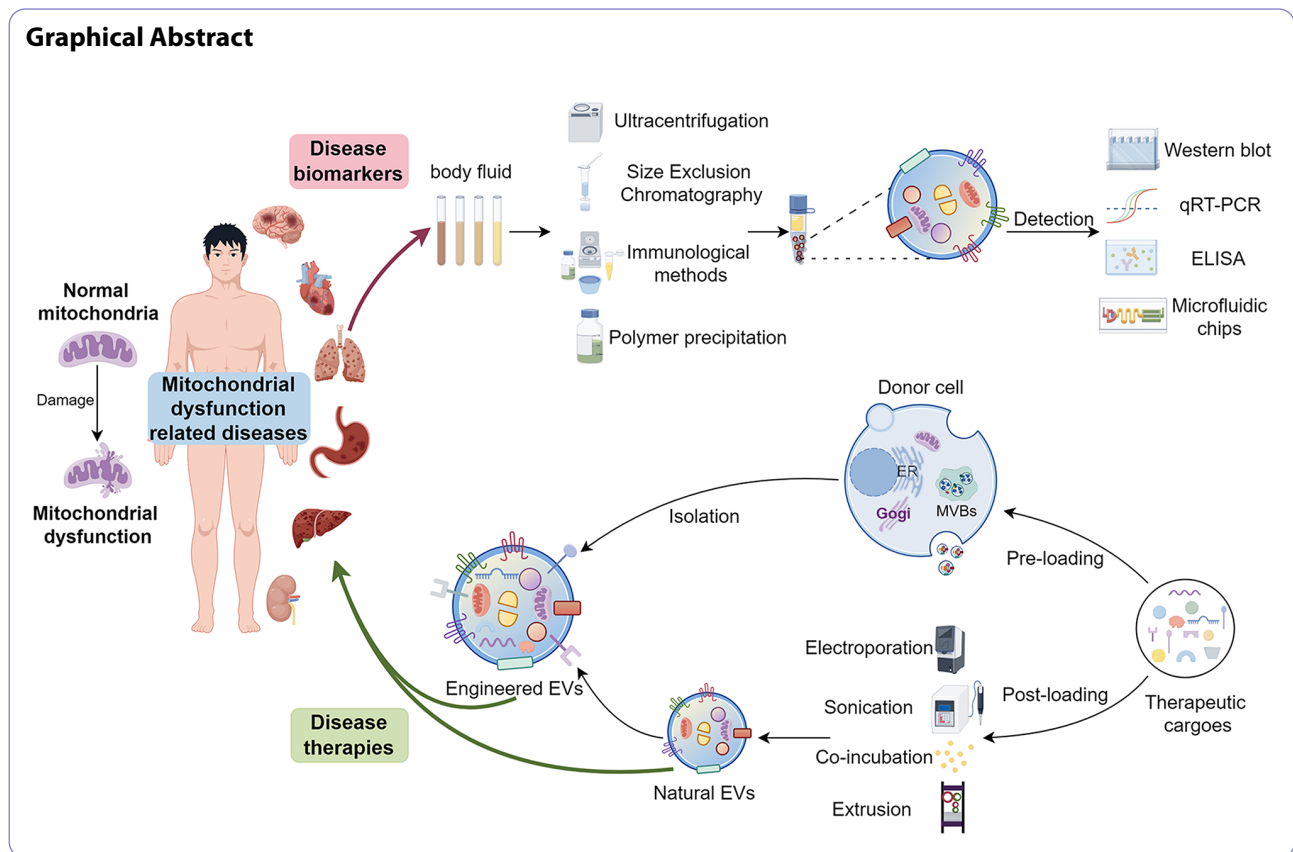
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targeting, there is a need to find more effective strategies for mitochondria-targeted therapy [7].

Extracellular vesicles (EVs) are nanoscale vesicles that cells release into the extracellular space, and these vesicles act as communication vehicles by carrying proteins, lipids, and nucleic acids for intercellular transfer [8]. Under physiological conditions, EVs are crucial for tissue growth, homeostasis maintenance, and tissue repair [9, 10]. More importantly, parental cells and the surrounding environment have a significant impact on the cargo and secretion of EVs. In a diseased state, they obtain pathological content through cellular processes closely related to the pathogenesis of the disease, thus becoming contributors to many diseases. For example, EVs are linked to the spread of neurotoxic proteins in Alzheimer's disease. Recent investigation has indicated that EVs are also enriched in mitochondria or mitochondrial components [11]. Under pathological conditions, the mitochondrial content of EVs released by donor cells into the circulatory system is altered, making EVs an important target for liquid biopsy [12]. On the other hand, healthy cell-derived EVs can rescue impaired mitochondrial function in recipient cells by transporting functional mitochondria (mitochondrial components) or other contents. By transferring mitochondria, some research showed that EVs enhanced heart function following myocardial infarction

[13] or lessened hepatic ischemia-reperfusion (I/R) injury [14]. In addition, another focus of EV research is to utilize them as carriers for delivering mitochondria-targeted therapeutic agents, such as mitochondria-targeted photosensitizers and sonosensitizers [15, 16]. These findings suggest that EVs may become a new and effective treatment approach for mitochondrial dysfunction related diseases. However, some key issues remain elusive, such as the detailed mechanism of integration between mitochondria (mitochondrial components) in EVs and the mitochondrial network in target cells and how different goods in EVs affect the mitochondrial function of target cells, which require further exploration.

In this review, we elucidate the significance of mitochondrial dysfunction in the occurrence and development of various diseases, as well as the evidence for the presence of mitochondrial components in EVs. We also discussed the potential utility of EVs as diagnostic indicators and therapeutic interventions for mitochondrial dysfunction related diseases, providing a theoretical basis for their clinical translation.

Mitochondrial dysfunction

Mitochondrial structure

Mitochondria are encapsulated by two membranes and are divided into four distinct regions: the outer

mitochondrial membrane (OMM), the intermembrane space (IMS), the inner mitochondrial membrane (IMM), and the mitochondrial matrix. The OMM contains pore proteins involved in material transportation and acts as a communication platform between mitochondria and other organelles [17]. The IMS contains cytochrome C (Cyt C) and apoptosis-inducing factor (AIF), which participate in apoptosis [18]. The IMM is a highly complex region that houses the electronic transport chain (ETC), ATP synthase, and transport proteins [19]. The IMM folds inward to form cristae, increasing the surface area of the IMM and thus maximizing the function of mitochondria in synthesizing bioenergy [20]. Within the mitochondrial matrix, the enzymes connected to the

tricarboxylic acid (TCA) cycle and fatty acid oxidation (FAO), as well as mtDNA, are present (Fig. 1).

Mitochondrial function

ATP synthesis

Known as the powerhouse, the principal role of mitochondria is to generate ATP to fulfill the energy requirements of cells for survival. This process involves the ETC, which oxidizes dihydronicotinamide adenine dinucleotide (NADH) and dihydroflavine adenine dinucleotide (FADH₂) produced by the TCA cycle and uses the energy thus gained to pump protons from the mitochondrial matrix into the IMS, thereby creating a proton gradient. Subsequently, as protons move back across the gradient

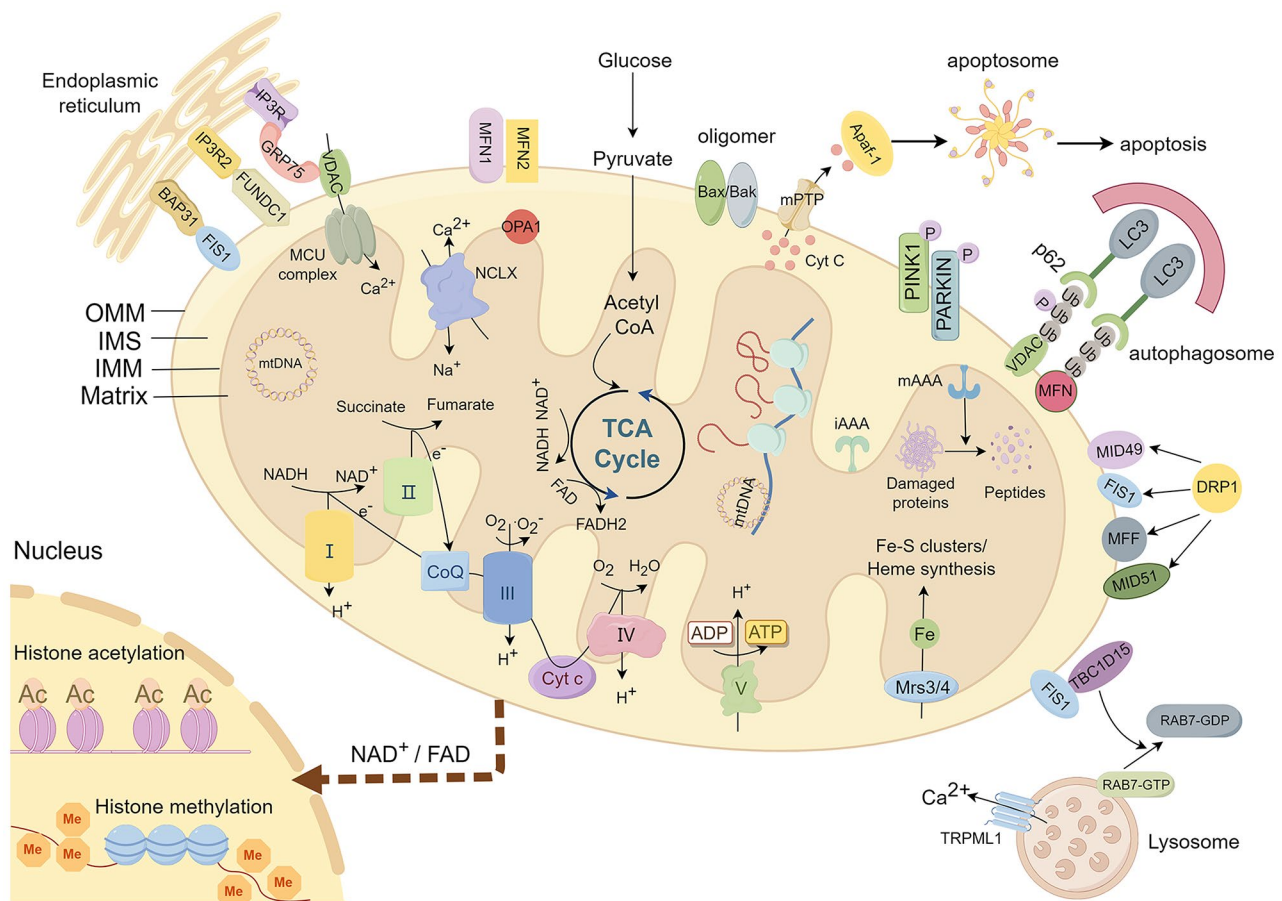


Fig. 1 Mitochondrial structure and function (by Figdraw). Mitochondria are composed of four functional regions: OMM, IMS, IMM, and mitochondrial matrix. Mitochondria are essential for multiple cellular processes, including ATP synthesis; protein, lipid, and nucleotide metabolism; calcium homeostasis maintenance; mitochondrial dynamics; iron-sulfur clusters and heme synthesis; mitochondrial autophagy; apoptosis; formation of mitochondria-associated membranes (MAMs) with endoplasmic reticulum and lysosomes. OMM, outer mitochondrial membrane; IMS, intermembrane space; IMM, inner mitochondrial membrane; MFN1, mitofusin 1; MFN2, mitofusin 2; OPA1, optic atrophy 1; DRP1, dynamin-related protein 1; FIS1, fission 1; MFF, mitochondrial fission factor; MID49, mitochondrial dynamics proteins of 49 kDa; MID51, mitochondrial dynamics proteins of 51 kDa; PINK1, PTEN-induced kinase 1; Ub, ubiquitin; P, phosphorylation; MCU, mitochondrial calcium transporter; NCLX, mitochondrial Na⁺/Ca²⁺ exchanger; APAF-1, apoptotic peptidase activating factor 1; mPTP, mitochondrial permeability transition pore; Cyt C, Cytochrome C; AAA, ATP-dependent proteases of the inner membrane; mtDNA, mitochondrial DNA; TCA, tricarboxylic acid; IP3R, inositol 1,4,5-trisphosphate receptor; GRP75, glucose-regulated protein 75; VDAC, voltage-dependent anion-selective channel; IP3R2, type 2 inositol 1,4,5-trisphosphate receptors; FUNDC1, FUN14 domain containing 1; BAP31, B cell receptor associated protein 31; TBC1D15, TBC domain family member 15; FAD, flavin adenine dinucleotide; FADH₂, dihydroflavine adenine dinucleotide; NAD⁺, nicotinamide adenine dinucleotide; NADH, dihydronicotinamide adenine dinucleotide

into the matrix, the enzyme ATP synthase is activated, catalyzing the synthesis of ATP [21].

