Journal of Translational Medicine

REVIEW Open Access

Targeting mitochondrial transfer: a new horizon in cardiovascular disease treatment

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Abstract
Cardiovascular diseases (CVDs) are the leading cause of mortality among individuals with noncommunicable diseases worldwide. Obesity is associated with an increased risk of developing cardiovascular disease (CVD). Mitochondria are integral to the cardiovascular system, and it has been reported that mitochondrial transfer is associated with the pathogenesis of multiple CVDs and obesity. This review offers a comprehensive examination of the relevance of mitochondrial transfer to cardiovascular health and disease, emphasizing the critical functions of mitochondria in energy metabolism and signal transduction within the cardiovascular system. This highlights how disruptions in mitochondrial transfer contribute to various CVDs, such as myocardial infarction, cardiomyopathies, and hypertension. Additionally, we provide an overview of the molecular mechanisms governing mitochondrial transfer and its potential implications for CVD treatment. This finding underscores the therapeutic potential of mitochondrial transfer and addresses the various mechanisms and challenges in its implementation. By delving into mitochondrial transfer and its targeted modulation, this review aims to advance our understanding of cardiovascular disease treatment, presenting new insights and potential therapeutic strategies in this evolving field.

Keywords Cardiovascular diseases, Mitochondrial transfer, Myocardial infarction, Cardiomyopathies, Therapeutic strategies

Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality among individuals with noncommunicable diseases worldwide [1, 2]. Recognized as a significant public health challenge, CVDs are characterized by a wide range of risk factors, complex pathophysiological mechanisms, and comorbidities, complicating their management [3]. Thus, there is a pressing need for effective strategies to address CVDs.

Mitochondria play a crucial role in the cardiovascular system, providing the energy necessary for normal cellular functions [4]. These organelles are known for their role in oxygen consumption and ATP production via the *Krebs* cycle [5]. The energy requirements of cardiovascular cell types vary, with a high demand for cardiomyocytes (CMs) and a lower demand for endothelial cells (ECs). Mitochondria constitute approximately 2–6% of

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the volume of ECs, whereas they constitute 28% of hepatocytes and 32% of cardiomyocytes [6]. Owing to the relatively low mitochondrial content in ECs, these cells rely primarily on anaerobic glycolysis for their energy needs instead of mitochondrial oxidative phosphorylation [7]. Thus, in the context of cardiovascular microcirculation, the role of mitochondria is largely in signal transduction rather than direct energy provision.

In addition to energy production, mitochondria are involved in several critical cellular processes, including signal transduction, maintaining redox balance, maintaining calcium homeostasis, the biotransformation of lipids and amino acids, and the regulation of necrosis and programmed cell death [8]. In addition, they can adapt to environmental changes to maintain energy homeostasis [9]. Given their importance in cardiac tissues, the quality of mitochondria is strictly regulated through the process of mitophagy, which ensures the removal of dysfunctional mitochondria. This process involves the autophagy machinery and a specific set of proteins that identify and target damaged mitochondria for degradation. In summary, mitochondria are essential for maintaining cardiovascular system homeostasis and function.

Mitochondrial Dysfunction in CVDs

Mitochondria are essential for the physiological function of the cardiovascular system, primarily because of their critical functions in energy generation and calcium regulation [10]. Mitochondrial dysfunction has been associated with metabolic pathologies such as diabetes, obesity, neurodegenerative diseases, CVDs, and cancer [11]. During mitochondrial dysfunction, inflammatory responses and the modulation of cell death processes are triggered, which play pivotal roles in the progression of cardiovascular disorders [12]. Notably, mitochondrial impairments have been implicated in the pathogenesis of numerous cardiovascular diseases, including myocardial infarction (MI), various cardiomyopathies, atherosclerosis, and hypertension [13].

Mitochondrial metabolism

Mitochondrial metabolism significantly varies between healthy individuals and those with CVD. Healthy cardiomyocytes predominantly utilize fatty acids, branched-chain amino acids, and, to a lesser extent, ketone bodies, which serve as an alternate energy source in certain conditions, to meet their increased energy demands and drive ATP production via the mitochondrial respiratory chain [14]. Conversely, pyruvate, a product of glycolysis, plays a limited role in providing energy in healthy hearts. The metabolic profile of mitochondria undergoes notable changes during the progression of various cardiac pathologies, such as heart failure or ischemia. For example, in conditions such as heart failure (HF), there is a decline in the bioenergetic reserve capacity within the myocardium. As myocardial remodeling progresses, there is a shift in metabolic preference from fatty acids to glucose, accompanied by a reduction in high-energy phosphate reserves, indicative of an energy-deficient state in the failing heart [15]. Like in the ischemic myocardium, the TCA cycle is enriched at intermediate succinate concentrations, which are mechanistically linked to oxidative damage during reperfusion [16]. Moreover, approximately six weeks post-MI, TCA activity is attenuated, potentially indicating a maladaptive response within the surviving cardiac tissues.

The mechanisms associated with the shift in metabolic profiles in the myocardium during the development of cardiac pathologies remain incompletely characterized. However, increasing evidence suggests the involvement of specific transcription factors. For example, nuclear receptor subfamily 2 group F member 2 (NR2F2) levels are elevated in patients with HF, and overexpression of NR2F2 has been shown to promote dilated cardiomyopathy and pathological metabolic remodeling [17]. Another transcription factor of significance is hypoxia-inducible factor 1α (HIF1α), which promotes the transcriptional upregulation of peroxisome proliferator-activated receptor-γ (PPARγ). This, in turn, leads to increased glucose uptake, increased lipid storage, apoptosis, and contractile abnormalities [18]. Similarly, deletion of HIF1α specifically in the ventricles of the heart can ameliorate pressure overload-induced cardiomyopathy in mice [19]. Moreover, miRNA also plays an important role in metabolic shift. In 2022, it was reported that epigenetic mechanisms contribute to metabolic shifts and are implicated in altered mitochondrial metabolism. Specifically, miRNA-21 regulates mitochondrial respiration and the ability of cells to select the most appropriate substrate for ATP production in specific environments [20].

Mitochondrial dynamics

The mitochondrial network remains in a constant state of dynamism due to the opposing actions of various proteins, including both fission and fusion proteins. Fission proteins such as mitochondrial fission factor (MFF), mitochondrial fission 1 protein (FIS1) and dynamin-1-like protein (DNM1L), as well as fusion proteins such as mitofusin 1 (MFN1), MFN2, and optic atrophy protein 1 (OPA1), orchestrate this intricate process (Fig. 1) [21] and are tightly regulated to ensure the optimal function of mitochondria under both physiological and pathological conditions, which is partially due to the mitophagic removal of dysfunctional mitochondria induced by inappropriate fission [22].

Mitochondrial dynamics, involving fusion and fission processes, are crucial for maintaining function. Defects in fusion proteins like OPA1, MFN1, and MFN2, along

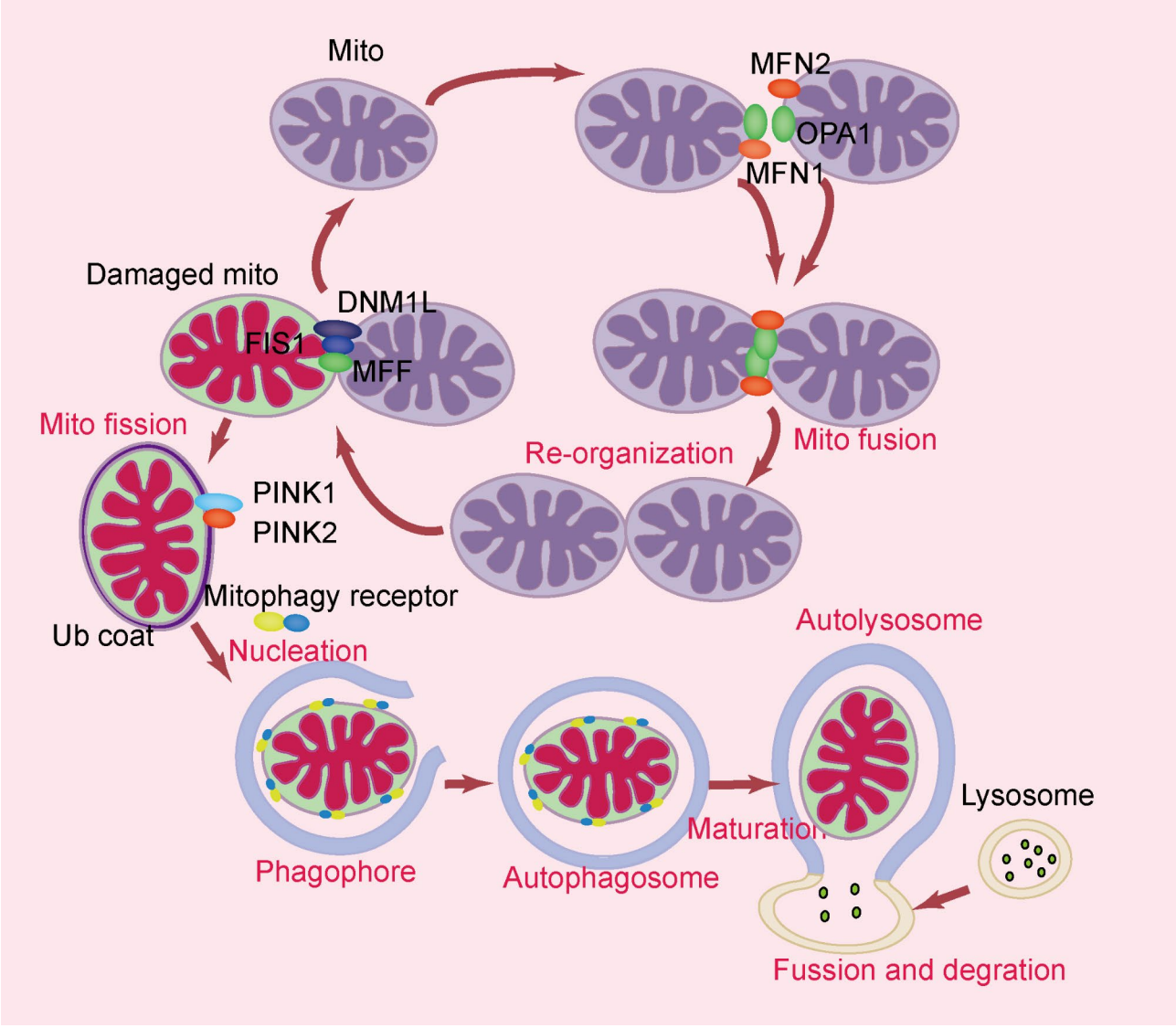

Fig. 1 Mechanism of Mitochondrial Dynamics. The mitochondrial network is constantly dynamic due to the antagonistic effects of several proteins, including fission proteins (i.e., MFF, FIS1 and DNM1L) and fusion proteins, such as MFN1, MFN2, and OPA1. It is tightly regulated to ensure the optimal function of mitochondria under both physiological and pathological conditions, which is partially due to the removal of dysfunctional mitochondria induced by inappropriate fission. Fission functions to segregate dysfunctional mitochondria, thereby enabling degradation by the autophagic machinery

with fission protein DRP1, contribute to mitochondrial dysfunction in cardiovascular diseases [3]. DRP1 dysregulation can lead to excessive fragmentation, worsening cardiac issues. Highlighting both processes helps explain how disruptions in mitochondrial dynamics lead to cardiovascular pathology.