Mediating apoptosis

The process of active cell death called apoptosis, which is regulated by particular genes, is crucial for embryonic growth and preserving internal homeostasis [22]. The pathways of apoptosis include intrinsic (or mitochondrial), extrinsic (or death receptor), and intrinsic endoplasmic reticulum pathways [23]. Several cellular stressors, including hypoxia, growth factor deprivation, and damage to DNA, can trigger the mitochondrial pathway of apoptosis. Under stress stimulation, the pro-apoptotic proteins Bax and Bak form oligomers embedded in the OMM, inducing mitochondrial outer membrane permeabilization (MOMP). This process facilitates the release of pro-apoptotic substances into the cytoplasm from the IMS, such as Cyt C [24]. Once in the cytoplasm, Cyt C binds to apoptotic peptidase activating factor 1 and procaspase-9 to form an apoptosome, initiating the caspase cascade reaction and leading to apoptosis [25].

Calcium homeostasis

Ca^{2+} is an intracellular second messenger, participating in many physiological processes, such as muscle contraction and cellular signal transduction [26]. As an important organelle for intracellular calcium regulation, mitochondria can take up and release Ca^{2+} . The passage of Ca^{2+} through the IMM is mediated by the mitochondrial calcium transporter, which functions as a highly selective ion channel [27]. There are three ways that Ca^{2+} can leave the mitochondrial matrix: the mitochondrial permeability transition pore (mPTP), the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger, and the mitochondrial $\text{H}^+/\text{Ca}^{2+}$ exchanger [28].

ROS generation

Mitochondria are the main source of intracellular ROS. These chemicals result from an incomplete reduction of oxygen during the electron transfer cascade of oxidative phosphorylation (OXPHOS) [29]. Under physiological circumstances, ROS are widely involved in cell signal transduction and life processes. For example, ROS are necessary for skeletal muscle contraction and ovarian cycle regulation [30, 31]. Appropriate concentrations of ROS drive cyclin-dependent kinase 2 phosphorylation to promote cell proliferation [32]. However, high levels of ROS damage cellular lipids, proteins, and nucleic acids, which can ultimately result in apoptotic or necroptotic cell death [33].

Other functions

Apart from the primary functions mentioned above, mitochondria also participate in the metabolism of

amino acids, lipids, and nucleic acids, the biosynthesis of heme and iron-sulfur (Fe/S) clusters, and the import and processing of precursor proteins synthesized on cytoplasmic ribosomes [34]. Additionally, the OMM can come into physical contact with other cellular organelles, forming mitochondria-associated membranes [35]. Mitochondria-endoplasmic reticulum contacts provide a platform for various cellular functions, including calcium homeostasis maintenance, autophagy, and lipid metabolism [36]. Similarly, mitochondria-lysosome contacts modulate mitochondrial and lysosomal dynamics and calcium signaling [37, 38].

The mechanism of mitochondrial dysfunction

Oxidative stress

The term “oxidative stress” implies an imbalance between oxidation and anti-oxidation caused by excessive ROS formation that exceeds the body’s ability to scavenge them. Overexposure to ROS causes mPTP to open, which in turn drives mitochondria to enlarge, rupture, and release Cyt C, resulting in mitochondrial malfunction and apoptosis [39]. Elevated ROS also directly harm mtDNA, and the buildup of compromised mtDNA can cause the destruction of mitochondria [40]. Furthermore, ROS induce Ca^{2+} into mitochondria, disrupting calcium homeostasis, which is another important contributing factor to mitochondrial dysfunction (Fig. 2) [41].

mtDNA mutation

Mitochondrial dysfunction also occurs when mtDNA mutations accumulate to a certain threshold [42]. Mitochondria have their own DNA and can independently replicate, transcribe, and translate some of the mitochondrial proteins. The proteins encoded are essential for participating in electron transfer and OXPHOS. Due to its naked nature, absence of histone protection, and direct exposure to high ROS, mtDNA is more prone to mutations than nuclear DNA (nDNA) [43]. The presence of mtDNA deletion mutations impedes OXPHOS, diminishes ATP synthesis, and increases oxygen radical production, all of which impair mitochondrial function [44].

Impaired mitochondrial quality control

Impaired mitochondrial dynamics Mitochondrial dynamics is the process by which mitochondria continually fuse and fission to preserve the quantity and quality of mitochondria [45]. Mitochondrial fusion promotes the interchange of mtDNA, proteins, and lipids within mitochondria. The fusion of normal and damaged mitochondria helps lessen cellular stress and maintain mitochondrial integrity [46]. Mitochondrial fission generates new mitochondria and isolates damaged mitochondria for degradation through mitophagy [47]. Mitofusin 1 and mitofusin 2 (MFN2) mediate OMM fusion, while IMM

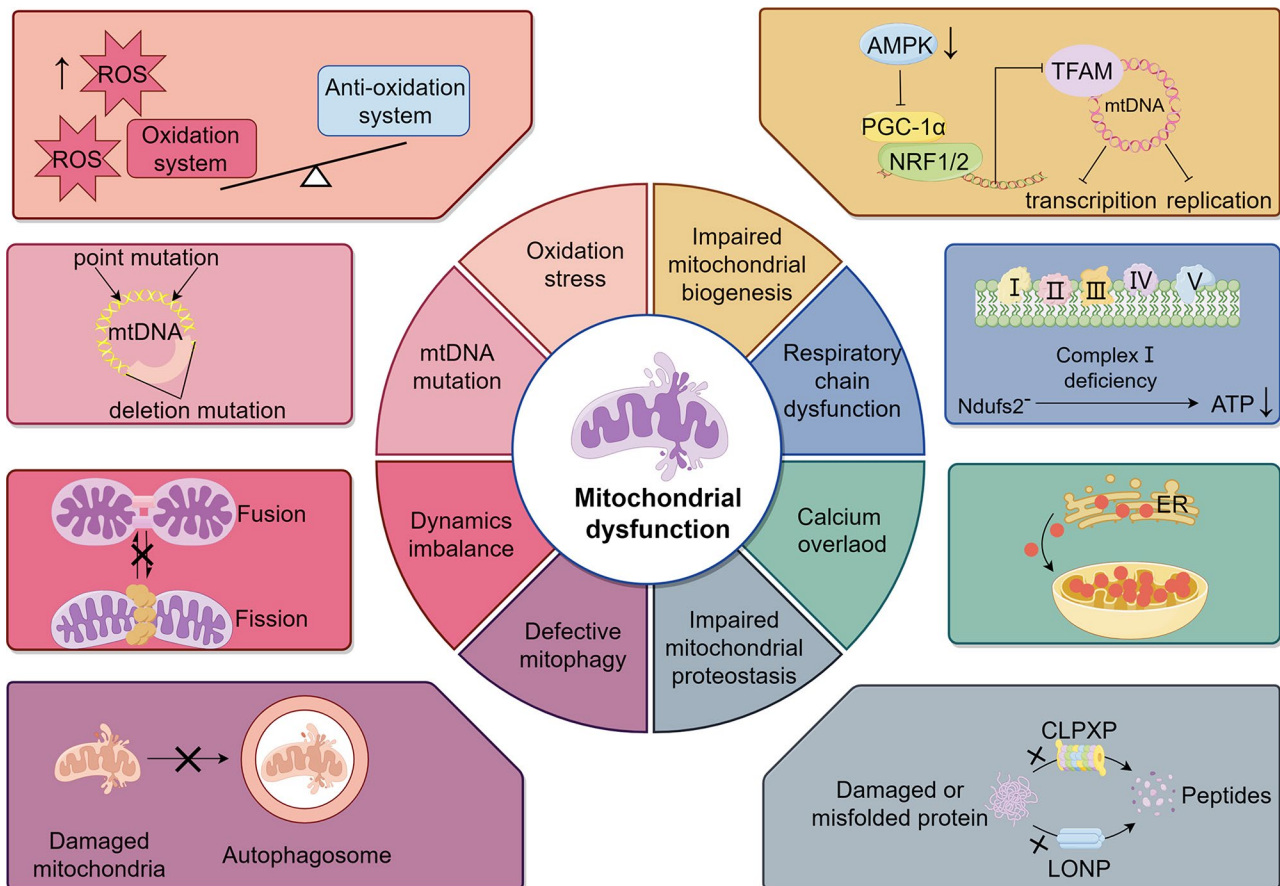


Fig. 2 The mechanisms of mitochondrial dysfunction (by Figdraw). Mitochondrial dysfunction can result from a range of mechanisms, primarily encompassing oxidative stress, mtDNA mutation, disturbances in mitochondrial dynamics, defects in autophagy, impaired mitochondrial biogenesis, reduced mitochondrial respiratory chain enzyme activity, calcium overload, and impaired mitochondrial protein homeostasis. ROS, reactive oxygen species; AMPK, AMP-activated protein kinase; PGC-1 α , peroxisome proliferator-activated receptor- γ coactivator-1 α ; NRF1/2, nuclear respiratory factor 1/2; TFAM, mitochondrial transcription factor A; mtDNA, mitochondrial DNA; LONP, Lon protease; ER, endoplasmic reticulum

fusion is mediated by optic atrophy 1. The primary proteins involved in fission are dynamin-related protein 1 (Drp1) and fission 1. Abnormal fission and fusion lead to excessive mitochondrial fragmentation and elongation, respectively, which upset the dynamic equilibrium of the mitochondria and impair their normal physiological processes [48].

Impaired mitochondrial biogenesis Peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) serves as an important regulator of mitochondrial biogenesis and has the capacity to stimulate the expression of genes related to FAO and OXPHOS by activating peroxisome proliferator-activated receptors and nuclear respiratory factor 1/2 [49]. AMP-activated protein kinase (AMPK) plays a pivotal role in upregulating the expression of PGC-1 α , thereby facilitating mitochondrial biogenesis and energy production [50]. Decreased

expression of PGC-1 α leads to a decline in biogenesis and ATP synthesis, causing mitochondrial dysfunction [51].

Impaired mitophagy Mitophagy, a crucial process for the control of mitochondrial quality, selectively eliminates excess or malfunctioning mitochondria to preserve intracellular and mitochondrial homeostasis [52]. The predominant mechanism for mitophagy is the PINK1/Parkin pathway. When mitochondria are under stress, the entry of PINK into the IMM is hindered, leading to the accumulation of PINK1 at the OMM and the activation and recruitment of Parkin to damaged mitochondria [53]. Activated Parkin ubiquitinates OMM proteins, which are subsequently recognized by autophagy receptors such as p62, and then degrades damaged mitochondria by binding to LC3 to form autophagosomes [54]. The buildup of impaired mitochondria arises from defective mitochondrial autophagy, triggering the generation of ROS and ultimately culminating in cell death.

Mitochondrial dysfunction related diseases

Neurodegenerative diseases

Alzheimer's disease (AD) The accumulation of Amyloid β ($A\beta$) and hyperphosphorylated tau proteins has traditionally been considered the main causes of AD. However, recent findings indicate that mitochondrial dysfunction represents an early stage in the development of AD and could potentially contribute to or exacerbate its progression [55]. Oxidative stress is a key contributor to the development and progression of AD. ROS have the ability to increase the generation of $A\beta$, thus intensifying the level of oxidative stress within the mitochondria and creating a detrimental feedback loop [56]. In the pathological conditions of AD, mitochondrial ROS (mtROS) and Ca^{2+} are abnormally elevated, promoting the opening of mPTP. Simultaneous inhibition of mitochondrial Ca^{2+} overload and mPTP opening effectively alleviated AD neuropathology and rescued cognitive deficits [57]. In addition, the equilibrium between fusion and fission is disrupted under the pathological circumstances of AD. Xie et al. [58] discovered that decreased CEND1 led to the overexpression of Drp1, which caused aberrant mitochondrial fission and accelerated the advancement of AD. Overexpression of CEND1 or the use of Mdivi-1 restored mitochondrial function and improved cognitive impairment.

Parkinson's disease (PD) In the substantia nigra of individuals with PD, there was a notable reduction in the activity of mitochondrial complex I and an elevated presence of mtDNA deletion. These findings suggested that mitochondrial dysfunction was implicated in the pathogenesis of PD [59]. Gonzalez-Rodriguez et al. [60] discovered that targeted suppression of the Ndufs2 gene, responsible for encoding the catalytic core subunit of mitochondrial complex I, led to the emergence of motor impairments linked to PD. In addition, mitochondrial dysfunction resulting from mutations in Parkin and PINK1 genes has been strongly linked to PD. In Parkin gene deletion mice, elevated levels of PARIS were observed, along with a decrease in its primary inhibitory factor, PGC-1 α [61]. The downregulation of PGC-1 α has been connected to changes in mitochondrial dynamics, an increase in ROS production, and a decrease in OXPHOS. The absence of Parkin in PD also destabilized the mitochondria-lysosome contact, and deficiencies of various amino acids in neuronal mitochondria contributed to mitochondrial dysfunction, which exacerbated the pathology of PD [62]. In dopaminergic neurons with PINK1 mutations, the ER-mitochondrial contact site was enhanced, and mitochondrial Ca^{2+} levels were raised, causing mitochondrial enlargement and neuronal death [63].

Cardiovascular disease

Myocardial infarction (MI) MI is myocardial ischemic-hypoxic necrosis brought on by a reduction in coronary artery blood flow. In the ischemic and hypoxic environments, mitochondrial membrane potential decreases, mitochondrial Ca^{2+} overload increases, and mtROS are produced excessively, leading to mitochondrial damage, which in turn enlarges the area of MI. Decreased Ndufs1 expression, along with mitochondrial impairment and disorganization of cristae, were evident in the cardiac tissue of the mice following MI [64]. Overexpression of Ndufs1 could restore mitochondrial respiratory capacity and reduce levels of mtROS. In addition, the mitigation of excess ROS has the potential to counteract mitochondrial damage in cases of MI. Zheng et al. [65] developed a ROS-responsive liposomal composite hydrogel that contained elamipretide (SS-31) that directly targeted mitochondria in damaged myocardium, inhibited ROS production, and improved mitochondrial dysfunction, thereby enhancing cardiac performance.

Myocardial ischemia-reperfusion injury (MI/R) MI/R refers to the phenomenon that the injury aggravates after a certain period of ischemic myocardial tissue restores blood supply. During the process of I/R, the opening of mPTP occurs continuously, causing mitochondrial swelling and OMM rupture, as well as the release of Cyt C and AIF, which induce myocardial cell apoptosis [66]. Li et al. [67] found that S100a8/a9, a key initiator molecule of MI/R, inhibited mitochondrial complex I, resulting in insufficient ATP synthesis and ultimately leading to cardiomyocyte death. Another study demonstrated that during MI/R, MMP2 was activated to catalyze the hydrolysis of MFN2, leading to compromised mitochondrial respiration and subsequent induction of cardiac inflammation [68]. Increased mitochondrial fission is a key contributor to MI/R injury, and the use of Mdivi-1 has been shown to effectively attenuate cardiomyocyte apoptosis, ameliorate myocardial mitochondrial dysfunction, and enhance cardiac function [69].

Cancer

Tumor development is a multifaceted process in which multiple signaling networks interact with and modulate each other. Various forms of mitochondrial dysfunction, such as TCA cycle enzyme defects, mtDNA mutations, and mitochondrial dynamics changes, have been observed across a broad range of cancers. Dysregulation of TCA cycle metabolites caused by deficiency of isocitrate dehydrogenase, succinate dehydrogenase (SDH), and fumarate hydratase (FH) drives the initiation and progression of cancer [70]. SDH mutations are commonly seen in pheochromocytomas, gastrointestinal

stromal tumors, and pituitary tumors [71]. A recent study has found a significant link between the lowering of SDHA/B and the advancement and poor prognosis of hepatocellular carcinoma [72]. Mutations in the FH gene can cause hereditary leiomyomatosis and renal cell carcinoma [73]. The excessive fumarate produced by FH mutations induced epigenetic changes in miR200 with anti-tumor metastasis effects, promoting epithelial-mesenchymal transformation (EMT) and the occurrence and spread of cancer [74]. Furthermore, the accumulation of fumarate inhibited the biological activity of PTEN through cysteine succinylation, thereby promoting the malignant progression of type 2 papillary renal cell carcinoma [75]. Additionally, there is a substantial functional association between mtDNA mutations and malignancies, as demonstrated by the confirmation of this relationship in eosinophilic tumors and prostate cancer [76]. Smith et al. [77] found that somatic mtDNA mutations resulted in OXPHOS deficiency, thus promoting metabolic remodeling, which in turn sped up the growth of colorectal tumors.

Lung diseases

Chronic obstructive pulmonary disease (COPD) COPD is a chronic lung disease typified by persistent airstream limitation, often linked to smoking and genetic determinants. The connection between COPD and mitochondrial dysfunction has been supported by numerous investigations. In a study involving bronchial epithelial cells exposed to aqueous cigarette smoke extract, it was observed that mitochondria were swollen, severely disrupted cristae, mitochondrial depolarized, and increased mtROS, indicating that cigarette smoke-induced damage to mitochondrial structure and function [78]. Furthermore, decreased levels of Parkin in COPD patients confirmed the involvement of impaired mitophagy in the progression of COPD [79]. The relationship between the mitochondrial-iron axis and COPD also has significant importance. In an inducible model of COPD, upregulation of IRP2 caused increased mitochondrial iron loading, which in turn resulted in impaired mitochondrial function and consequent death of epithelial cells [80].

Acute lung injury (ALI) ALI is acute hypoxic respiratory failure syndrome following severe infection, trauma, or shock. Cen et al. [81] observed that stimulation of lung microvascular endothelial cells with lipopolysaccharides (LPS) resulted in ROS generation and apoptosis, accompanied by mitochondrial swelling, reduced matrix density, cristae loss, and a decrease in mitochondrial membrane potential, suggesting impaired mitochondrial function during ALI. Treatment with the antioxidant mitoQ was found to reverse LPS-induced mitochondrial dysfunction

by eliminating excessive ROS and reducing the mitochondrial apoptotic pathway. In another model of LPS-induced ALI, it was reported that alveolar epithelial cells (AECs) had a significantly higher citrate content in response to LPS stimulation. The accumulated citrate drove mitochondrial fission through the recruitment of Drp1 by FUN14 domain containing 1 (FUNDC1), leading to excessive autophagy and causing necroptosis, which ultimately initiated and promoted the development of ALI [82].

Kidney diseases

Chronic kidney disease (CKD) CKD is characterized by the gradual and ongoing destruction of the structure and function of the kidneys, which can be caused by various factors including diabetes, hypertension, glomerulonephritis, and hereditary or cystic diseases, with a duration of more than three months. Coughlan et al. [83] discovered that aberrant mitochondrial bioenergetics occurred before the onset of albuminuria and renal histologic changes in the development of diabetes to diabetic kidney disease (DKD) in rats, thereby providing evidence in favor of the theory that mitochondrial damage is an important factor in DKD. In individuals with CKD and in mouse models, there was a decrease in the expression of LONP1, a protein responsible for preserving the stability of mitochondrial proteins. This downregulation resulted in the impairment of mitochondrial function, thereby exacerbating the progression of CKD [84].

Acute kidney injury (AKI) AKI is a clinical condition brought on by an abrupt loss of renal function due to I/R, sepsis, or nephrotoxic substances [85]. Experimental investigations revealed a breakdown between the oxidative and antioxidant systems in the I/R-induced AKI model, where inadequate ROS scavenging by renal tubular mitochondria led to ROS accumulation [86]. An additional study indicated that mtROS contributed to mtDNA deletion and heightened cytokine release by reducing mitochondrial transcription factor A (TFAM) abundance, which consequently caused mitochondrial dysfunction and inflammation [87]. Additionally, mtDNA leakage was observed in the AKI model caused by cisplatin [88], and clinical studies showed a correlation between the severity and prognosis of the illness in AKI patients with elevated circulating mtDNA levels [89].

Liver disease

Alcohol-associated liver disease (ALD) ALD is a persistent liver condition resulting from prolonged alcohol consumption. In ethanol-fed mice, the expression of Parkin and p62 was reduced, indicating compromised mitophagy [90]. AMPK could minimize the damage induced by alcohol by increasing levels of mitophagy, which might be a

promising strategy for treating ALD [91]. In the context of ALD, an increase in ROS was observed, concomitant with a decrease in the levels of superoxidase dismutase (SOD) and glutathione (GSH) [92]. The synthesis of ROS was also found to activate receptor-interacting protein kinase 1, causing necrotic apoptosis [93]. Moreover, mtDNA release into the cytoplasm and activation of the cGAS-STING signaling pathway were triggered by alcohol-induced liver injury, and these events ultimately resulted in liver inflammation [94].

Nonalcoholic fatty liver disease (NAFLD) Triacylglycerol (TG) buildup in the liver is the hallmark of NAFLD, which affects approximately one-quarter of the world's population [95]. One important factor in the onset and course of NAFLD has been identified as impaired mitochondrial β -oxidation, leading to dramatically raised ROS levels [96]. Furthermore, studies showed that a marked decrease in proliferator-activated receptor alpha (PPAR α) was present in NAFLD patients' livers, together with impaired mitochondrial oxidative capacity and hepatic steatosis [97]. The overexpression of PPAR α has been

found to promote mitochondrial biogenesis while reducing TG accumulation. Abnormal mitochondrial fission also encouraged the growth of NAFLD, and targeted inhibition of hepatic Drp1 expression shielded mice from obesity induced by a high-fat diet (HFD) (Fig. 3) [98].

Summary of EV biology

Classification and biogenesis of EVs

EVs are lipid bilayer-encapsulated nanoparticles released from cells and are highly heterogeneous [99]. According to the International Society for Extracellular Vesicles (ISEV) position MISEV2018 and MISEV2023, these vesicles can be further categorized into small EVs (sEVs, <200 nm) and large EVs (lEVs, >200 nm) based on their particle size [100, 101]. Whereas researchers have typically classified EVs into exosomes (30–150 nm) and microvesicles (MVs, 50–1000 nm) based on their biogenesis in previous studies [102–104]. Exosomes originate in the endosomal system and are formed by the fusion of multivesicular bodies (MVBs) with the cytoplasmic membrane to release contained intraluminal vesicles (ILVs) [105]. MVs are produced by budding directly from the plasma membrane [8]. In addition to exosomes and

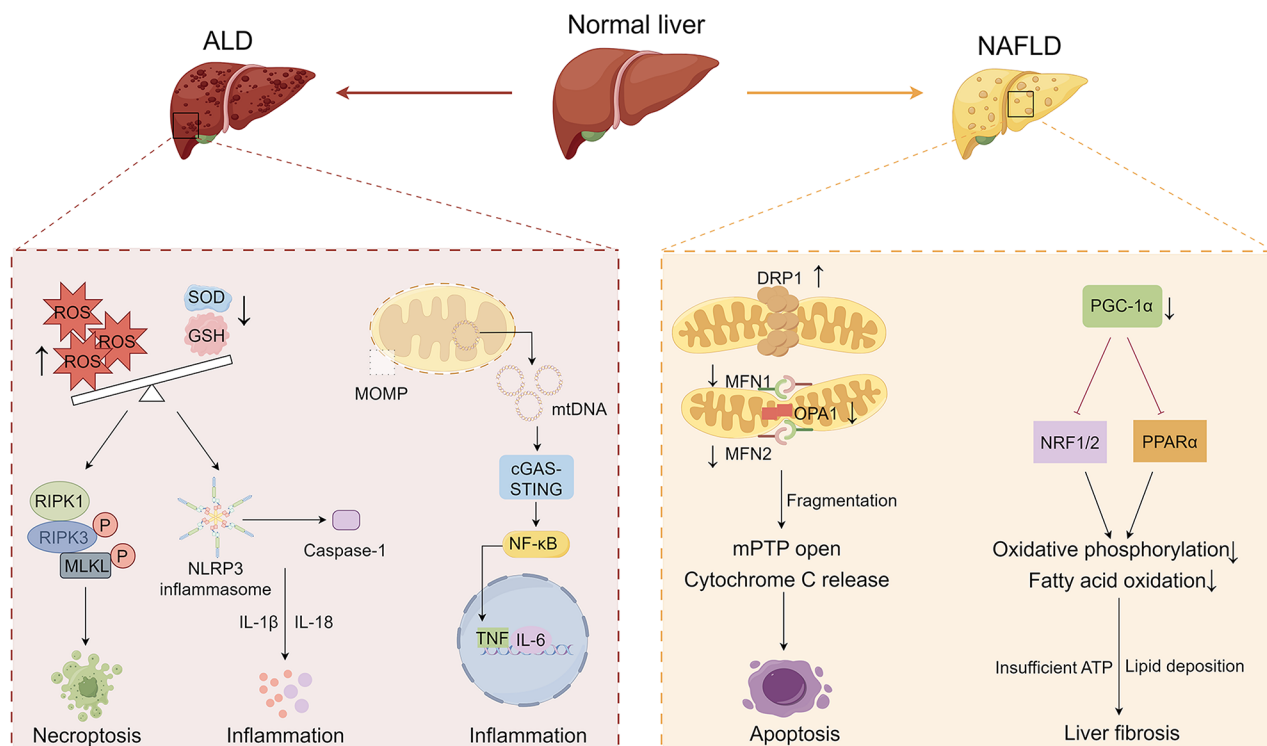


Fig. 3 The roles of mitochondrial dysfunction in liver diseases (by Figdraw). In alcohol-associated liver disease and nonalcoholic fatty liver disease, mitochondrial dysfunction, including oxidative stress, mtDNA leakage, reduced FAO, and altered mitochondrial dynamics, exacerbates the progression of liver fibrosis and non-alcoholic steatohepatitis. ROS, reactive oxygen species; SOD, superoxidase dismutase; GSH, glutathione; RIPK1, receptor-interacting protein kinase 1; RIPK3, receptor-interacting protein kinase 3; MLKL, mixed lineage kinase domain-like protein; NLRP3, NOD-like receptor thermal protein domain associated protein 3; MOMP, mitochondrial outer membrane permeabilization; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; DRP1, dynamin-related protein 1; MFN1, mitofusin 1; MFN2, mitofusin 2; OPA1, optic atrophy 1; PGC-1 α , peroxisome proliferator-activated receptor- γ coactivator-1 α ; NRF1/2, nuclear respiratory factor 1/2; PPAR α , peroxisome proliferator-activated receptor alpha