As a consequence, defects in these mitochondrial dynamic genes are closely linked to CVDs in different animal models [23]. The myocardium of *Opa1*$^{+/-}$ mice displays irregular mitochondrial morphology with tangled cristae and decreased mitochondrial DNA (mtDNA) levels, and *Opa1*$^{+/-}$ mice are more sensitive to hypertrophic pressure than their wild-type littermates are [24]. Cardiac deletion of *Opa1* induced by Yme1l1 ablation promoted mitochondrial fragmentation and metabolic impairment, which can lead to HF [25]. Interestingly, cardiomyopathy induced by angiotensin II favors OPA1 acetylation and mitochondrial fragmentation [26]. In addition, the double deletion of *Mfn1* and *Mfn2* in cardiomyocytes significantly blocks mitochondrial fusion, which induces cardiac dysfunction manifesting as acute progressive dilated cardiomyopathy that cannot be fully abrogated by concomitant deletion of *Dnm1l* (Fig. 1) [27]. Moreover, *Mfn1*$^{-/-}$*Mfn2*$^{-/-}$ hearts are less susceptible to ischemia–reperfusion injury, potentially owing to mitigated calcium overload [28].

Mitophagy

Mitophagy plays a prominent role in preserving mitochondrial homeostasis in both healthy and pathological cardiovascular systems (Fig. 2) [29]. Thus, deficiencies in mitophagy are closely linked to the development of CVDs, as previously demonstrated in various experimental models. Specifically, the protein levels of PINK1 were diminished in end-stage human heart failure, indicative of a reciprocal relationship between PINK1 activity and heart failure. Although normal at birth, the *Pink1*$^{-/-}$ mice develop cardiac hypertrophy and left ventricular dysfunction by 2 months of age. This phenotype was accompanied by increased oxidative stress and mitochondrial dysfunction (Fig. 1) [30]. Furthermore, the depletion of Park2, an E3 ubiquitin ligase crucial for mitophagy, in mice myocardial tissue has been shown to induce mild cardiac disorders and continuous defective mitochondrial metabolic functions, suggesting that autophagic removal of mitochondria is only partially recovered in the absence of *Park2*, or that Park2 may play other beneficial roles in cardiac mitochondria beside mitophagy [31]. Conversely, ablation of Park2 in the myocardium of neonatal mice results in the early onset of severe cardiomyopathy, ultimately leading to premature and acute lethal outcomes accompanied by impaired mitochondrial maturation [32].

Ca2+ homeostasis

Mitochondria in cardiomyocytes are involved in calcium flux [33]. The depolarization of the plasma membrane facilitates the activation of voltage-dependent L-type Ca^{2+} channels, leading to an influx of Ca^{2+} into the cytosol, which is mediated by ryanodine receptor 2 (RYR2) [34]. Cytosolic Ca^{2+} is predominantly removed by the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) family and by solute carrier family 8 member A1 (SLC8A1) [35]. Mitochondrial calcium handling involves the calcium uniporter protein mitochondrial (MCU) for calcium influx and the Na^{+}/Ca^{2+} antiporter SLC8B1 for calcium efflux. Mild and transient increases in mitochondrial calcium levels support oxidative phosphorylation. Mitochondrial Ca^{2+} activates key enzymes involved in the TCA cycle, of which, isocratic dehydrogenase and α-ketoglutarate dehydrogenase are activated in a Ca^{2+}-dependent manner. Ca^{2+} also activated the F1/F0 ATPase, which promotes increased conversion of nicotinamide adenine dinucleotide (NAD^{+}) to reduced NADH, transferring electrons from complex I to complex

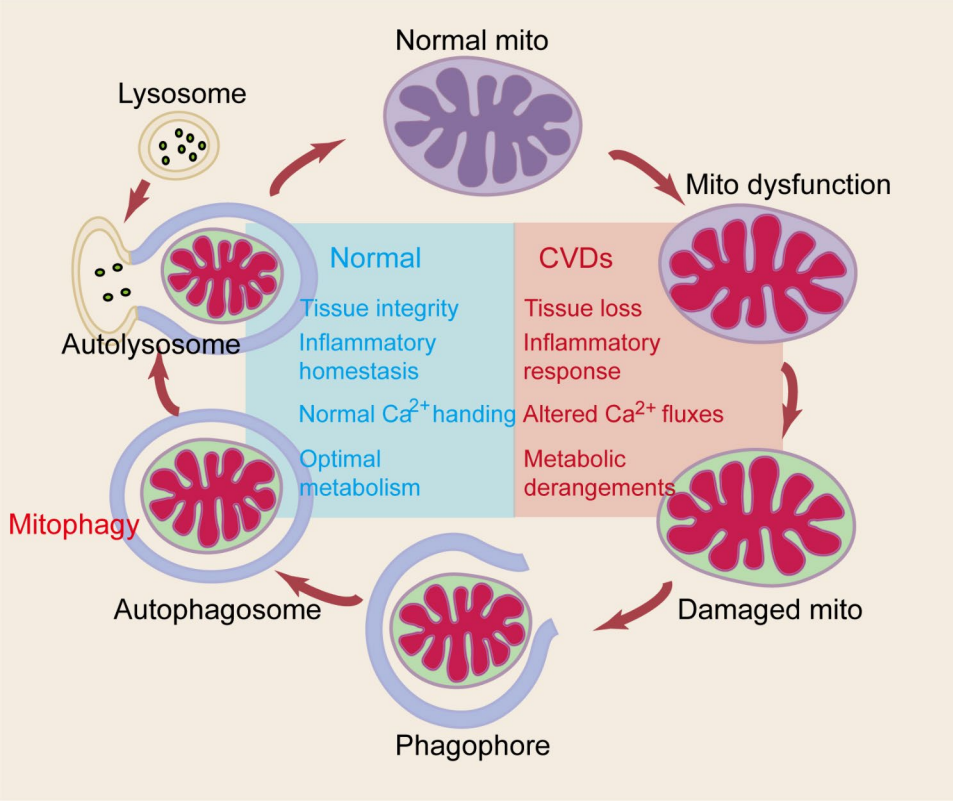

Fig. 2 Mitochondrial dysfunction in cardiovascular diseases. Mitochondria are essential for the physiological function of the cardiovascular system because of their important roles in energy production and calcium flux. Upon mitochondrial damage, dysfunctional mitochondria activate inflammatory responses and regulate cell death, ultimately contributing to the progression of cardiovascular disorders. In CVD, mitochondrial dysfunction causes tissue loss, an inflammatory response, Ca^{2+} flux alterations, and metabolic rearrangement

IV. Protons are pumped into the intermembrane space by complexes I, III, and IV, creating a proton motive force by which compound V is driven to convert ADP into ATP [36]. However, acute and persistent calcium overload can trigger the mitochondrial permeability transition (MPT). MPT can be triggered by excessive ROS production, leading to the opening of the mitochondrial permeability transition pore (mPTP) [37]. This results in a sudden loss of membrane potential, impaired ATP synthesis, and, ultimately, cell death during ischemia-reperfusion injury. In this context, the overexpression of the leaky variant of RYR2 exacerbates ischemia-reperfusion injury and induces calcium overload in cardiomyocytes (Fig. 2) [38].

Inflammation

The innate immune response induced by mitochondria plays a major role in CVD (Fig. 2) [39]. Mitochondrial components, including ROS, mtDNA, ATP and cardiolipin, can act as damage-associated molecular patterns (DAMPs) upon release and activate the immune response. ROS and mtDNA promote the inflammatory response by stimulating the release of IL-1β, IL-18 and type I interferon upon activation of the inflammasome and stimulator of interferon genes protein (STING) [4]. Additionally, mtDNA can induce granulocyte degranulation by binding to Toll-like receptor 9 (TLR9) [40]. Regulated cell death-induced ATP release can act both as a chemoattractant and an immunostimulant for myeloid cells [41]. The ability of cardiolipin to activate γδ T lymphocytes is dependent on CD1D [42]. Although not all of these processes are directly implicated in the pathophysiology of CVDs, these components can trigger deleterious immune responses in the cardiovascular context. Experimental evidence supports the impact of immune-related pathways on CVD outcomes. For example, depletion of cyclic GMP-AMP synthase (CGAS) in mice led to improved survival, reduced cardiac immune infiltration, and attenuation of pathological tissue remodeling following MI [43]. Similarly, specific deletion of NLRP3 substantially enhanced myocardial function and reduced infarct size in the context of ischemia–reperfusion injury [44].

Regulated cell death

The death of irreparably damaged cells is a prominent etiological mechanism underlying various CVDs, including MI, HF and atherosclerosis [45]. Widespread and irreversible mitochondrial damage, leading to mitochondrial membrane permeabilization, plays a pivotal role in processes such as apoptosis, a form of programmed cell death that happens when cells naturally self-destruct or die, MPT-induced necrosis, an accidental or unprogrammed cell death that causes tissue death, and parthanatos, collectively contributing to pathological tissue loss in CVD patients (Fig. 2) [46]. Consequently, genetic manipulations targeting components involved in regulated cell death offer potential interventions for multiple CVD pathologies. For example, *Bbc3*$^{-/-}$ mice, which display defects in one of the upstream activators of apoptosis, demonstrate increased resistance to ischemia–reperfusion injury compared with their wild-type counterparts [47]. Similarly, the overexpression of the apoptosis regulator Bcl2 in mice has been shown to attenuate myocardial infarction in the context of ischemia–reperfusion injury [48].