MVs, apoptotic bodies (50–5000 nm) were also one of the most extensively studied subtypes of EVs, formed by bubbling the plasma membrane and enveloping the cytoplasm, organelles (mitochondria), and nucleic acid fragments after cell apoptosis [106]. Moreover, mitochondrial-derived vesicles (MDVs, 70–150 nm) are a specific category of EVs that are generated following minor mitochondrial impairment, which are then released into the extracellular milieu as EVs subsequent to merging with MVBs [107]. The vesicles of mitochondrial origin were initially considered as a quality control mechanism to compensate for the insufficient maintenance of mitochondrial homeostasis when the mitochondrial autophagy system is impaired [108]. The biogenesis of MDVs is slightly complex. Several molecules are involved in the sprouting of damaged mitochondrial fragments to MDVs, including Parkinson's disease-associated proteins PINK1, Parkin, and the vacuolar sorting protein 35 (Vps35) [109–112]. Mitochondrial membrane proteins are oxidized under mild oxidative stress. ROS and oxidative stress initiate local activation of PINK1 and Parkin, leading to the budding of oxidized membrane proteins into vesicles [109, 113]. The mutation of Vps35 impaired the formation of MDVs, which proved that Vps35 played an important role in the formation of MDVs [111]. In addition, Matheoud et al. [113] found that Rab9 and sorting nexin 9 (SNX9) need to be recruited for MDV biogenesis, but the specific mechanism remains to be explored. The destinations of MDV transport include lysosomes, peroxisomes, bacterial phagosomes, and EVs. The transport of MDV-containing oxidized protein to the lysosome is mainly affected by the PINK1/Parkin pathway [109, 110, 114, 115]. The transport of MDVs to the peroxisome is related to mitochondrial-anchored protein ligase (MAPL) [116]. In addition, the transport of MDVs to phagosomes containing bacteria can play an antibacterial defense role [117]. However, there are still many unclear aspects of MDVs that need further exploration (Fig. 4). In various literature, researchers have used different names to describe EVs based on specific biogenesis, diameter, or content. However, ISEV recommends using the umbrella term “extracellular vesicles” to encompass all types of membrane-derived vesicles and can be further divided into sEVs and lEVs based on their size [101]. Therefore, in this review, the term EV will be used primarily to refer to all nanoparticles unless a specific name is applied according to the context of the study.

Contents of EVs

EVs are rich in a variety of biologically active substances, such as proteins, nucleic acids, and lipids. Due to the different plasma membrane origins of the different subtypes of EVs, their protein expression is also different. lEVs express Cav-1, CK18, and GAPDH, whereas sEVs

are characterized by biological proteins (Alix, TSG101), tetraspanins (CD9, CD63, and CD81), and heat-shock proteins (HSP70 and HSP90) [118]. In addition, many studies have shown that different mitochondrial contents are enriched in some EV subgroups, and sEVs contain mitochondrial components such as mtDNA, mtRNA, and mitochondrial protein, while lEVs contain structurally intact mitochondria [119]. By shotgun proteomics analysis of EVs derived from human brain endothelial cells (BECs), it was found that lEVs were rich in 89 kinds of mitochondrial proteins (such as SOD2, MRPS22, MRPL13, and ATP5A1), while sEVs contained only one kind of mitochondrial protein [120], indicating that the size and type of EVs may be closely related to the content of mitochondria. In addition, the complete mitochondrial genome was also detected in circulating EVs of patients with hormone therapy-resistant metastatic breast cancer [121]. Abundant data shows that EVs are rich in mitochondrial components, but due to the high heterogeneity of EVs and the limitations of separation technology, the specific relationship between the type and size of EVs and the content of mitochondria is still elusive (Fig. 5).

Isolation and characterization of EVs

Based on the properties of EV size, density, and surface charge, various separation methods have been developed. Ultracentrifugation is one of the most commonly used methods to isolate EVs. However, this method requires a large amount of cell culture fluid and high-speed centrifugation equipment and is prone to introduce contamination. Other conventional separation methods include density gradient centrifugation, size exclusion chromatography, coprecipitation, and field flow fractionation. The advantages and disadvantages of these separation methods have been well-reviewed [122, 123]. In recent years, to improve separation efficiency and specificity, microfluidic, contactless sorting, immunoaffinity enrichment, and other new EV enrichment technologies have emerged. However, due to the overlap of different EV subtypes in size and biogenetic pathway and the presence of contamination of non-mitochondrial EV content after ultracentrifugation, the existing separation methods cannot meet the specific separation of EVs rich in mitochondrial components. In this context, D'Acunzo et al. [124] have developed a new method to isolate EVs from brain tissue based on iodixanol-based high-resolution density gradient centrifugation. Compared with sucrose gradient centrifugation, it can better separate mitochondrial component-enriched EVs and other sEVs. But this method still needs 0.22 μm filtration to further purify EVs, which may lead to the loss of lEVs containing the entire mitochondria. In addition, a study reported that chromatography on immobilized heparin could separate MDVs from free mitochondria, which could not be achieved by

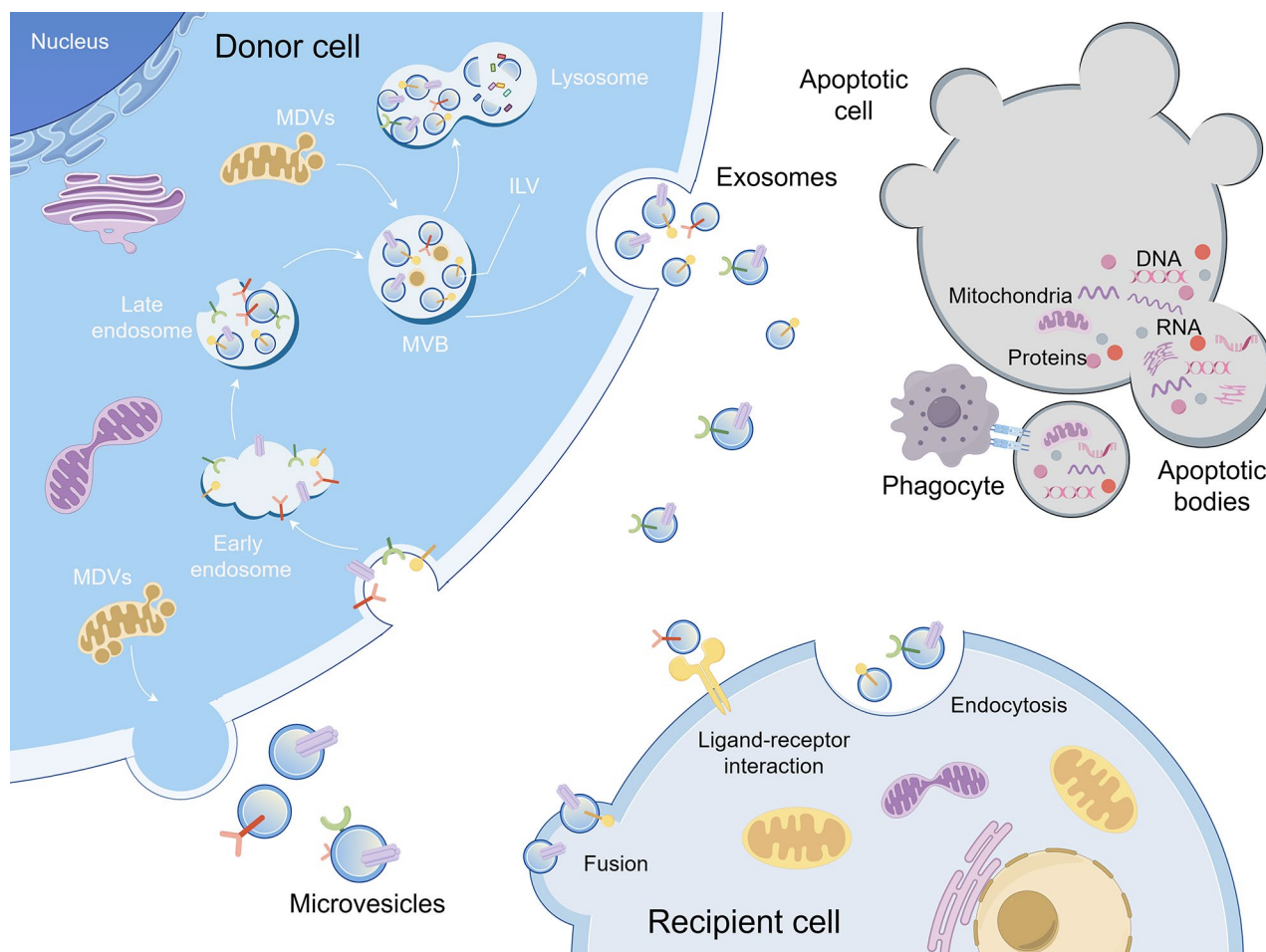


Fig. 4 Classification, biogenesis and uptake of EVs (by Figdraw). Exosomes are produced through the endosomal pathway, while vesicles and apoptotic bodies are directly generated by plasma membrane budding. Exosomes and MVs can be taken up by recipient cells through various pathways such as membrane fusion, ligand-receptor binding, and endocytosis, and apoptotic bodies can be phagocytosed by phagocytes. MDVs are mainly released into the extracellular space through the MVB-mediated pathway and the microvesicle pathway. MVB, multivesicular bodies; ILV, intraluminal vesicles. MDVs, mitochondrial-derived vesicles

size exclusion chromatography [125]. Combining different separation methods may be a better option for separating EVs.

Conventional characterization methods for EVs include atomic force microscopy, electron microscopy, nanoparticle tracking analysis, and western blotting, and these methods have been well reviewed previously [126, 127]. In addition, flow cytometry has become one of the commonly used characterization methods for EVs due to its ability to characterize multiple parameters on a single particle in a high-throughput manner. However, conventional flow cytometry has a low detection limit, may exclude a large number of smaller EVs present from analysis, and has the potential to detect multiple EVs as a single event, making its applicability in the field of EV research remains controversial [128, 129]. To address the limitations of conventional flow cytometry in EV characterization, many improvements have been

made, including replacing photodiodes with photomultiplier tubes on the light-scattering channels and adding reduced wide-angle forward scatter/medium-angle light scatter collection, which has facilitated the development of high-resolution flow cytometers with detection limits lower than those of conventional flow cytometers [130–133]. Currently, the use of high-resolution flow cytometry has become more and more common in EV research [134, 135]. Similarly, for mitochondrial components in EVs, RT-PCR and western blotting can be used to detect the presence of mtDNA, mtRNA, and mitochondrial proteins [136]. Through the observation of the transmission electron microscope, we can see that there are mitochondria with complete structure in EVs [13]. Integrity EVs can be labeled with calcein, and intact mitochondria can be labeled with MitoTracker. A double-positive event by flow cytometry detection of calcein and MitoTracker is considered to be EVs containing functional mitochondria

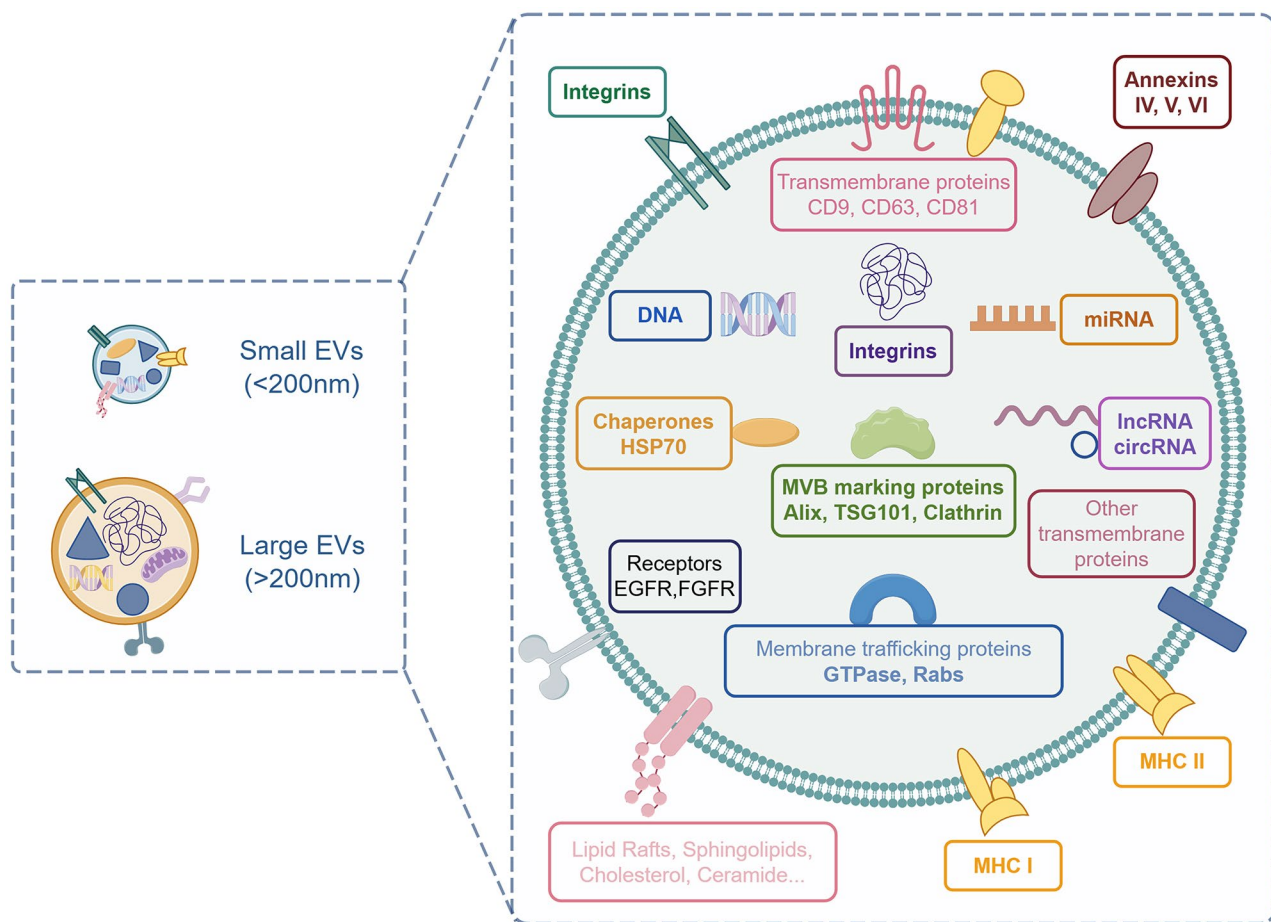


Fig. 5 Components of EVs (by Figdraw). EVs have lipid bilayers, heterogeneous components, and highly expressed tetraspanins (CD9, CD81, and CD63). In addition, large amounts of DNA, RNA, enzymes, and other functional proteins are encapsulated. sEVs may contain mitochondrial components such as mtDNA, mtRNA, and mitochondrial protein, while lEVs may contain structurally intact mitochondria. HSP70, heat shock protein 70; MVB, multivesicular body; Alix, ALG-2-interacting protein X; TSG101, tumor susceptibility gene 101; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; EVs, extracellular vesicles; sEVs, small extracellular vesicles; lEVs, large extracellular vesicles

[137]. In addition, Zhang et al. [135] stained respiring mitochondria in EVs using MitoTracker and examined the presence and activity of functional mitochondria in EVs of different sizes by high-resolution multicolor flow cytometry, and found that the percentage of EVs containing functional mitochondria and the relative activity of respiring mitochondria was highest in lEVs, and lower in sEVs. This result again demonstrates that the mitochondrial content and function within EVs correlate with the size and type of EVs. For mitochondrial function within EVs, Peruzzotti-Jametti et al. [138] assessed the function of mitochondria in the crude EV preparations by examining the activity of ETC in maintaining a mitochondrial transmembrane potential and respiration using a JC1 assay and high-resolution respirometry, respectively. However, further research is needed on more specific, effective, and convenient separation and detection techniques.

The early detection and treatment effects of EVs on mitochondrial dysfunction related diseases

Mitochondrial damage significantly contributes to the pathogenesis of many diseases. Damaged cells or tissues released damaged-mitochondria enriched-EVs can be used as biomarkers for diagnosing diseases. More importantly, EVs can not only directly transport mitochondria (or mitochondrial components) to target cells to restore mitochondrial function but also play a therapeutic role by modulating mitochondrial function in target cells through their unique internal components originating from parental cells. In addition, modifying EVs to load substances targeted for mitochondrial therapy may be one of the future development directions. These important findings motivate our research into EVs and their functional relevance to mitochondria in immune regulation and tissue damage repair and offer novel perspectives on the potential utility of EVs as a tool for the

diagnosis and treatment of mitochondrial dysfunction related diseases (Fig. 6).

Neurological disorders

EVs as promising non-invasive biomarkers for the diagnosis of neurological disorders

Aging caused by mtDNA mutations and oxidative damage is a key risk factor for neurodegenerative diseases, and mitochondrial damage occurs earlier, which is causally related to the pathogenesis of neurodegenerative diseases [139]. Therefore, EVs rich in mitochondria or mitochondrial components are considered potential biomarkers for detecting and diagnosing neurodegenerative diseases. Yao et al. [140] discovered that ATP synthase and mitochondrial ETC complex I/III/IV were lower in EVs obtained from plasma neurons of AD patients than in normal. Kim et al. [141] found that mtRNA was significantly elevated in plasma EVs in individuals with mild cognitive impairment and AD. This may be because the

toxic environment in the AD brain stimulates brain cells to secrete mtRNA-rich EVs across the blood-brain barrier (BBB) into the plasma. It is worth noting that some researchers have developed more novel isolation methods to obtain a specific subgroup of mitochondrial EVs called mitovesicles [124]. D'Acunzo et al. [142] obtained mitochondrial EVs from the extracellular matrix of the brain through high-resolution density gradient separation and demonstrated that brain-derived mitochondrial vesicles contained specific mitochondrial components, and their levels and contents may change in Down syndrome. Furthermore, EVs derived from plasma neurons in patients with multiple sclerosis have higher mitochondrial complex IV activity and lower mitochondrial complex V activity, which may be associated with more rapid whole-brain, brain substructure, and retinal atrophy [143]. These findings prove that mitochondrial content in EVs is strongly associated with neurodegenerative

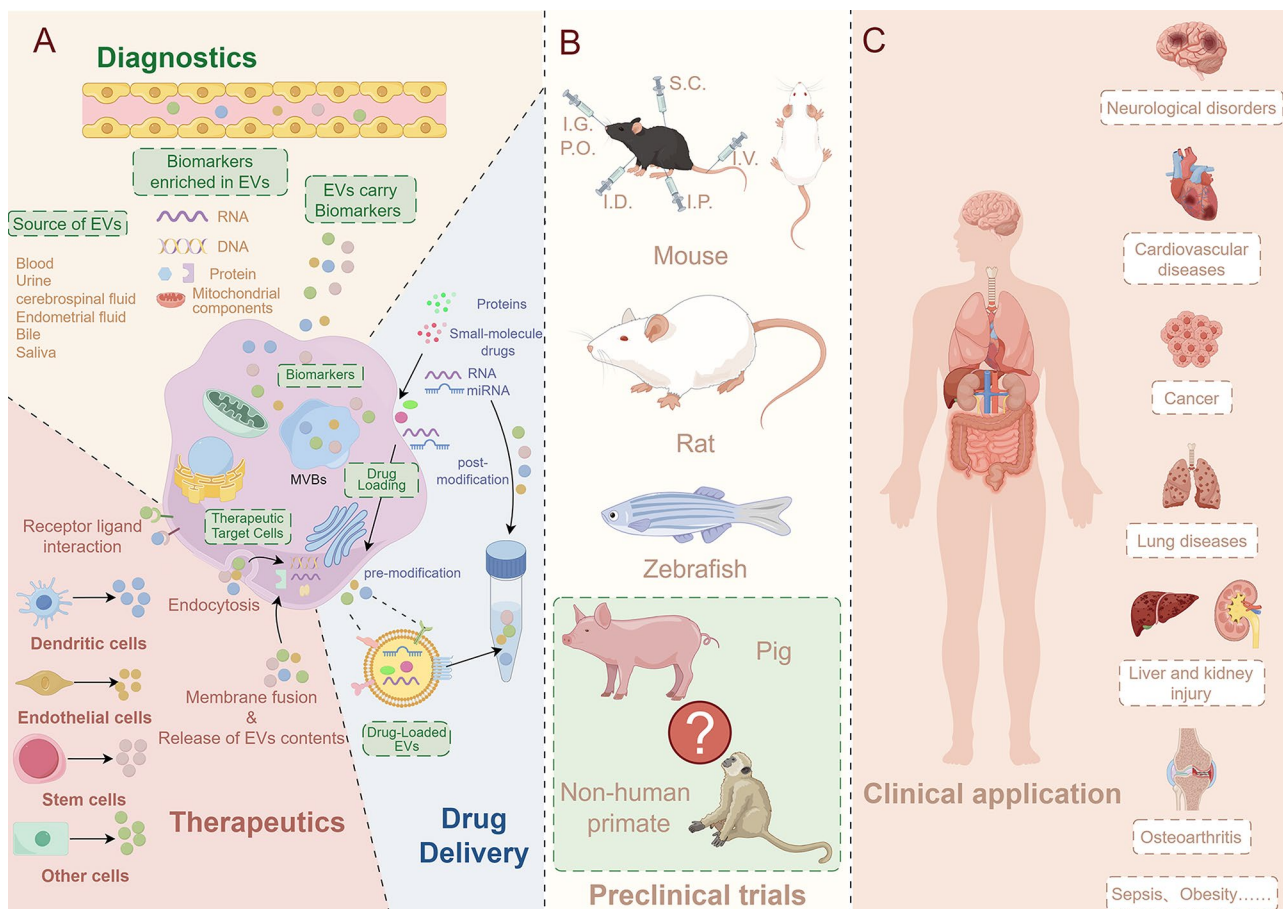


Fig. 6 Clinical application potential of EVs (by Figdraw). **(A)** EVs are abundant in various bodily fluids, and their content levels reflect the type and activation status of their parent cells. This makes them valuable biomarkers for diagnosing mitochondrial dysfunction related diseases. Moreover, natural EVs contain a variety of bioactive substances that can enter receptor cells through multiple pathways to regulate the mitochondrial function of target cells, making them promising therapeutic agents for mitochondrial dysfunction related diseases. Furthermore, EVs are also high-quality drug nanocarriers that can be engineered to deliver therapeutic drugs in a targeted manner. **(B)** Different preclinical models have been developed to evaluate the biology of endogenous or injected EVs in vivo. **(C)** Prospects for clinical applications of EVs

diseases and that its decrease or increase may reflect the extent of mitochondrial damage (Table 1).

The utilization of EVs from various sources for the therapeutic management of neurological disorders

Due to the selective penetration of BBB, according to statistics, only 2% of therapeutic compounds can pass through BBB and achieve their therapeutic goal [157]. Researchers are actively developing safer, more efficient, and more accurate drug delivery systems to promote better treatment for the brain. Compared with other drug delivery systems, EVs, as natural nanovesicles, have the significant advantages of optimal biocompatibility, biodegradability, and low immunogenicity. sEVs can effectively penetrate the physical barrier formed by the tight junction of BBB. In addition, the phospholipid bilayer structure of EVs can protect the goods in EVs from the influence of enzyme barrier in brain parenchyma. More importantly, EVs have been shown to play an important role in brain cell communication. Therefore, EVs derived from brain cells have become excellent brain-targeted cargo delivery carriers because of their natural biological effects and the rich parental cell surface proteins loaded during biogenesis. Receptor-mediated transcytosis is the most widely recognized effective way EVs penetrate the BBB. EVs from brain cells attach to BBB cell receptors through surface proteins derived from parental cells. After endocytosis by BECs, they accumulate in endosomes and achieve transcellular trespassing through the formation of MVBs and exocytosis to the brain parenchyma [158]. Then EVs can interact with glial cells and neurons in brain parenchyma.