Mitochondrial transfer

Mitochondria have ancient origins through an endosymbiotic process in which proteobacteria are engulfed and adapted for energy production and cellular metabolism [27]. These organelles undergo separation during cell division and differentiation, ensuring the vertical inheritance of mitochondria as mtDNA is passed from parent to daughter cells. However, emerging evidence suggests that mitochondria from certain cells can be transferred to unrelated cells via intercellular mitochondrial transfer or horizontal mitochondrial transfer (HMT) [49]. The first documented functional instance of mitochondrial transfer was reported in 2006, when cells that were pretreated with ethidium bromide so that the mtDNA became mutated and depleted and the cells became incapable of aerobic respiration and growth (A549 ρ° cells), were rescued through the transfer of mitochondria from neighboring cells in a coculture system [50]. Recent studies have revealed the occurrence of mitochondrial transfer in various tissues through diverse mechanisms under both physiological and pathological conditions. In the following sections, we delve into the mechanisms of mitochondrial transfer, the known functions of HMT, its implications in CVD, and how it is used for potential CVD treatments.

Mitochondrial Transfer Mechanisms

Currently, several approaches underlying HMT have been revealed in numerous studies and can be grouped into three categories: (1) Transient cellular connections through which mitochondria can be transferred across different cells; (2) Expelled mitochondria from extracellular vesicles for delivery into recipient cells; (3) The direct release of mitochondria into the extracellular environment, where they are subsequently absorbed by recipient cells (Fig. 3).

Transient cellular connections

Transient cellular connections involving tunneling nanotubes (TNTs) and/or connexin 43 (Cx43)-mediated gap junctional channels (GJCs) are the most commonly reported mechanisms in HMT (Fig. 3) [49]. TNT was

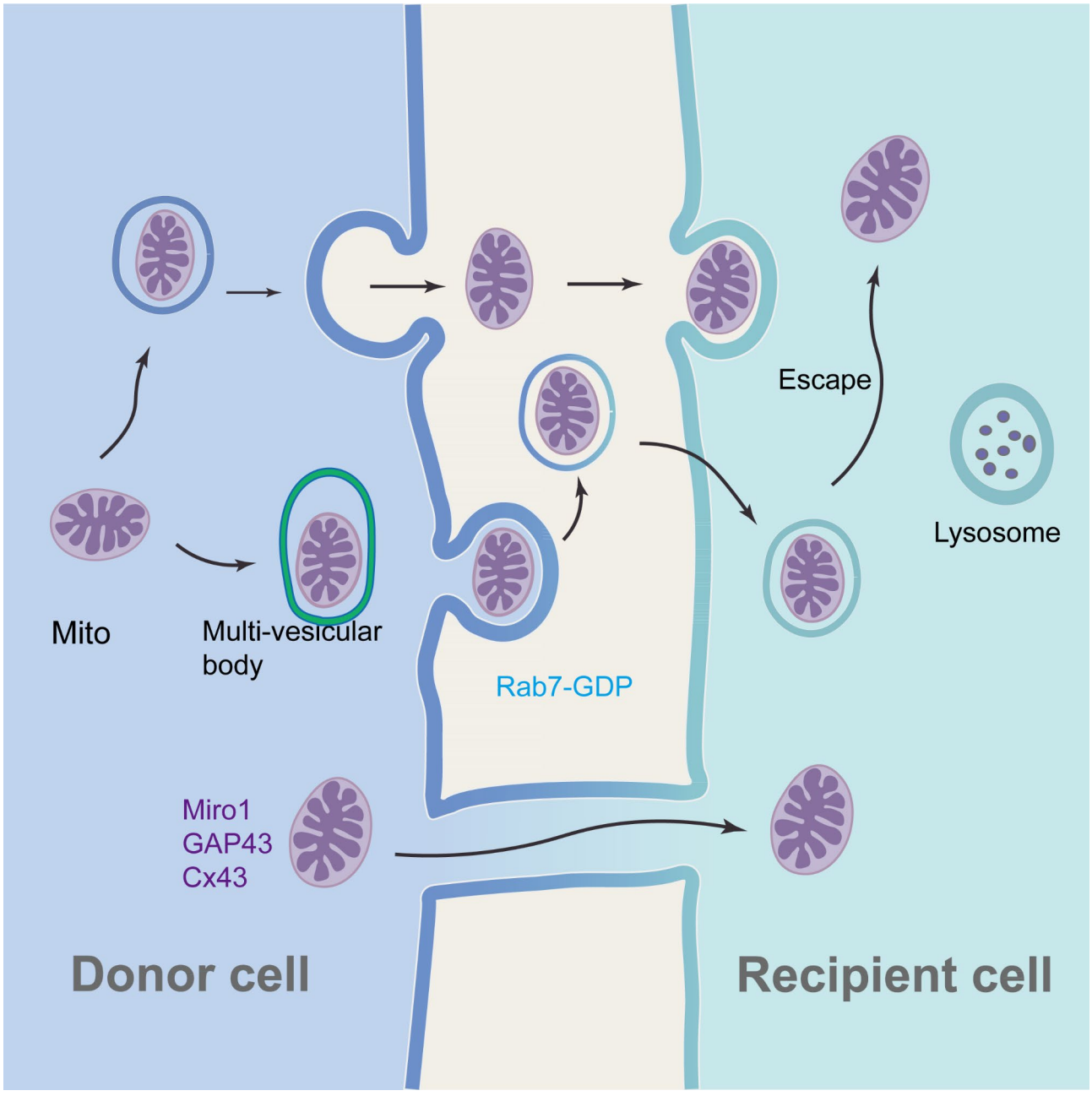

Fig. 3 Mechanisms of mitochondrial transfer. Mitochondrial transfer can be grouped into three categories: (1) Transient cellular connections through which mitochondria can be transferred across different cells; (2) Expelled mitochondria from extracellular vesicles for delivery into recipient cells; (3) The direct release of mitochondria into the extracellular environment, where they are subsequently absorbed by recipient cells. Mitochondrial transfer occurs through transient cellular connections, such as TNTs, mediated by the Cx43, GAP43 and Miro1 shuttle vesicles, which are vesicular bodies that are released as extracellular vesicles in a Rab7-GDP-dependent manner for capture by recipient cells. The mitochondria captured by the recipient cell are degraded via the lysosome

first observed in cultured rat pheochromocytoma PC12 cells, human embryonic kidney (HEK) cells, and normal rat kidney cells. TNTs were later discovered by Koyanagi et al. to be capable of transferring mitochondria between neonatal rat cardiomyocytes and endothelial progenitor cells. These structures have been observed in nerve, muscle, and cancer cells as well as immune cells. Mitochondrial transfer mediated by TNT can be unidirectional or bidirectional. Unidirectional transfer generally occurs from healthy to damaged cells, whereas the amount of bidirectional mitochondrial transfer depends on the degree of cell type specificity, resulting in different effects on damaged cells [51]. Some studies suggest that connexins, particularly Cx43, may exert partial control

over TNT formation [52]. These structures mutually enable the transfer of cytosolic and plasma membrane constituents. The initial study revealed that mitochondrial transfer involves human mesenchymal stem cells and rat cardiomyocytes in culture, which were distinguished through electron microscopy on the basis of their structural disparities [53]. The occurrence of TNT-mediated intercellular mitochondrial transfer has been widely documented across various systems, including the cardiovascular system, immune system, tumors, and nervous system. TNT formation involves the participation of growth-associated protein 43 (GAP43) and potentially Cx43, while mitochondria are conveyed along the actin–microtubule pathway via the Rho-GTPase Miro1 into the recipient cell cytoplasm (Fig. 4) [54–56]. Given that TNTs facilitate the exchange of multiple cellular components, attributing outcomes solely to intercellular contact or mediated by mitochondrial exchange remains a complex challenge.

Extracellular vesicle-associated mitochondria

Intercellular mitochondrial transfer can also occur through the uptake of vesicle-associated mitochondria. Extracellular vesicles containing mitochondria (EVMs) can be categorized on the basis of their size and mitochondrial cargo. Small vesicles (diameter, 100–200 nm) typically carry oxidatively damaged mitochondrial components and are released by white and brown adipocytes [57, 58], which are characterized by the presence of tetraspanins CD63, CD9 and CD81 (Fig. 4) [58].

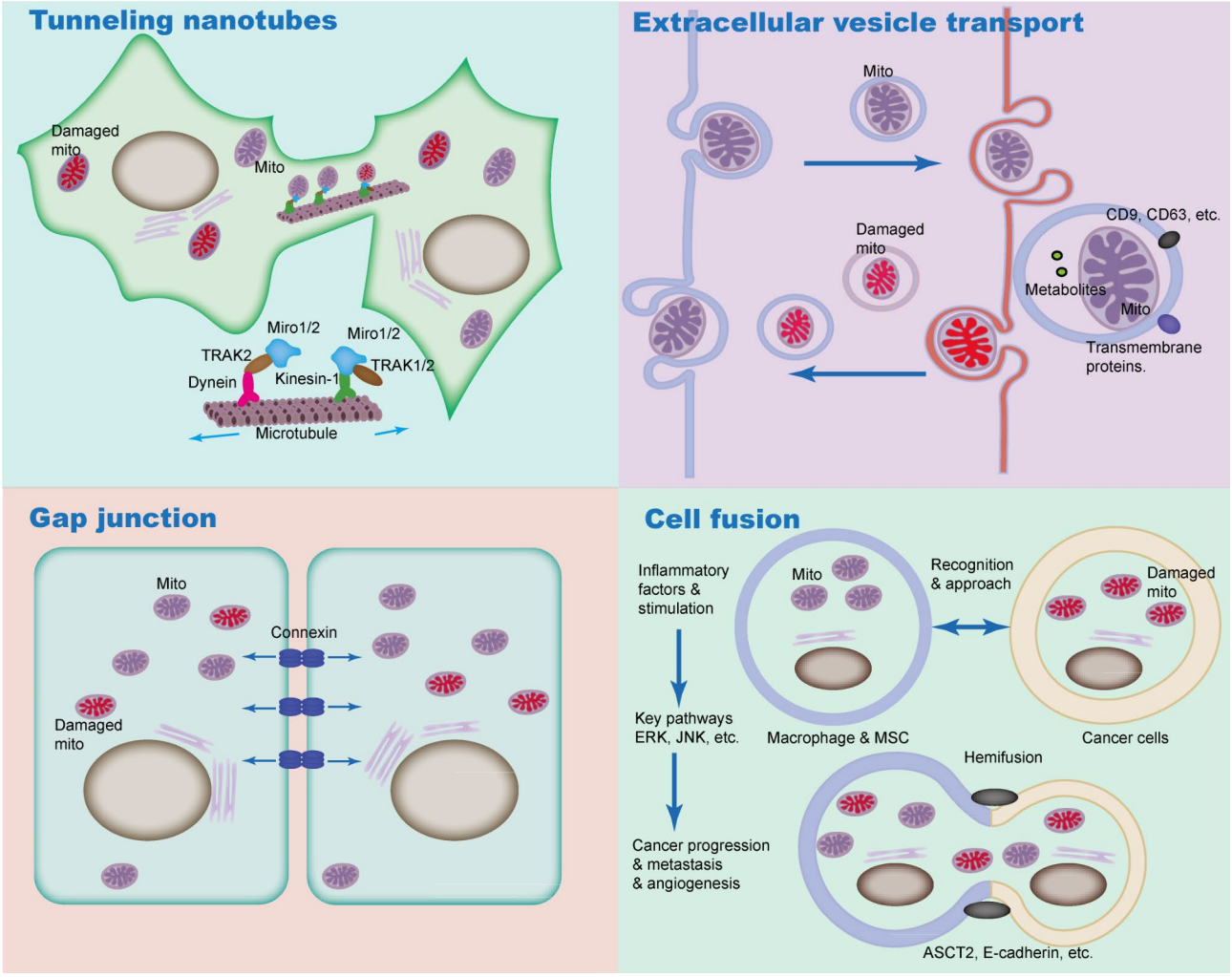

Fig. 4 Models of mitochondrial transfer. Mitochondrial transfer can be mediated by TNTs, extracellular vesicle transport, gap junctions, and cell fusion. TNTs are normally formed from F-actin filaments. During intercellular mitochondria transfer, microtubules are also involved in TNTs and are thus able to transfer bulkier structures propelled by dynein and kinesin motor complexes consisting of several adaptor proteins, such as Miro1 or Miro2. The transfer of mitochondria via extracellular vesicles involves small double-membrane structures formed by blebbing of the plasma membrane. These vesicles can be grouped by size and mitochondrial cargo; gap junctions contain connexin structures, which form pores connecting two neighboring cells, allowing for bidirectional transport of whole mitochondria; and cell fusion allows cells to share mitochondria from the two original cell lines. During this process, several signaling pathways are triggered, resulting in increased tumorigenesis and increased metastasis of cancer cells

Extracellular vesicles (EVs), which act as natural cell-derived carriers for delivering proteins, nucleic acids, and organelles between cells, can be secreted by most cells.

Cardiac mitochondria are expelled via vesicles, giving rise to two distinct types of EVMs. The first type comprises larger mitochondria, measuring 3–4 μm in size, known as exophers, characterized by the presence of LC3 on their surfaces, suggesting their origin within the autophagosomal system (Fig. 4) [59]. Exophers predominantly emerge from cardiomyocytes as a result of damaged autophagic processes and are subsequently engulfed by cardiac macrophages for mitochondrial degradation. The second type of mitochondrial vesicle is smaller (diameter, 300–600 nm) and can be released through multivesicular bodies [60]. These vesicles rely on the activity of the small GTPase Rab7, which is closely associated with lysosomal fusion dynamics. The activation of Rab7 leads to lysosomal degradation of mitochondria, while Rab7-GDP inactivation facilitates the extracellular release of these mitochondria within vesicles (Fig. 3) [61]. Importantly, not all mitochondria within these vesicles are necessarily damaged, contrary to the prevailing assumption.

Some studies have revealed that intact and functional mitochondria can also be exported from approximately 1 μm diameter extracellular vesicles via multivesicular bodies or budding from the plasma membrane [62]. EV-mediated mitochondrial transfer was found in multiple tissues as part of essential cellular bioprocesses. For example, astrocytes release EVMs into hypoxic neurons to maintain normal neuronal mitochondrial function following ischemic stroke in the brain [63]. This process is achieved by CD38, and ablation of CD38 exacerbates stroke pathology in mice [63]. Future investigations may shed light on the precise mechanisms underlying the docking of EVMs to recipient cells and the subsequent intracellular processing following EVM internalization. EVs are highly heterogeneous and have different scales, contents, and specific functions. The poor yield and unscalable production of EVs remain major issues for the application of EV-mediated mitochondrial transfer. Various chemical, physical, and biological stimuli have emerged as promising strategies to improve EV production and facilitate the clinical translation of EV-based therapies.

Free mitochondrial release and capture

The final main method of mitochondrial transfer is the ejection of free or naked mitochondria, which are subsequently captured by recipient cells [64]. These mitochondria are initially observed in the bloodstream and are partially generated by activated platelets, which produce both EVMs and free mitochondria. Free mitochondria contain full-length mtDNA and maintain the membrane potential. In this context, fresh human plasma can consume oxygen but only in the presence of mitochondria. The sources of these free mitochondria in the bloodstream are diverse, with platelets being one source and adipocytes being another [65, 66]. The release of free mitochondria is dependent on mitochondrial fission proteins such as DRP1 and FIS1, although the precise underlying mechanism remains to be elucidated [67]. The capture of free mitochondria is facilitated through processes such as phagocytosis and possibly micropinocytosis (Fig. 4). Heparan sulfate has been identified as a key player in the capture of free mitochondria, as revealed through genome-wide CRISPR screening [68]. The sulfation of the heparan sulfate chain at the 6-O position is a prerequisite for effective mitochondrial capture [69]. Notably, treatment with the anticoagulant heparin, which is a highly sulfated form of heparan sulfate in mice, partially impedes mitochondrial transfer from adipocytes to macrophages. This observation indicates that this form of mitochondrial transfer is indeed functional in vivo and can be influenced by commonly used anticoagulants [70]. Following capture by recipient cells, the fate of free mitochondria remains unclear.

Intercellular mitochondrial transfer activation signals

A fundamental requirement for intercellular mitochondria transfer lies in the capacity of cells to perceive numerous environmental cues and subsequently execute processes such as absorption, trafficking, processing, and integration [71]. Identifying the precise signals that instigate mitochondrial transfer is important for advancing our theoretical understanding and therapeutic applications. Mitochondrial transfer can be triggered by an array of intracellular and extracellular signals in recipient cells, including hypoxia, oxidative stress and inflammation, which have the potential to attract donor cells and induce mitochondrial ejection [72]. Interestingly, damaged mitochondria within recipient cells may themselves emit danger signals that stimulate the transfer of mitochondria from donor cells. For example, prior to caspase-3 activation, damaged mitochondria release cytochrome C, which initiates TNT formation [73]. While caspase-3 is not directly associated with TNT formation, treatment with caspase inhibitors effectively impedes microtubule entry or assembly during TNT formation [74].

Additionally, in response to oxidative stress, depolarized mitochondria are recruited to the plasma membrane by mesenchymal stem cells (MSCs). These compromised mitochondria are then encapsulated within vesicles, which, in turn, are engulfed and processed by aggregating macrophages [75]. Notably, damaged mitochondria can serve as DAMPs, inducing the expression of heme oxygenase-1 (HO-1) and promoting mitochondrial biogenesis within MSCs, which enhances mitochondrial transfer

and ultimately restores the functional capacity of MSCs in damaged cells (Fig. 5) [76].

Intercellular mitochondrial transfer molecular mechanism

TNFaip2/M-Sec, a ubiquitously expressed protein in mammalian cells, can recruit active RalA, which induces membrane deformation and TNT formation with the aid of the exocyst complex and Lst1 (Fig. 5) [77]. Pretreatment of corneal epithelial cells (CECs) with rotenone induces ROS production, resulting in the expression of M-Sec through the activation of the transcription factor nuclear factor kappa B (NF-κB), which contributes to the formation of TNTs in CECs and enhances mitochondrial transfer from MSCs to CECs [78]. Furthermore, tumor necrosis factor-alpha (TNF-α) can promote the expression of M-Sec in MSCs by activating NF-κB, facilitating TNT formation, and augmenting mitochondrial transfer from MSCs to injured cells (Fig. 5) [79, 80]. The mitochondrial outer membrane (MOM) proteins Miro1 and Miro2, which function as Rho-GTPases, serve as adaptor proteins in coordinating microtubule motor proteins and are crucial for integrating cellular signals [81]. During the process of mitochondrial transfer, Miro proteins directly bind to the kinesin motor protein KIF5 in conjunction with accessory proteins such as TRAK1, TRAK2, MYO10, and MYO19, forming a motor–adaptor complex that facilitates the mobility of mitochondria along microtubules (Fig. 4) [82]. The overexpression of Miro1 in MSCs effectively enhances mitochondrial transfer efficiency and mitigates rotenone-induced cell