At present, the main focus of natural EV treatment is mitochondrial transfer. As an illustration, neural stem cell-derived EVs restored mitochondrial dynamics and cellular metabolism by transferring mitochondrial proteins and structurally intact functional mitochondria to the target cell, as well as inhibited the pro-inflammatory state of the cell, and these effects were co-determined by the mitochondrial activity and content of mitochondria released from the parental cell [138]. In addition, endothelial- and macrophage-derived EVs also carried polarized mitochondria, and because of the natural affinity for BECs, endothelial-derived EVs enabled a higher degree of mitochondrial transport, leading to an increase in ATP. Interestingly, it was the transfer of larger EV fractions, rather than the transfer of smaller EVs, that led to increased mitochondrial function [159]. Similarly, Dave et al. [119] found that while BECs-derived sEVs contained mitochondrial proteins, only lEVs contained intact mitochondria and that lEVs, but not sEVs, transferred loaded mitochondria to BECs, increased the relative ATP levels and mitochondrial function, and reduced the size of cerebral infarcts in mice. These results suggest that the sizes

and types of EVs may be related to their mitochondrial content and function and that EVs carrying structurally intact mitochondria may have better therapeutic effects.

In addition to delivering mitochondria or mitochondrial components, EVs may also modulate mitochondrial function within target cells by delivering cellular contents originating from their parent cell specifics. For example, although the sEVs secreted by astrocytes in different regions of the nigrostriatal system all could protect the activity of mitochondrial complex I damaged by neurotoxin MPP⁺, only the sEVs secreted by astrocytes in the ventral midbrain could improve the ATP production of neurons damaged by MPP⁺, indicating that EVs from different sources may contain specific cellular contents to regulate mitochondrial function, but further research is needed to determine this conclusion [160]. Moreover, there have been multiple reports indicating that EVs derived from adipose stem cells possess the ability to specifically target mitochondrial dysfunction, restore proper mitochondrial function, and mitigate cell apoptosis regulated by mitochondria in neurodegenerative diseases such as amyotrophic lateral sclerosis and Huntington's disease [161–164]. Through proteomics, it was found that several molecules in EVs may have the effect of counteracting neurodegenerative mechanisms, including *SOD1*, ribonuclease RNase 4, the insulin-like growth factors IGF1 and Akt, but the specific mechanism of their role is still unclear [163] (Table 2).

Cardiovascular diseases

The roles of EVs in the diagnosis of cardiovascular disease

Under physiological conditions, intercellular mitochondrial transfer in the cardiovascular system can maintain normal cardiac homeostasis. Under pathological conditions, damaged cells seek help by releasing EVs rich in damaged mitochondria. Therefore, detecting mitochondrial components in EVs may be an effective strategy for diagnosing CVD. An existing study showed that in the plasma EVs of patients with coronary artery disease, the mtRNA (*MT-COI*) level of patients with new cardiovascular events was lower, which proved that circulating EVs rich in mitochondrial components were a possible predictor of CVD [144]. The specific cargo characteristics and stability of EVs make them a valuable diagnostic tool that needs more research (Table 1).

Therapeutic application of EVs in cardiovascular disease

In ischemic cardiomyopathy and other pathological conditions, damaged cells can use exogenous functional mitochondria to save their mitochondrial network. EVs have great potential as a mitochondrial delivery system, which may contribute to the uptake of mitochondria by recipient cells and improve the vitality and stability of mitochondria [13]. In addition, EVs from different culture

Table 1 EVs and their cargoes as biomarkers of mitochondrial dysfunction related diseases

| Diseases | Nano vesicles | Sources of EVs | Biomarkers | References |
|------------------------------|---------------------------|----------------------------|---|------------|
| AD | sEVs (70–118 nm) | Plasma neuron | The levels of ATP synthase and mitochondrial ETC complex I/III/IV↓ | [140] |
| AD | EVs (~200 nm) | Plasma | <i>MT-ND1-6, MT-ND4L, MT-ATP6, MT-ATP8, MT-CYTB, MT-CO1, MT-CO2, MT-CO3</i> mRNAs, and <i>MT-RNR1</i> rRNA↑ | [141] |
| DS | Mitovesicles (~50–200 nm) | Post-mortem brain matrix | Mitochondrial proteins (VADC, COX-IV, and PDH-E1α) ↑ | [142] |
| MS | EVs (Undefined) | Plasma | Mitochondrial ETC complex IV activity↑ Mitochondrial ETC complex V activity↓ | [143] |
| CAD | sEVs (~110 nm) | Plasma | <i>MT-CO1</i> mRNA↓ | [144] |
| Ovarian cancer | sEVs (~150 nm) | Plasma | mtDNA↑ | [145] |
| Ovarian cancer | EVs (40–300 nm) | Plasma | <i>MT-CO2</i> and <i>COX6c</i> ↑ | [146] |
| Oral squamous cell carcinoma | sEVs (~100 nm) | Plasma | mtDNA↑ | [147] |
| early PDAC | EVs (Undefined) | Serum | mtDNA mutation↑ | [148] |
| Pancreatic Cancer | sEVs (~50 nm) | Serum | mtDNA/nDNA↑ | [149] |
| Melanoma | EVs (40–300 nm) | Plasma | <i>MT-CO2</i> and <i>COX6c</i> ↑ | [146] |
| Breast cancer | EVs (40–300 nm) | Plasma | <i>MT-CO2</i> and <i>COX6c</i> ↑ | [146] |
| ALI | EVs (< 1 μm) | Alveolar epithelial cells | Tom20↓ | [150] |
| ALD | EVs (Undefined) | Hepatocyte | mtDNA↑ | [151] |
| ALD | sEVs (~30–200 nm) | Hepatocyte | mtDNA↑ | [152] |
| Liver fibrosis | EVs (Undefined) | Hepatocyte and serum | mtDNA↑ | [153] |
| OA | sEVs (40–110 nm) | IL-1β-treated chondrocytes | mitochondrial proteins↓ | [154] |
| Sarcopenia | sEVs (< 100 nm) | Serum | <i>ATP5A, NDUFS3</i> and <i>SDH-B</i> ↓ | [155] |
| Aging | sEVs (~150 nm) | Plasma | mtDNA↓ | [156] |
| Aging | EVs (< 6 μm) | Plasma | mtDNA and mitochondria↓ | [135] |

conditions and sources are rich in specific contents from parental cells, and these non-mitochondrial cargoes also play a beneficial role in the treatment of CVD. In the long run, EVs can also be modified to transport goods in a highly targeted manner to target mitochondria. Therefore, EVs have great potential as a cell-free strategy for the treatment of CVD. EV-mediated treatment of CVD has a variety of mechanisms, including the following points. (1) Improvement of mitochondrial biogenesis. Mitochondria-rich EVs from human-induced pluripotent stem cells (iPSCs)-derived cardiomyocytes (iCMs) (M-EVs), which could restore intracellular ATP production and improve the contractile properties of iCMs by transporting mitochondria to hypoxic damaged iCMs. In addition, non-mitochondrial cargo (mRNA promoting mitochondrial biosynthesis) in M-EVs could also enhance intracellular bioenergetics by activating mitochondrial biosynthesis [13]. (2) Enhancement of antioxidant capacity. Hypoxia-conditioned human mesenchymal stem cells (hMSCs)-derived EVs, which were abundant in Parkinson disease protein 7, exhibited a more potent inhibitory impact on myocardial hypertrophy compared to normoxia-conditioned hMSC-derived EVs. They could serve as antioxidants to alleviate mitochondrial dysfunction and excessive production of mtROS. The potential mechanism underlying the observed phenomenon involves the inhibition of proteasome subunit beta type 10 activity by Parkinson disease protein 7 through direct physical interactions to reduce the ubiquitination degradation of AT1R-related protein to inhibit the signaling pathway mediated by angiotensin II type 1 receptor leading to myocardial hypertrophy [165]. (3) Reduction of apoptosis. Hypoxic/serum-deprivation stimulated neonatal mice cardiomyocytes treated with EVs derived from bone marrow mesenchymal stem cells (BMSCs) overexpressing macrophage migration inhibitory factor (MIF) showed reduced mitochondrial fragmentation and cell apoptosis [166] (Table 2).

Cancers

EVs as attractive candidates for diagnosing of various types of cancers

Timely detection of cancer is of paramount importance to achieve favorable treatment outcomes in the ongoing fight against this ailment. The transfer of mitochondria (or mitochondrial components) mediated by EVs can occur in tumor cells or between tumor cells and other cell types, and this effect is involved in regulating tumor differentiation, tumor microenvironment, tumor drug resistance, and tumor invasion [121, 147, 167–170] (Fig. 7). Furthermore, EVs exhibit remarkable stability while present in various bodily fluids, therefore indicating their considerable promise as biomarkers for cancer detection. For instance, previous research has demonstrated

Table 2 Therapeutic effects of EVs from different sources in mitochondrial dysfunction related diseases

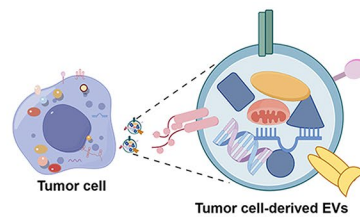
| Diseases | EV origins | Types, sizes, and contents | Models | Effects of EVs | References |
|---------------------|--|--|--|---|------------|
| AE | NSCs | EVs: 80–1000 nm; Mitochondrial proteins, mtDNA and mitochondria. | MOG-induced mice. | Ameliorated neuroinflammation. | [138] |
| Ischemic stroke | hCMEC/D3 | EVs: 150–250 nm; Mitochondria and ATP5A. | OGD-treated BECs. | Increased overall mitochondrial function and cellular ATP levels. | [159] |
| Ischemic stroke | hCMEC/D3 | IEVs: 200–500 nm; Mitochondria. | A mouse middle cerebral artery occlusion model of ischemic stroke; Oxygen glucose-deprived primary human brain microvascular endothelial cells. | Increased the relative ATP level and mitochondrial function of receptor cells; Reduced the area of cerebral infarction in mice. | [119] |
| PD | Nigrostriatal astrocytes | sEVs: ~100 nm; Undefined. | SH-SY5Y cells damaged by H ₂ O ₂ /neurotoxin MPP ⁺ . | Anti H ₂ O ₂ induced activation of cystatin 3; Rescued neuron mitochondrial complex I function damaged by neurotoxin MPP ⁺ . | [160] |
| ALS | Adipose stem cells | sEVs: 30–120 nm; Undefined. | NSC-34 cell line which overexpressing human SOD1(G93A) mutant protein. | Restored complex I activity, coupling efficiency, and mitochondrial membrane potential. | [161] |
| ALS | Adipose stem cells | EVs: Undefined; Undefined. | Neuronal cells from G93A ALS mice model. | Reduced the aggregation of superoxide dismutase 1; Increased phosphorylation CREB/CREB ratio and PGC-1 α expression level. | [162] |
| ALS | Adipose stem cells | sEVs: 50–150 nm; SOD1, ribonuclease RNase 4, the insulin-like growth factors IGF1 and Akt. | NSC-34 cells. | Reduced mitochondrial regulated cell apoptosis. | [163] |
| HD | Adipose stem cells | EVs: Undefined; Undefined. | R6/2 mice-derived neuronal cells. | Reduced mHtt aggregates; Reduced cell apoptosis. | [164] |
| MI | hiPSCs-derived cardiomyocytes | EVs: 100–600 nm; Mitochondria, <i>PGC-1α</i> and <i>ERRγ</i> mRNA. | Murine myocardial infarction model; Hypoxia-induced iCM. | Improved in vitro intracellular energetics; Enhanced cardiac function after myocardial infarction. | [13] |
| MI | BMSCs overexpressing MIF | EVs: ~10–400 nm; MIF. | A rat model of MI; Hypoxia/serum deprivation stimulated neonatal mice cardiomyocytes. | Enhanced cardiac function; Reduced cardiac remodeling; Reduced mitochondrial debris, ROS generation, and cell apoptosis in myocardial cells. | [166] |
| Cardiac hypertrophy | hMSCs | sEVs: 30–150 nm; Parkinson disease protein 7. | A mouse model of transverse aortic constriction; Neonatal rat cardiomyocytes after angiotensin II stimulation. | Reduced the cardiomyocytes size and the mRNA levels of BNP, ANP, and β -MHC; Reversed the increase in LVPW thickness and HW/BW ratio. | [165] |
| Cancer | Dendritic cells | EVs: 100–400 nm; AIE-photosensitizer. | Orthotopic breast tumor models; 4T1 tumor-bearing mice; Subcutaneous CT26 colorectal tumor-bearing mouse model; 4T1 and CT26 tumor cells. | Activated T cell; Induced superior immunogenic cell death. | [177] |
| Breast cancer | Breast cancer cells | sEVs: ~65 nm; TPP/P53. | Balb/c mice inoculated with 4T1 cells to construct a mouse breast cancer model; MCF-7 (ER ⁺ , PR ⁺ , HER2 ⁻ , P53 ^{wild}) and SKBR-3 (ER ⁻ , PR ⁻ , HER2 ⁺ , P53 ^{mut}). | Downregulated anti-apoptotic protein (Bcl-2); Upregulated proapoptotic protein (Bax). | [171] |
| Breast cancer | MSCs | sEVs: 30–100 nm; miR-34a. | MDA-MB-231 cell. | Downregulated anti-apoptotic protein (Bcl-2). | [172] |
| Breast cancer | Human embryonic kidney HEK-293T cells | sEVs: ~100 nm; T-Ce6 and glycolysis inhibitors (FX11). | MCF-7-xenograft mice; MCF-7 human breast cancer cells. | Increased ROS production and promoted cell apoptosis; Suppressed ATP production in mouse tumors. | [16] |
| Liver cancer | Hepatocellular carcinoma (HepG2) cells | EVs: ~200 nm; ASO-G3139. | Hepatocellular carcinoma (HepG2) cells. | Downregulated anti-apoptotic protein (Bcl-2). | [173] |