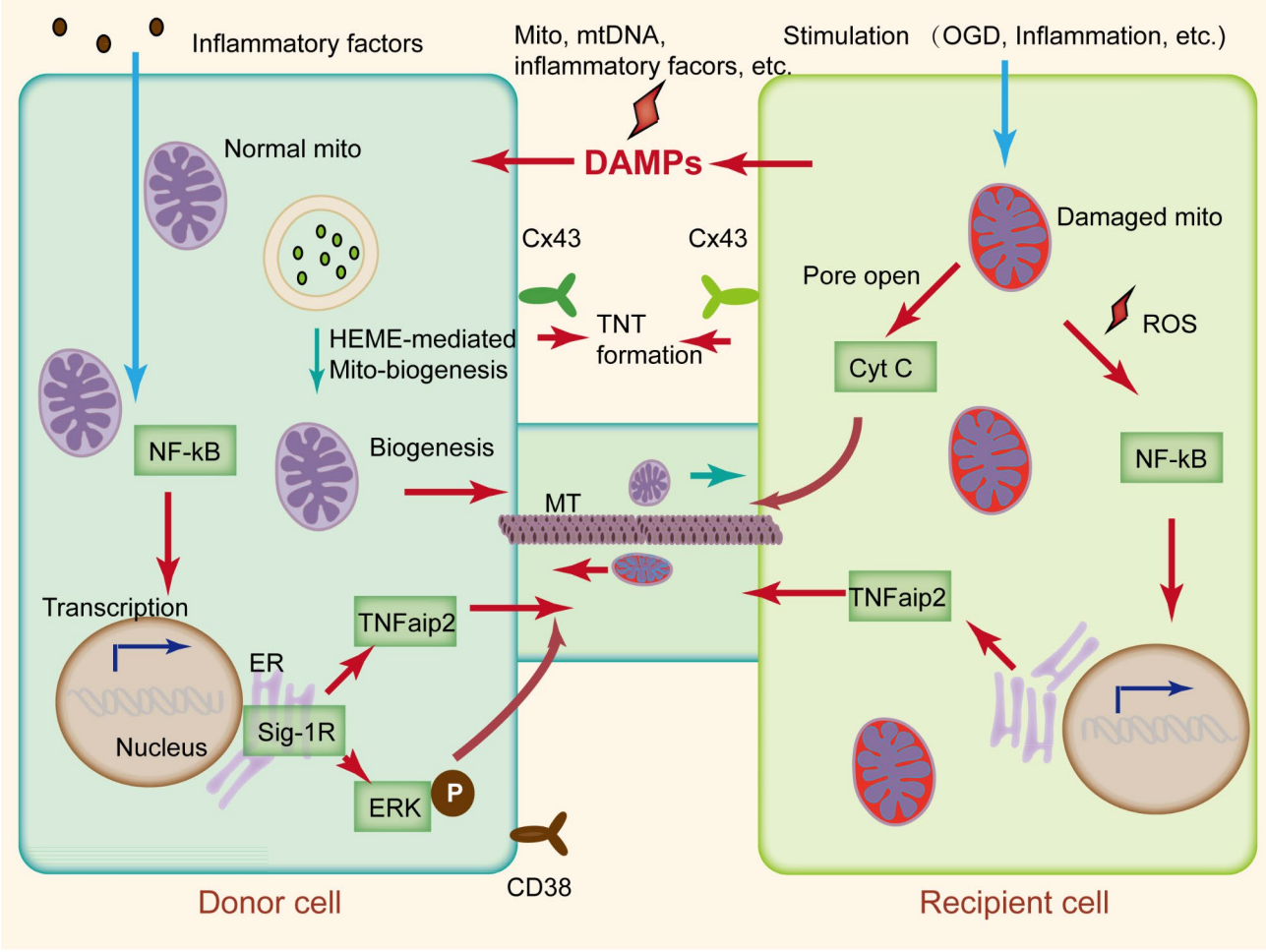

Fig. 5 The cellular and molecular mechanisms of mitochondrial transfer. Mitochondrial transfer can be triggered by various intracellular and extracellular signals in recipient cells, including hypoxia, oxidative stress and inflammation, and the latter can recruit donor cells to induce mitochondrial ejection. Damaged mitochondria in recipient cells may elicit danger signals to induce mitochondrial transfer from donor cells, act as DAMPs and induce the expression of HO-1 and mitochondrial biogenesis in MSCs, increasing mitochondrial transfer and restoring MSC function to damaged cells. CECs were pretreated with rotenone to induce ROS production, resulting in the expression of M-Sec through the activation of NF-κB, which contributed to the formation of TNTs in CECs and increased mitochondrial transfer from MSCs to CECs. Both Sig-1R and calcium ions modulate mitochondrial transfer. During the process of mitochondrial transfer, Miro proteins directly bind to the kinesin motor protein KIF5 in conjunction with accessory proteins such as TRAK1, TRAK2, MYO10, and MYO19, forming a motor–adaptor complex that facilitates the mobility of mitochondria along microtubules

injury, whereas the knockdown of Miro1 in MSCs diminishes mitochondrial transfer efficiency and impairs tissue repair capacity [83]. Moreover, Miro1 serves as a Ca^{2+} sensor and suppresses MFN function at elevated [Ca^{2+}]m. Miro1 functions as a coordinated Ca^{2+} responder by limiting mitochondrial transport while suppressing network fusion. Modulation of the interaction between Miro1 and MFN is a potential intervention for modulating network homeostasis. The loss of Miro1-directed mitochondrial transfer results in osteoclastic bone loss, which is similarly observed in neuron disease caused by Miro1 deficiency. Accordingly, the overexpression of Miro1 in MSCs (MSCmiroHi) results in increased mitochondrial transfer and recovery from epithelial injury, whereas Miro1 knockdown (MSCmiroLo) results in decreased efficacy [84]. Compared with control MSC, treatment with MSCmiroHi was associated with greater therapeutic efficacy in mouse models of rotenone (Rot)-induced airway injury and allergic airway inflammation (AAI).

CD38, a transmembrane glycoprotein, plays a pivotal role in both the synthesis and degradation of cyclic ADP-ribose (cADPR) [85]. CD38 is instrumental in facilitating the transfer of mitochondria between bone marrow-derived mesenchymal stem cells (BMSCs) and multiple myeloma cells, primarily by mediating the formation of TNTs [86]. Depletion of CD38 has been shown to impede mitochondrial transfer both in vitro and in vivo. Moreover, CD38 and cADPR are involved in the calcium-dependent release of extracellular mitochondrial particles by astrocytes, further supporting their role in intercellular mitochondrial transfer [87]. Inhibition of CD38 has been demonstrated to hinder mitochondrial transfer from astrocytes to neurons, and the CD38-cADPR axis has been implicated in cell endocytosis processes [88]. Notably, the expression of CD38 can be augmented by the endoplasmic reticulum-resident transmembrane protein sigma-1 receptor (Sig-1R), which in turn promotes mitochondrial transfer in astrocytes [89].

Sig-1R is located on ERs and is a calcium storage organelle [1]. Given that both Sig-1Rs and calcium ions have been implicated in the regulation of mitochondrial transfer, it is plausible that the ER plays a mediating role in this process (Fig. 5). Notably, the ER is closely associated with mitochondria through the formation of ER-mitochondria tethers, often referred to as mitochondria-associated membranes (MAMs) [90]. MAMs are recognized for their involvement in mediating mitochondrial replication, division, and distribution. In the context of mitochondrial transfer between osteocytes, MAMs have been reported to play a modulatory role, with the MAM-localizing protein MFN2 facilitating the transfer of mitochondria [91]. The mechanism underlying MFN2 in mitochondria transfer is mitochondrial contact with microtubules, which serve as sites of α-tubulin acetylation MFN2-mediated recruitment of α-tubulin acetyltransferase 1 (ATAT1) [92]. This activity is critical for MFN2-dependent regulation of mitochondrial transport, and axonal degeneration caused by CMT2A MFN2-associated R94W and T105M mutations may depend on the inability to release ATAT1 at sites of mitochondrial contact with microtubules. Our findings reveal a function for mitochondria in α-tubulin acetylation and suggest that disruption of this activity plays a role in the onset of MFN2-dependent CMT2A. Additionally, CD38 has been shown to induce contact between mitochondria and the ER, which may contribute to increased energy production and redox homeostasis during the process of mitochondrial transfer in astrocytes [93].

Extracellular mitochondrial entry mechanisms

Mitochondria are captured by endocytosis, but the type of endocytosis involved remains debatable. Pacak et al. investigated this process in cardiomyocytes and proposed that mitochondria are captured through actin-dependent endocytosis [94]. In contrast, Kesner et al. shed light on the mechanisms employed by HepG2 cells and fibroblasts and reported that these cells primarily internalize mitochondria through micropinocytosis, with minimal involvement of clathrin-mediated endocytosis (Fig. 6) [95]. Micropinocytosis is a specific form of endocytosis that relies on actin and results in the formation of large vacuoles [96]. Interestingly, macropinocytosis, which is a widely conserved process, is utilized predominantly by hepatocytes and MSCs to internalize isolated mitochondria [97]. Notably, macropinocytosis does not depend on specific interactions between particles and receptors on the cell membrane and occurs in a nonselective manner. However, Kesner et al. reported that intact mitochondria are specifically captured in HepG2 cells and fibroblasts through a process contingent on the presence of the cellular proteoglycan heparan sulfate [95]. Following internalization, mitochondria are transported to endosomes and lysosomes. In cardiomyocytes, exogenous mitochondria can escape from these cellular compartments and integrate into the endogenous mitochondrial network, which necessitates the coordinated actions of MFN1, MFN2 and OPA1 to facilitate mitochondrial fusion in cardiomyocytes and fibroblasts (Fig. 6) [98]. However, the specific mechanisms underlying the escape of mitochondria from endosomes and lysosomes remain to be fully elucidated.

Functions of mitochondrial transfer

To date, the understanding of the function of mitochondrial transfer has evolved from the notion that it merely supports energy metabolism in recipient cells to the

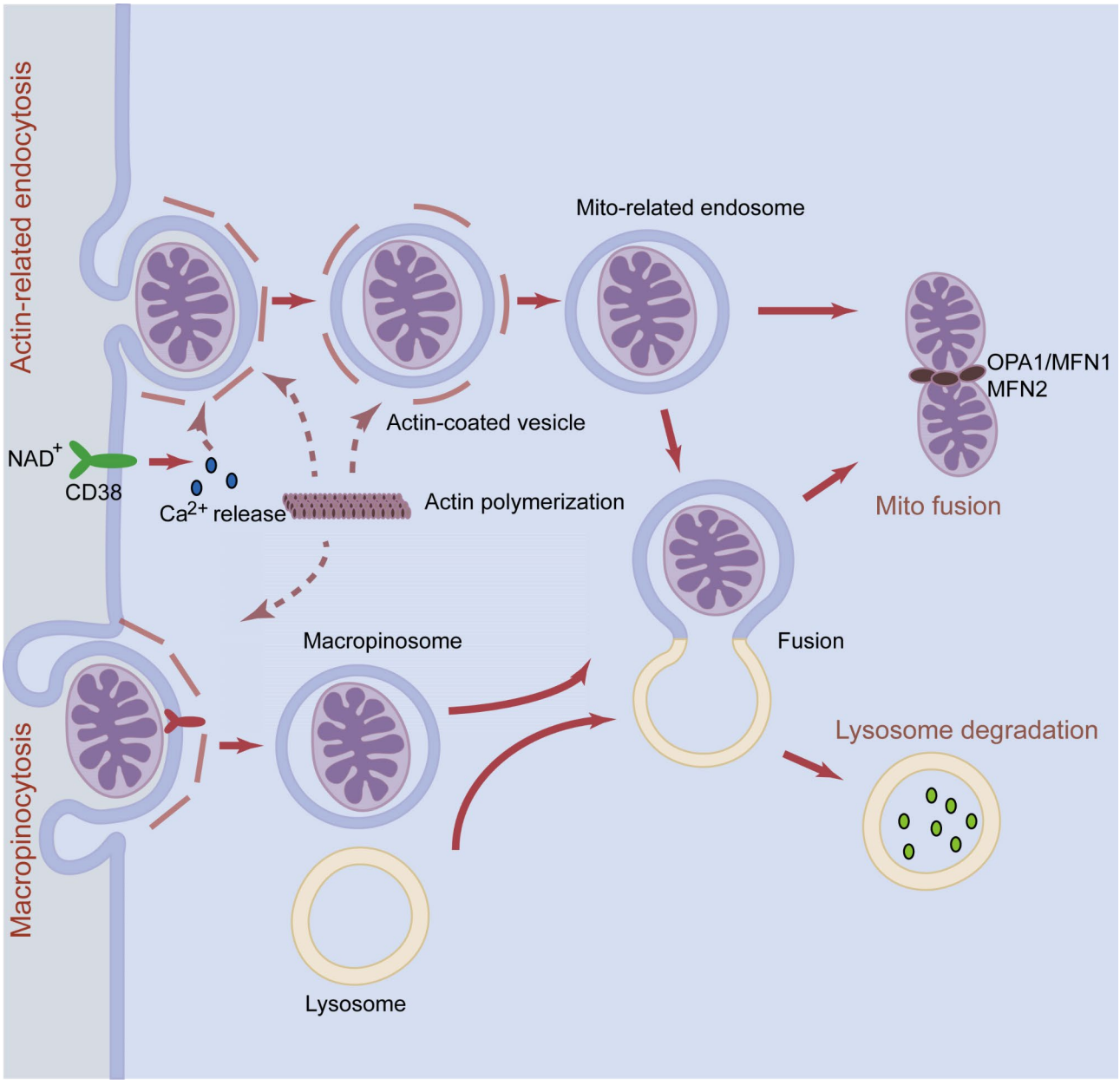

Fig. 6 Potential mechanism by which cells capture and internalize exogenous mitochondria. Exogenous mitochondria are internalized into the cell via actin-related endocytosis or macropinocytosis. Macropinocytosis occurs in a nonselective manner. Following internalization, mitochondria are transported to endosomes and lysosomes. In cardiomyocytes, exogenous mitochondria can escape from these cellular compartments and integrate into the endogenous mitochondrial network, which necessitates the coordinated actions of MFN1, MFN2 and OPA1 to facilitate mitochondrial fusion in cardiomyocytes and fibroblasts. Some mitochondria are degraded by fusion with lysosomes and endosomes. Most exogenous mitochondria can escape from endosomes or lysosomes and fuse with the endogenous mitochondrial network through a process mediated by MFN1, MFN2, and OPA1

recognition that it plays a pivotal role in preserving the functions of various organs.

Recipient cell metabolism maintenance

The first evidence demonstrating the support of recipient cell metabolism revealed that ρ0 cells can take up mitochondria from cocultured cells, thereby enabling oxidative phosphorylation and the restoration of cell division functionality (Fig. 7) [50], which was also evident when ρ0 cells were cultured with purified mitochondria. Furthermore, the introduction of wild-type mitochondria into macrophages lacking NDUFS4, a nuclear protein essential for mitochondrial complex I activity, resulted in the complete restoration of mitochondrial function in peritoneal macrophages [99]. In addition, both wild-type and NDUFS4-deficient cells exhibited similar capacities

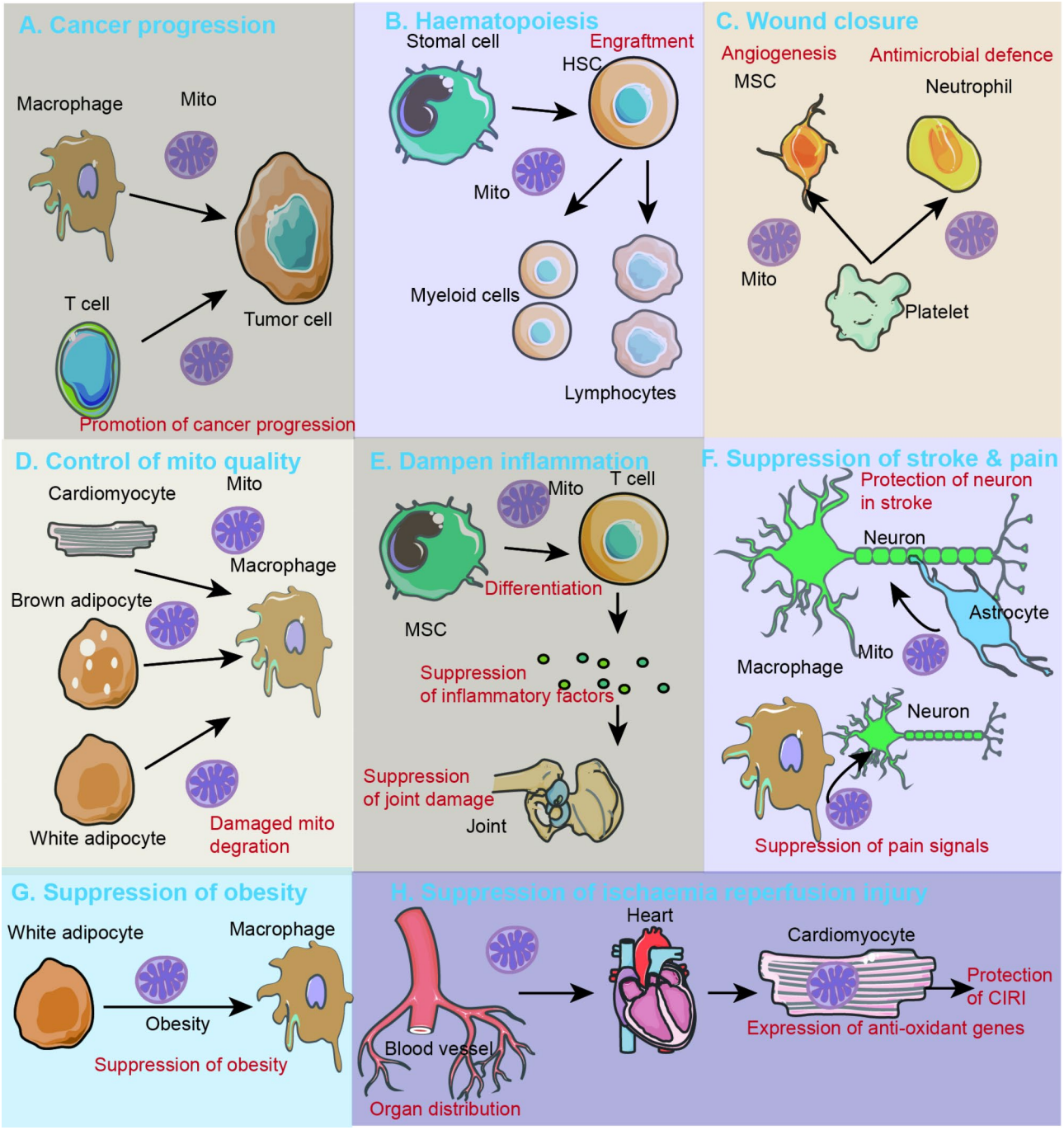

Fig. 7 Mitochondrial transfer functions. **A**, Cancer cells receive mitochondria from immune cells such as T cells and macrophages, inhibiting antitumor immunity, maintaining cancer cell metabolic demands and driving cancer progression. **B**, Mitochondrial transfer promotes hematopoietic stem cell (HSC) engraftment and contributes to hematopoiesis. **C**, During wound closure, activated platelets donate their mitochondria to MSCs to promote angiogenesis and wound closure, as do neutrophils to initiate antimicrobial defense. **D**, Adipocytes and cardiomyocytes transfer damaged mitochondria to macrophages for degradation and maintenance of mitochondrial quality. **E**, Mitochondrial transfer from MSCs to T cells promotes regulatory T-cell differentiation and reduces proinflammatory cytokine production. **F**, During ischemic stroke in the central nervous system (CNS), astrocytes transfer mitochondria to neurons to support their survival, whereas macrophages deliver mitochondria to neurons in the dorsal root ganglia to inhibit pain signal propagation in the peripheral nervous system. **G**, In white adipose tissue, adipocytes transfer mitochondria to macrophages to maintain tissue homeostasis and prevent obesity. **H**, Mitochondria from blood vessels are transported into the heart, where they elicit an antioxidant response that protects the heart from ischemia–reperfusion injury

for acquiring exogenous mitochondria. However, macrophages with NDUFS4 deficiency appear to retain these acquired mitochondria for extended durations, suggesting potential selective retention of healthy mitochondria by defective cells [99].

Exogenous mitochondria do not typically influence metabolism in healthy macrophages, indicating that cells might resort to the use of exogenous mitochondria for energy production, primarily under conditions of energy scarcity, which is supported by findings that BV2 cells, under standard conditions, do not increase aerobic respiration with purified mitochondria. However, they can use these mitochondria to recover respiration in the face of mitochondrial dysfunction [66], suggesting that while cells inherently rely on their endogenous mitochondria for energy, they may incorporate and use exogenous mitochondria during energy shortages. Notably, in ischemic cardiomyocytes and neurons, the uptake of exogenous mitochondria plays a crucial role in sustaining cell viability [100].

In response to ischemic stimulation, astrocytes actively release EVMs and facilitate the transfer of mitochondria to neighboring neurons, which is mediated by CD38 signaling [63]. Disrupting this process with an anti-CD38 monoclonal antibody resulted in exacerbated outcomes in a mouse model of ischemic stroke [101]. Similarly, the administration of purified mitochondria to ischemic animals led to the accumulation of mitochondria in the infarct zone, resulting in the rescue of ATP levels and a reduction in the size of the infarct zone [102]. These findings confirmed the role of free mitochondria in the metabolic processes of ischemic models.

The process of mitochondrial transfer, while pivotal in maintaining cellular energy levels, can also contribute to undesirable outcomes. Notably, ρ0 tumor cells demonstrate an increased rate of cell division upon acquisition of whole mitochondria and mtDNA from adjacent cells, thereby sustaining their capacity for tumorigenesis [103]. The specific mechanisms by which mitochondria facilitate the proliferation of cancer cells remain elusive but may involve the production of ROS, which not only deliver mitochondria to cancer cells but also activate pathways that promote cell growth. The implications of mitochondrial transfer in tumor environments are multifaceted and potentially influence cancer cell proliferation, invasiveness, immune system evasion and resistance to treatment [104, 105]. Moreover, the origins of mitochondria acquired by cancer cells are varied and include mesenchymal stem cells/stromal cells, cancer-associated fibroblasts, T cells, macrophages, and astrocytes, each of which potentially impacts the destiny of the recipient cells [106]. In light of the intricate role of mitochondrial transfer in cancer, gaining a more comprehensive understanding of the various pathways through which mitochondrial transfer influences cancer progression and therapy resistance is crucial for the development of innovative cancer treatments.