Table 2 (continued)

| Diseases | EV origins | Types, sizes, and contents | Models | Effects of EVs | References |
|-----------------------|------------------------|--|--|---|------------|
| Ovarian cancer | OVCAR-8 cells | EVs: 100–300 nm; B2C. | A mouse tumor xenograft model; Human MDR ovarian cancer cells (OVCAR-8/MDR). | Depleted GSH to induce oxidative stress; Inhibited ATP production; Induced mitochondrial dysfunction; Induced cell apoptosis. | [175] |
| GBM | Brain endothelial cell | sEVs: 50–130 nm; T-Ce6. | Orthotopic GBM-xenografted mice; The in vitro BBB model composed of bEnd.3 and U87MG cells. | Produced cytotoxic ROS; Induced mitochondrial damage and cell apoptosis. | [176] |
| ARDS | MSCs | EVs: <4 μ m; Mitochondria. | MDMs stimulated by LPS or BALF from patients with ARDS. | Inhibited the secretion of pro-inflammatory cytokines; Enhanced the phagocytic ability of macrophages; Reduced lung injury. | [178] |
| ARDS | MSCs | EVs: 100–700 nm; Mitochondria. | LPS-induced lung injury model; HPMECs and HSAECs and human PCLSs were stimulated with endotoxin or plasma samples from patients with ARDS. | Reduced lung injury; Relieved mitochondrial dysfunction; Restored mitochondrial respiration; Restored the integrity of the epithelial and endothelial cell barrier. | [182] |
| ALI | AdMSCs | sEVs: 50–150 nm; mtDNA. | LPS-induced ALI model; LPS-stimulated MH-S mouse macrophage cells. | Restored mtDNA levels in recipient macrophages; Restored mitochondrial function; Suppressed inflammatory response. | [179] |
| ALI | BMSCs | IEVs: 1–2 μ m; Mitochondria. | LPS-induced ALI mice. | Reduced alveolar leukocytosis and protein leakage; Increased the production of alveolar ATP. | [181] |
| Severe emphys-ema | BMSCs | EVs: ~150–250 nm; Mitochondria. | Murine model of elastase-induced severe emphysema. | Reduced the neutrophil count, the mean linear intercept, and IL-1 β and TGF- β levels; Reduced pulmonary arterial hypertension; Increased the right ventricular area. | [180] |
| Ischemic renal injury | STC-like cells | EVs: 100–300 nm; Mitochondria. | Induction of 2-week unilateral renal artery stenosis in mice; AMA-induced injured proximal TEC (PK1 cells). | Reduced oxidative stress in TECs; Inhibited Drp1 activity; Improved perfusion; Relieved renal fibrosis. | [187] |
| AKI | MSCs | EVs: ~50–500 nm; <i>TFAM</i> mRNA, mtDNA, ATP5a1, COX IV, Tom20. | A renal IRI model; HK-2 cells treated with H ₂ O ₂ . | Reversed mtDNA deficiency and mitochondrial OXPHOS deficiency in damaged renal tubular cells; Alleviated kidney damage formation, mitochondrial damage, and inflammation in AKI mice. | [136] |
| AKI | iPSCs | EVs: 32–229 nm; Undefined. | A bilateral renal arterial clamping model; RPTEC damaged by hypoxia-reoxygenation. | Reduced cell death; Maintained functional mitochondria; Reduced oxidative stress after IRI to promote renal protection. | [184] |
| AKI | Macrophage | EVs: 10–500 nm; IL-10. | A murine model of renal I/R injury; Cisplatin-induced AKI. | Promoted mitochondrial autophagy; Reduced renal tubular atrophy, TEC flattening and detachment, and fibrosis. | [190] |
| Renal IRI | BMSCs | sEVs: ~71.25 nm; miR-223-3p. | Renal I/R injury model; TCMK-1 cells were subjected to hypoxia/reoxygenation. | Reduced cell apoptosis and activation of inflammasomes; Promoted mitochondrial autophagy. | [188] |
| Renal IRI | hWJMSCs | EVs: Undefined; miR-30. | Animal models of unilateral renal IRI. | Reduced activation of Drp1 and mitochondrial fragmentation. | [189] |
| Liver IRI | hUC-MSCs | EVs: 100–700 nm; Mitochondria. | A mouse liver IRI model; Neutrophils from the bone marrow of C57BL/6 mice. | Restricted the release of pro-inflammatory cytokines and NETs formation of neutrophils in the liver during early reperfusion. | [14] |

Table 2 (continued)

| Diseases | EV origins | Types, sizes, and contents | Models | Effects of EVs | References |
|----------|---------------------|---|---|--|------------|
| OA | Primary chondrocyte | sEVs: 40–110 nm; Mitochondrial protein. | A mouse OA model; IL-1-β stimulated chondrocytes. | Restored the structure of abnormal mitochondria; Increased ATP levels and mitochondrial mass. | [154] |
| OA | BMSCs | IEVs: 800–1100 nm; Mitochondrial. | A rat OA model; IL-1-β stimulated chondrocytes. | Inhibited chondrocyte apoptosis; Inhibited the expressions of iNOS and MMP13; Improved mitochondrial function. | [194] |
| Sepsis | Neutrophils | IEVs: 2–4 μm; Mitochondrial and SOD2. | A murine model of LPS-induced sepsis. | Improved obstructive blood perfusion induced by microthrombosis; Reduced the accumulation of ROS. | [195] |
| Obesity | BAT | sEVs: ~100 nm; Mitochondrial protein. | Obese mice induced by HFD feeding. | Reduced body weight, blood sugar, and lipid accumulation; Restored abnormal cardiac function. | [197] |



| | Differentiation | ECM remodeling | | Angiogenesis | Thrombosis | Immune escape | Drug resistance | Invasion | EMT |
|----------------------|-----------------------|---------------------------------|------------------------------------|-------------------------------|---|--|---|----------------|----------------------------|
| Effective substances | Mitochondrion | TGF-β | Proteinase MMPs Tetraspanins | miRNA Tetraspanins DLL4 | Histone Tissue factor Heat shock proteins | mtDNA PD-L1 | miRNA LncRNA mtDNA Mitochondrion | mtDNA miRNA | MMPs Integrins EpCAM |
| Recipient cells | Tumor cells | Fibroblasts | | Endothelial cells | Platelets | TME cells | Drug-sensitive cells | Tumor cells | Endothelial cells |
| Effects | Tumor differentiation | Myfibroblasts ECM remodeling | | Angiogenesis | Thrombosis | Macrophages Cytokines Cytotoxic T cells Tumor cells | Drug-resistance cells | Tumor invasion | Mesenchymal cells |

Fig. 7 The roles of tumor cell-derived EVs in tumor (by Figdraw). Tumor cell-derived EVs are loaded with a variety of substances, which are involved in the processes of tumor development, invasion, and metastasis through diverse mechanisms. mtDNA, mitochondrial DNA; DLL4, Delta-like 4; ECM, extracellular matrix; TGF-β, transforming growth factor beta; MMPs, matrix metalloproteinases; TME, tumor microenvironment; EMT, epithelial-mesenchymal transition; EpCAM, epithelial cell adhesion molecule

a notable rise in the copy number of plasma EV mtDNA in individuals diagnosed with ovarian cancer [145]. The mtDNA levels within plasma EVs of patients with oral squamous cell carcinoma are higher than those of healthy subjects and positively correlate with circulating IFN-γ and PD-L1 levels, which can be used to assess the efficiency of clinical anti-PD-L1 therapy [147]. Similarly, patients with early pancreatic ductal adenocarcinoma (PDAC) exhibited a higher frequency of mtDNA

mutations in serum EVs than healthy subjects, and these mutations were unique, demonstrating that EVs carrying mitochondrial cargoes can be used as a reliable tool for diagnosing PDAC [148]. Intriguingly, in addition to detecting changes in mtDNA in EVs, Rasuleva et al. [149] also developed a rapid method combining immunoprecipitation and qPCR quantification (EvIPqPCR) for the detection of mtDNA/nDNA ratios in serum EVs of tumor origin as a cell-specific indicator of mitochondrial

enrichment characteristics, and elevated mtDNA/nDNA ratios can be used as a biomarker for pancreatic cancer. Moreover, Jang et al. [146] also discovered that melanoma tissue-derived EVs exhibited a higher abundance of mitochondrial membrane proteins when compared to EVs derived from non-melanoma sources. These studies suggest that EVs enriched with mitochondrial cargoes could be a complementary approach to cancer screening, but their clinical translational applications require further validation in large cohorts (Table 1).

EVs target mitochondrial therapy for cancers

Dysfunction in cancer cell energy is a hallmark of cancer, and mitochondrial dysfunction is related to multiple aspects of cancer progression. Moreover, considering the significance of mitochondrial reprogramming in tumor survival by promoting metabolic adaptation and anti-apoptotic properties, the implementation of targeted mitochondrial therapy emerges as a potentially effective strategy for cancer treatment. EVs, as natural nanocarriers, can deliver molecules specifically targeting mitochondria. EVs originating from neoplastic cells have a substantial abundance of biological constituents, akin to those found in their corresponding parental cancer cells. EVs can be internalized by parental cells, hence facilitating the identification of the specific cell type from which they originate. This particular attribute provides significant advantages within the realm of molecular-targeted therapy. For instance, EVs originating from breast cancer cells exhibited the capacity to selectively deliver lipophilic triphenylphosphonium (TPP)-modified therapeutic recombinant P53 proteins (TPP/P53) to the mitochondria of breast cancer cells and induced death in both P53 mutant cancer cells and P53 wild-type cells through P53-mediated endogenous mechanisms, presenting a promising avenue for breast cancer treatment in the future [171]. Furthermore, other tactics involving EVs center on the reprogramming of mitochondria through the regulation of the Bcl-2 family, resulting in the induction of cellular apoptosis [172, 173].

Currently, drug resistance in cancer cells is also one of the main reasons for cancer treatment failure. It has been found that EVs can transport mitochondria with mutated DNA to sensitive cancer cells to make them chemoresistant, and blocking this process may be one of the strategies for cancer treatment [174]. Meanwhile, tumor cell-derived EVs, as naturally occurring nanoparticles that can bypass and evade the exocytosis system and can achieve tumor targeting without the need for surface modification, also have great potential for overcoming drug resistance and improving targeting. Tumor-derived sEVs loaded with pro-oxidants can bypass the efflux pump and deliver pro-oxidants to the cancer cell cytoplasm, depleting GSH to induce oxidative stress,

inhibiting ATP production, and inducing mitochondrial dysfunction and thus apoptosis in cancer cells [175].