Mitochondrial quality control

In addition to its crucial role in maintaining cellular metabolism, mitochondrial transfer also plays a significant role in preserving mitochondrial health within donor cells (Fig. 7). As previously discussed, damaged mitochondrial components expelled by brown adipocytes and cardiomyocytes are subsequently acquired and degraded by neighboring macrophages [58]. This process has implications for cardiac function and thermogenesis in brown adipocytes [107]. In cardiomyocytes, loss-of-function mutations in LAMP2, a lysosomal protein, result in an increased presence of EVMs, highlighting a compensatory mechanism that prevents the accumulation of damaged mitochondria when mitophagy is insufficient [108]. Consistently, mitochondrial oxidative stress can trigger the export of damaged mitochondria from zebrafish retinas to Müller glia for degradation, suggesting that impaired mitochondria are dispatched from irreplaceable cells to be cleared by macrophages or other cell types, thus serving as a mechanism for mitochondrial quality control. This process, observed in both fish and mammals, is referred to as 'licensed mitophagy' [109].

Wound healing

Following physical injury, epithelial cells initiate a complex healing process aimed at preventing microbial invasion and restoring the integrity of the barrier surface. Platelets are mobilized to the site of injury to facilitate healing, a process during which the mitochondria within the platelets migrate from the central part of the cell to the inner surface of the plasma membrane [110]. During platelet degranulation, mitochondria are released into the extracellular environment, and the platelets that release mitochondria play distinct roles in the wound healing process (Fig. 7) [111]. Initially, they can adhere to neutrophils, triggering an anti-inflammatory response. These mitochondria can subsequently be taken up by subendothelial mesenchymal stem cells, which in turn secrete proangiogenic factors, thereby promoting a healing response.

Furthermore, mitochondria from various cell types may also contribute to the wound healing response. Endothelial cell progenitors can transfer mitochondria to mature endothelial cells within the brain, resulting in increased angiogenesis and improved endothelial barrier function [112]. Additionally, endothelial cells can receive mitochondria from mesenchymal stem cells, reinforcing antioxidant defense mechanisms and mitigating cell senescence and myocardial infarction [113]. These findings suggest that mitochondrial transfer to endothelial

cells plays a role in promoting angiogenesis, which is advantageous for the revascularization of injured tissues.

Immune system

Mitochondria bear some resemblance to their bacterial ancestors, characterized by circular genomic DNA with hypomethylated CpG residues and formylated N-terminal methionine peptides in mtDNA-encoded proteins. These molecular features can be recognized by the immune system as damage-associated molecular patterns (DAMPs), which interact with Toll-like receptor 9 (TLR9) and formyl peptide receptors 1–3 (FPR1-3), and can initiate proinflammatory immune responses [114, 115]. Consequently, owing to the presence of these DAMPs, free mitochondria are believed to trigger an immune response. However, mitochondrial transfer to immune cells can exhibit both proinflammatory and anti-inflammatory effects.

Mitochondrial transfer to neutrophils is proinflammatory, assisting in the removal of foreign antigens from wounds [116]. In lung transplantation, allografts reject mitochondria released into the alveolar space and blood [117, 118], and the released mitochondrial components elicit an FPR1-dependent neutrophil response and aggravate engrafted tissue rejection in mice [119]. Moreover, the presence of free mitochondria or mtDNA in bronchoalveolar lavage fluid or blood from lung transplant patients is positively correlated with early rejection, the severity of lung allograft dysfunction, and elevated levels of circulating cytokines, including IL-6, IL-8, IFNγ, and IL-1-RA [120]. Consequently, mitochondrial release following tissue damage appears to have proinflammatory consequences, although the specific mechanisms governing mitochondrial release may be complex in modulating immune system activation.

Mitochondrial transfer from stromal cells to progenitor cells initiates a leukocyte proliferative response that provides protection against acute bacterial infection at distant bone marrow sites [121], suggesting that mitochondrial transfer may play a role in recruiting the host defense response. Conversely, mitochondrial transfer can also serve as an anti-inflammatory process [122]. Initially, believed to reduce inflammation in lung epithelial cells during conditions such as asthma and acute lung injury, mitochondrial transfer was subsequently shown to have an anti-inflammatory effect on the adaptive immune system, particularly on T cells. This transfer is more likely to occur in $CD4^+$ T cells and has been shown to regulate T-cell function through the increased expression of IL-10 in models of graft-versus-host disease and arthritis (Fig. 7) [123]. Further research is needed to elucidate how intercellular mitochondrial transfer precisely regulates immune cell activation, differentiation, and function during the normal immune response, as well as how dysregulation of this process may contribute to inflammation.

Metabolic homeostasis maintenance

Intercellular mitochondrial transfer modulates local and systemic metabolic homeostasis, especially in adipose tissues. Mitochondrial transfer from adipocytes to macrophages in white adipose tissue occurs under steady-state conditions and is partially mediated by heparan sulfate on the macrophage surface (Fig. 7) [68]. Following diet-induced obesity, macrophages exhibit reduced heparan sulfate levels on the macrophage surface despite an increase in phagocytic capacity [124]. Macrophage-specific deletion of exostosin-1 (EXT1), which encodes a glycosyltransferase required for heparan sulfate chain elongation, contributes to decreased mitochondrial transfer and metabolic disorders, including weight gain and an increased probability of developing obesity [125]. These results support the importance of mitochondrial transfer from adipocytes to macrophages in maintaining metabolic homeostasis.

Upon stimulation with palmitic acid, adipocytes release an increased quantity of extracellular vesicles, many of which carry mitochondrial proteins. These vesicles containing mitochondria-related components disperse to various organs, including the heart, where they exert antioxidant effects. This distribution to the heart contributes to the organ's adaptation to protect against metabolic disruptions and ischemia–reperfusion injuries [57]. Additionally, mitochondria derived from other tissues, such as skeletal muscle, also play a protective role against ischemia–reperfusion injuries in the heart [126–128]; however, the precise mechanisms involved remain unclear. Notably, individuals with obesity exhibit higher levels of extracellular vesicles containing mitochondria-related components in their bloodstream than healthy individuals do, suggesting the functionality of this pathway in individuals with obesity [129]. Interestingly, dietary long-chain fatty acids (LCFAs) inhibit the uptake of mitochondria by macrophages in adipose tissues, leading to the transport of mitochondria derived from adipocytes to the heart. However, low dietary LCFAs do not seem to impact mitochondrial transfer from adipocytes to macrophages, as observed in an age-related high-fat diet with medium-chain fatty acids [130], indicating that macrophages can detect changes in dietary metabolites and modulate whether adipocyte-derived mitochondria are locally absorbed or released into the bloodstream for transfer to distant organs.

Mitochondrial transfer in CVDs

Cardiac cell types exhibit varying levels of energy dependence, with some, such as CMs, having high energy demands, whereas others, such as ECs, have lower energy

requirements. Despite these differences, both CMs and ECs possess mitochondria, suggesting that mitochondrial transfer is not limited to high-energy-producing cell types. These findings highlight the importance of mitochondrial transfer in enhancing stress resistance. A previous study demonstrated the spontaneous transfer of mitochondria from CMs to ECs through transient nanotube-like structures [131]. These mitochondria can subsequently be internalized by CMs or other cell types under conditions such as cardiomyopathy and ischemia.

MSCs also play a role in mitochondrial transfer, donating mitochondria that can be captured by both CMs and ECs, thereby providing protection against ischemia-induced cell death. In a hypoxia/reoxygenation coculture model, mitochondria are transferred unidirectionally from human MSCs to rat CMs or from myofibroblasts to damaged CMs, effectively attenuating CM apoptosis [132]. Furthermore, bidirectional mitochondrial transfer has been observed between cardiac fibroblasts and CMs, as well as between MSCs and CMs or ECs, which is particularly relevant in cases of neonatal cardiomyopathy, where mitochondrial dysfunction plays a pivotal role, and mitochondrial transfer has been shown to restore CM bioenergetics and viability in rats with pregestational diabetes [133].

In ischemic cardiomyopathy, damaged cells not only release dysfunctional mitochondria but also capture exogenous mitochondria to restore the mitochondrial network. Notably, the beneficial effect of mitochondrial transfer can be sustained for a long time (at least 28 days) in ischemic CMs, which is quite different from what occurs in healthy CMs (short-term improvement) [134]. Mitochondrial transfer has beneficial effects on preventing oxidative damage in cardiac cells and endothelial cells.

MSC transfer to a doxorubicin-induced cardiomyopathy model can relieve cardiac inflammation through mitochondrial transfer [135]. The protective effect might be due to the overexpression of HO-1, which has anti-inflammatory and antioxidant effects. Under stress conditions (i.e., hypoxia, chemical treatment and metabolic stress), CMs and ECs are susceptible to apoptosis, and mitochondrial transfer can relieve apoptosis in injured cells. The underlying mechanisms might be the decreased ratio of Bax/Bcl-2 and the inhibited activity of caspase-3. Overall, numerous studies have emphasized the importance of mitochondrial transfer in the cardiovascular system.

Under physiological conditions, frequent bidirectional intercellular mitochondrial transfer also frequently occurs between cardiac fibroblasts and cardiomyocytes to maintain normal cardiac function, which was also observed in an in vitro model but was not confirmed in vivo. Defective mitochondrial transfer from CMs to macrophages is important for the fitness of CMs. When they contain donor cell information, mitochondria can induce a cascade of responses when internalized by recipient cells, which ultimately influences the donor cells. For example, upon ischemia/reperfusion injury, CMs and ECs transmit mitochondria to MSCs as signals for help [136]. After being internalized by mitochondria, MSCs promote mitochondrial biogenesis, enhance the antiapoptotic response, and subsequently deliver functional mitochondria to damaged cells.

Therapeutic applications and challenges

Mitochondrial transfer from healthy cells to damaged cells is beneficial for the repair and restoration of normal cells. Thus, targeting mitochondrial transfer is suggested as a potential strategy for CVD treatment. The most commonly used approaches for CVD treatment are cell-mediated therapy and cell-free therapy, including naked mitochondria transplantation and EV-based transplantation.

Cell-based mitochondrial transfer

MSCs and donor cells are considered excellent candidates for mitochondrial donation in CVD treatment because of their abundant mitochondria and high respiratory activity. Transplanting MSCs has proven effective in repairing ischemic cardiomyopathy-induced myocardial damage. The protective effects primarily result from mitochondrial transfer, as demonstrated in both in vitro and in vivo studies. For example, MSCs can protect endothelial cells from ischemia–reperfusion-induced injury by increasing aerobic respiration and reducing apoptosis through mitochondrial delivery [137]. Mitochondrial transfer from MSCs to CMs can mitigate oxidative stress-induced cell damage by restoring mitochondrial respiration activity. In an animal model of anthracycline-induced cardiomyopathy, MSC transplantation alleviated cardiac fibrosis, reduced left ventricular dilation, and restored cardiac function through mitochondrial transfer [138]. MSCs for clinical use can be obtained from various sources. Dental pulp (DP)- and Wharton's jelly (WJ)-derived MSCs exhibit lower mitochondrial transfer activity but higher respiratory capacity than MSCs from bone marrow and adipose tissue [139]. Compared with bone marrow-derived MSCs, human-induced pluripotent stem cell-derived MSCs also exhibit greater mitochondrial transfer efficiency because of increased expression of Miro1 and TNFaIP2. Hence, it is essential to carefully consider the varying capacities and effectiveness of mitochondrial transfer in CVD treatment.