Photodynamic therapy and sonodynamic therapy have demonstrated significant advantages over traditional radiotherapy, and the use of nanotechnology in conjunction with them has led to the development of new photosensitizers and sonosensitizers, as well as improved efficacy in combination with other therapies. Mitochondria-targeted photosensitizers were transported into glioblastomas via brain endothelium-derived EVs, and light conditions triggered the production of cytotoxic ROS by the mitochondria-targeted photosensitizers to selectively kill infiltrating malignant brain tumors [176]. Similarly, Nguyen Cao et al. [16] recently developed bio-reducible EVs of loaded mitochondrial targeted sonosensitizers and glycolysis inhibitors that exhibited GSH/US dual response drug release, which effectively damaged mitochondria and promoted cell apoptosis. Furthermore, Cao et al. [177] constructed dendritic cells (DCs)-derived sEVs (DEVs)-mimicking aggregation-induced emission (AIE) nanoparticles, which, in conjunction with photodynamic therapy, exhibited the potential to enhance the immune response against tumors by promoting mitochondrial targeted immunogenic cell death, highlighting the promising prospects of developing clinical nano-vaccines with anticancer properties. These studies suggest that using EVs as a cross-cutting natural nano-platform for cancer mitochondrial targeting is one of the major directions for future cancer therapy (Table 2).

Lung diseases

EVs may be potential biomarkers for diagnosing lung diseases

Streptococcus pneumoniae (Spn) infection may cause serious complications, including ALI and acute respiratory distress syndrome. It has been shown that treatment of A549 cells with pneumolysin (PLY), a major virulence factor of Spn, resulted in a decrease in the mitochondrial content of EVs released by the cells, which might be attributed to the inhibition of mtROS due to PLY stimulation, which interfered with the packaging of mitochondria within EVs [150]. This study suggests that mitochondrial content within EVs may be a potential biomarker of lung disease, but its clinical translational potential does need to be demonstrated by additional trials and innovative results (Table 1).

EVs treat lung disease by transferring mitochondria or mitochondrial components

Mesenchymal stem cell (MSC) therapy has shown a good therapeutic effect in lung injury disease models by secreting EVs to transfer mitochondria or mitochondrial components. On the one hand, the therapeutic effect of MSC-EVs is mainly through regulating the metabolic

state and phenotype of macrophages. For example, MSC-EVs enhanced OXPHOS, promoted phagocytosis of macrophages, and inhibited the secretion of proinflammatory cytokines by transferring functional mitochondria to macrophages [178]. Similarly, adipose-derived MSCs (AdMSCs)-derived EVs transferred mitochondrial components (mtDNA) to alveolar macrophages, resulting in improved mitochondrial integrity and OXPHOS levels in macrophages [179]. In addition, the delivery of mitochondria by MSC-EVs in vitro could also improve the oxygen consumption rate in alveolar macrophages [180].

On the other hand, MSC-EVs regulate the metabolic state of cells in other lung tissues by transferring functional mitochondria. MSC-EVs could transport mitochondria to AECs to restore ATP production and LPS-induced alveolar leukocytosis and protein leakage [181]. In addition, Dutra Silva et al. [182] found that the mitochondria of MSC-EVs could be integrated into the mitochondrial network of recipient cells, thereby restoring normal levels of mitochondrial autophagy and mitochondrial biosynthesis and restoring significant damage to the integrity of the alveolar-capillary barrier. At present, relevant studies mainly focus on the role of MSC-EVs. Whether EVs from other cells, especially lung tissue cells, can also play a role in treating lung diseases by transferring mitochondria or mitochondrial components remains a big research space. EVs derived from homotypic healthy cells of injured lung tissue may have better-targeted therapeutic effects (Table 2).

Kidney injury

Opportunities and challenges of EVs in the diagnosis of kidney injury

The kidney, being a highly energy-consuming organ, is ranked second in mitochondrial mass and oxygen consumption, following only the heart [183]. The involvement of mitochondrial damage is of considerable importance in the pathogenesis of AKI and CKD. In the state of disease, EVs carrying damaged mitochondria are released from donor cells into the extracellular space and enter the circulatory system. Meanwhile, during the progression of DKD, aberrant mitochondrial bioenergetics occurred before proteinuria and renal histological changes. Therefore, the content of mitochondrial cargo in circulating EVs may be an early diagnostic marker of kidney disease, but there is no relevant research at present.

EVs play a therapeutic role by regulating mitochondrial homeostasis in multiple pathways

Previous studies have shown that EVs can play a therapeutic role in kidney injury by maintaining the quality of functional mitochondria in the receptor cells and restoring mitochondrial homeostasis, but the specific way in which EVs affect mitochondria has not been evaluated

[184–186]. Recent studies have shown that healthy mitochondria can be packaged in EVs and transferred to recipient cells to play a therapeutic role. For example, renal scattered tubular-like cells-derived EVs carried mitochondria and transferred them to injured tubular epithelial cells (TECs) to inhibit Drp1 activity, thus partially restoring mitochondrial function and alleviating kidney injury [187]. MSC-EVs carried mtDNA and ETC proteins to restore TFAM signal transduction and mtDNA stability in damaged TECs [136]. In addition, EVs could also regulate the mitochondrial bioenergetics, biogenesis, mitochondrial autophagy, and kinetics of target cells through the non-mitochondrial cargo carried by EVs. BMSC-derived EVs directly targeted NLRP3 via miR-223-3p to inhibit inflammasome activation and enhanced mitochondrial autophagy to alleviate renal I/R injury [188]. Similarly, MSC-EVs played an anti-apoptotic role by inhibiting Drp1 activation and mitochondrial fission through miR-30 [189]. EVs are enriched with a variety of miRNAs that may be key mediators for rescuing mitochondria. Recently, EVs have attracted strong research interest as natural drug delivery systems. EVs loaded with IL-10 not only improved the stability of IL-10 but also effectively targeted kidney injury, inhibited mammalian target of rapamycin (mTOR) activity, and thus promoted mitochondrial autophagy to maintain mitochondrial health in TECs [190]. Therefore, an EV-based drug delivery system may be a strong candidate for the treatment of kidney injury in the future (Table 2).

Liver injury

EVs as a biomarker in liver injury

Liver biopsy is still the gold standard diagnostic tool for evaluating the staging of liver disease. The lack of non-invasive diagnostic methods is a major obstacle in the clinical management of liver disease. Circulating EVs carry information from parent cells or tissues and become a new target for liquid biopsy. Long-term excessive alcohol intake could promote the release of inflammatory mtDNA-enriched EVs from hepatocytes [151]. Similarly, hepatocytes exposed to alcohol released more EVs containing mitochondrial double-stranded RNA (mtdsRNA) [152]. The changes in mitochondrial content in EVs may reflect the severity of the disease, which provides a new way to predict the progress of liver disease (Table 1).

The application of EVs in liver injury

At present, the implementation of immunomodulatory strategies aimed at reducing neutrophil extracellular traps (NETs) is widely regarded as a primary therapeutic avenue for addressing hepatic I/R injury and may hold the potential to improve the prognosis of individuals undergoing liver transplantation [191]. MSC-EVs,

as nanoparticles, can emulate the abilities of MSCs in modulating diverse immune cells [192]. Lu et al. [14] found that MSC-EVs could inhibit the formation of NETs in liver tissue and subsequently ameliorate I/R injury in the liver through transferring functional mitochondria to intrahepatic neutrophils and repairing their mitochondrial function. The utilization of EV-based therapy presents a compelling strategy for immunomodulation in the context of liver injury (Table 2).

Additional diseases

Osteoarthritis

Mitochondrial dysfunction is one of the main characteristics of osteoarthritis (OA). Proteomic analysis showed that, compared with the normal primary chondrocyte EVs, the decrease in the number and score of mitochondrial proteins in the EVs of chondrocytes after treatment with IL-1 β indicated that this may be a potential direction for the detection of OA [154].

OA predominantly features wear in articular cartilage, synovial inflammation, and joint space constriction, all of which contribute to compromised joint mobility [193]. More and more evidence showed that EVs might play an important and complex role in the pathogenesis, diagnosis, and treatment of OA. Zheng et al. [154] found that primary chondrocyte EVs cultured in a normal environment restored mitochondrial dysfunction, thereby repairing damaged chondrocytes. This effect may be related to the rich mitochondrial proteins in EVs. In addition, BMSCs-derived EVs have also been reported to significantly ameliorate osteoarthritis, and larger EV fractions had a greater impact on enhancing the mitochondrial function of chondrocytes, potentially due to their ability to transport intact and healthy mitochondria [194]. The type and mitochondrial content of EVs may affect their therapeutic efficacy, and EVs carrying intact mitochondria have better therapeutic effects (Table 2).

Sepsis

Sepsis is a critical medical condition characterized by a systemic inflammatory response, posing a significant threat to the patient's life. Despite ongoing research efforts, the development of a definitive and effective therapy for sepsis remains an unresolved challenge. In a model of sepsis, circulating neutrophils transmitted mitochondria-rich EVs, which could prevent the accumulation of endothelial ROS and alleviate endothelial dysfunction after LPS attack through superoxide dismutase 2 contained in mitochondria [195]. This research provides new directions for treating mitochondrial dysfunction in critical diseases such as sepsis (Table 2).

Obesity

There has been a significant increase in the occurrence of obesity and its related ailments, making it a serious global public health issue. Remarkable progress has recently been made by researchers in treating obesity by using brown adipose tissue transplants [196], but two primary challenges persist: the challenge of procuring donor tissue and the issue of immune rejection. Hence, it is crucial to explore alternative approaches for addressing obesity-related ailments that do not involve transplantation. Mitochondrial components were significantly enriched in brown adipose tissue-derived EVs and were effective in alleviating metabolic syndrome in obese mice, including hyperglycemia and hepatic lipid accumulation, at least in part, by transferring mitochondria-associated functional proteins to the liver and facilitating energy expenditure [197]. EVs have shown potential as a viable approach for addressing the challenges associated with obesity and its associated disorders (Table 2).

Conclusions and perspectives

Notably, EVs have been shown their involvement in the regulation of several mitochondrial dysfunction related diseases and have displayed considerable promise as both diagnostic and therapeutic agents for such conditions. EVs not only deliver mitochondria or mitochondrial constituents to the target cells to repair impaired mitochondrial function but also modulate mitochondrial function by transferring specific cellular contents that are representative of their parent cells. It is worth noting that the sizes and types of EVs may relate to their mitochondria contents (full unit or partial) and function. EVs carrying structurally intact mitochondria may have better therapeutic effects. Moreover, the transportation of mitochondria through EVs can take place in both physiologic and pathologic states. Therefore, blocking the transfer of damaged mitochondria carried by EVs in pathological states might be a promising therapeutic strategy for a wide range of diseases, but there are currently no specific inhibitors of EVs for in vivo use. Another way to inhibit the spread of EV-mediated diseases is to block the uptake of EVs by recipient cells, but the strategy remains to be thoroughly investigated due to the diversity and complexity of the mechanisms by which EVs are taken up. Furthermore, the therapeutic potential of EVs can be increased by loading therapeutic agents or surface modifications to enhance mitochondrial targeting. Considering the aforementioned benefits, EVs possess the potential to serve as effective nanotherapeutics for mitochondrial dysfunction related diseases.

Some studies on EVs are now in clinical trials, such as DEVs as an alternative to DC vaccines are in clinical phase I and phase II trials, but challenges remain in meeting clinical requirements. Due to the limitations of

EV separation methods, most current research focuses on the functions of mixed EV subgroups. Therefore, it is difficult to confirm which EV subgroups are rich in mitochondrial components and the impact of their types on mitochondrial function. In addition, it is still unclear whether other mechanisms affect the mitochondrial content in EVs, in addition to the selective elimination of damaged mitochondria through the release of EVs through mitochondrial quality control. In fact, the limited comprehension of EVs, the absence of standardized quality management protocols, the variability in EVs derived from different tissue sources, donor cells, and preparation techniques, and the lack of consensus on optimal concentrations and intervention strategies for EVs in various diseases pose significant obstacles to the successful translation of EVs in research and practical applications. Moreover, despite the promise of EVs as biomarkers for various diseases, there are few relevant research reports at present, and further clinical evidence is needed to determine whether their changes are indeed related to disease progression and prognosis.

Despite the challenges, EVs have made useful achievements in several fields in recent years. The ongoing advancements in isolation, purification, and characterization techniques have led to a growing interest in investigating the therapeutic applications of EVs as drugs or drug delivery vehicles, and EVs hold great promise as potential diagnostic and therapeutic candidates for clinical mitochondrial dysfunction related diseases.

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Author contributions

ZXS conceived and revised the paper. JLL and TRW drafted the manuscript and prepared the figures and tables. XMH, YL, JXZ, WHB, and HQ performed manuscript reviewing and editing. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

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