Naked mitochondria transplantation

Another approach to mitochondrial transfer involves the transplantation of isolated and free mitochondria to

the injured site through the circulatory system or local injection. The use of naked mitochondria for treatment was initially conducted in 2009 when McCully's laboratory discovered that intramyocardial injection of naked mitochondria could reduce infarct size and restore post-ischemic function in an animal model of heart ischemia-reperfusion [126]. In this study, New Zealand White rabbits ($n=52$) underwent 30 min of equilibrium and 30 min of regional ischemia (RI) induced by snaring the left anterior descending coronary artery. At 29 min of RI, the RI zone was injected with vehicle (sham control or RI vehicle) or vehicle containing mitochondria (7.7×10^6 +/- 1.5×10^6/ml) isolated from donor rabbit left ventricular tissue (RI-Mito). The snare was released at 30 min of RI, and the hearts were reperfused for 120 min. The proper mitochondrial dose for preventing ischemia–reperfusion injury is 2×10^5 to 2×10^8/g wet weight. The internalization of autologous mitochondria by CMs usually takes a few minutes. Although exogenous mitochondria can escape degradation by lysosomes or autophagosomes, it takes more than 8 hours for CMs to internalize. The first clinical use of mitochondrial transfer occurred in 2017, when five pediatric patients with cardiac ischemia–reperfusion injury received this treatment [140]. In all patients, the mediastinum was accessed, and epicardial echocardiography was performed to identify regions of myocardial akinesis or hypokinesis. A 6×6-mm piece of healthy rectus abdominis muscle was harvested from the inferior aspect of the field via sharp dissection. Autologous mitochondria ($1\times10^8 \pm 1\times10^5$) were isolated under sterile conditions and suspended in 1 mL of respiration buffer. Ten 100-μL injections containing $1\times10^7 \pm 1\times10^4$ mitochondria each were delivered by direct injection with a 1-mL tuberculin syringe (28-gauge needle) to the myocardium affected by ischemia-reperfusion, as identified by epicardial echocardiography [141]. Epicardial echocardiography was performed at the conclusion of the procedure to assess the presence of myocardial hematoma related to the injections. Patients did not experience adverse short-term complications related to mitochondrial injection (arrhythmia, intramyocardial hematoma, or scarring), and all patients demonstrated improvement in ventricular function within several days after treatment. Mitochondrial therapy is most advantageous if it is delivered as soon after ischemic injury as possible, as evidenced by studies in animal models. The patients in this series, however, were selected because they showed no recovery of myocardial function despite 1–2 days of ECMO support, and spontaneous recovery of ventricular function did not seem likely. Future studies investigating the optimal timing of therapy are necessary.

Despite the demonstrated effectiveness and safety of intramyocardial injection of mitochondria for treating myocardial ischemia injury, several challenges hinder its broader application. One limitation is the ability to inject only a small fraction of mitochondria into the myocardium at a time [142]. The percentage of mitochondria successfully internalized at a single site is also restricted, typically ranging from approximately 3–7% [127, 143]. Therefore, multiple injection procedures are necessary to ensure the even distribution of donor mitochondria throughout the ischemic heart. Additionally, the requirement for thoracotomy as a prerequisite for intramyocardial injection of mitochondria could be a barrier to the broader implementation of mitochondrial transfer in relevant diseases [144].

Recently, intracoronary delivery has emerged as a promising alternative for mitochondria transplantation [145]. This approach allows for the widespread distribution of both autologous and exogenous mitochondria throughout the entire heart within a mere 10 min. Furthermore, the efficiency of intracoronary delivery for internalizing mitochondria into cardiac myocytes is notably greater (approximately 23%) than that of intramyocardial injection [145]. In animal models of regional ischemia/reperfusion, intracoronary injection of autologous mitochondria, either before or after ischemic injury, resulted in a reduced infarct size, improved blood flow, and enhanced cardiac function. Importantly, intracoronary mitochondria transplantation has proven to be both safe and highly effective, positioning it as an ideal therapeutic approach for myocardial infarction [146].

Compared with intramyocardial or intracoronary approaches, the intravenous administration of mitochondria offers a more convenient clinical delivery method, and this method has shown promise in the treatment of conditions such as fatty liver disease and *Parkinson's disease* [147, 148]. Notably, intravenous injection of intact mitochondria over three weeks has been demonstrated to improve right ventricular function in an animal model of pulmonary hypertension [149]. After systemic injection, mitochondria can be identified in various tissues, including the heart. The stability of naked mitochondria and their efficacy in tissue-specific delivery are critical factors that determine the success of this approach under both ischemic and nonischemic cardiac conditions.

Furthermore, mitochondrial transfer has the potential to mitigate the progression of heart failure and rejuvenate myocardial metabolism in the offspring of diabetic mothers [149, 150]. While the effectiveness of naked mitochondria transplantation has been established, obtaining high-quality mitochondria remains a challenging endeavor. The efficiency of mitochondrial uptake by specific cells and the preservation of mitochondrial viability are two pivotal considerations for the clinical implementation of naked mitochondria transplantation.

EV-based mitochondrial transfer

Another avenue of cell-free mitochondrial transfer involves mitochondria-rich EVs, which are recognized as potent carriers for mitochondrial transfer [151, 152]. MSCs derived from various tissues serve as the primary source of these EVs. While MSC-derived EVs have shown potential as treatments for CVD, it remains unclear whether their protective effects are attributed to their cargo function. EVs derived from cardiomyocytes generated from human induced pluripotent stem cells have diameters ranging from 98 to 677 nm. Compared with free mitochondria, the mitochondria enclosed within EVs are more stable, as the outer lipid bilayer of EVs serves as a protective barrier, preserving mitochondrial morphology and function [151].

Transplantation of EVs has been shown to restore cellular bioenergetics, inhibit postischemic ventricular remodeling, and enhance myocardial contractility. These EVs typically have diameters of less than 10 μm, enabling safe intravenous or intracoronary injection without causing microvascular obstruction. Unlike MSCs, intramyocardial transplantation of mitochondria-containing EVs in the peri-infarct region does not induce cardiac arrhythmias, making this approach a safer alternative. The composition of EVs, including their mitochondria, nucleic acids, lipids, and proteins, varies depending on their source and isolation method. These complex compositions exert different effects on various diseases through distinct mechanisms. A study by Ikeda and colleagues revealed that the beneficial effects of EVs are attributed not only to their mitochondrial cargo but also to their nonmitochondrial components [153]. To address the heterogeneity of EV therapy, a standardized method for EV isolation is imperative. Additionally, improving the targeting specificity of EVs is a critical challenge that warrants further investigation.

Therapeutic practices involving mitochondrial transfer for CVD treatment

Therapies aimed at mitigating mitochondrial dysfunction have shown promising results in slowing CVD progression, underscoring the importance of robust mitochondrial transfer as a critical approach for cardioprotection, which has been supported by numerous studies across various cardiovascular conditions. The therapeutic potential of mitochondrial transfer in the cardiovascular system during ischemia/reperfusion injury was first applied. Function-competent mitochondria were isolated from healthy tissues and directly administrated into the ischemic region of rabbit hearts during early reperfusion, which significantly ameliorated myocardial damage and cell viability.

In addition, the autologous local mitochondria transfer protected from ischemia-reperfusion damage in cardiomyocytes and prolonged cold ischemia prior to heart transplantation. In pediatric patients with congenital heart disease, mitochondria transfer in the coronary artery reduced the infarct size and improved heart function. In response to energetic stress, respiration-competent mitochondria released by adipocytes are internalized by cardiomyocytes, where they induced transient mitochondrial oxidative stress leading to pre-conditioning, and therefore protecting against ischemia/reperfusion injury [134]. Moreover, mitochondrial transfer is also proved beneficial in the liver and lung of animal models of ischemia/reperfusion, indicating that mitochondrial transfer by isolated mitochondria or mitochondria-containing ECVs has the potential to provide clinical benefit.

Myocardial I/R injury

Mitochondrial transfer for the treatment of CVDs was implemented by the McCully group, who transplanted mitochondria derived from the left ventricle of donor rabbit sham controls or from regional ischemia into the ischemic site of the rabbit heart before reperfusion [126]. They reported that mitochondria from healthy controls dramatically promoted cardiac recovery and cell viability after ischemia [127]. Allotransplantation was also successfully performed by transferring human mitochondria to a rabbit cardiac I/R model. The therapeutic efficacy of mitochondrial transfer through the coronary vasculature and direct mitochondrial injection in human fibroblasts was compared.

Transplantation through the coronary vasculature increased the concentration and distribution of mitochondria. Mitochondrial delivery through coronary arteries before and during reperfusion resulted in temporarily restored coronary blood flow and a reduced myocardial infarct size. Intracoronary mitochondria delivery is thought to be a safe and effective method for preventing myocardial I/R injury. The source of mitochondria can be derived from pectoralis major muscle cells as well as gastrocnemius myocytes. Transplantation of mitochondria derived from gastrocnemius muscles into the coronary artery before donor heart harvest revealed prolonged cold ischemia time, improved graft function and alleviated graft tissue injury [154].

Cardiomyopathy

Diabetes-related cardiomyopathy is the most common cardiovascular disorder [155]. Human adipose MSCs transfer mitochondria into human islet β cells under symbiotic conditions, leading to enhanced bioenergetics and insulin secretion in damaged β cells, suggesting that mitochondrial transfer is a promising approach for the treatment of diabetes. Mitochondrial transfer also improved diabetic myocardial function, suggesting the therapeutic value of mitochondrial transfer for

cardiac damage in diabetic patients [156]. As shown by another study, mitochondrial transfer was more effective at preventing sepsis-induced cardiomyopathy than was simply relieving mitochondrial function. Decreases in mitochondrial function, biogenesis and kinetics are associated with the SIRT-1/PGC-1α network [157]. Intramyocardial injection of mitochondria into anthracycline-induced cardiomyopathy patients significantly increased mitochondrial respiration and cardiomyocyte viability. These results indicated that mitochondrial transfer could improve anthracycline-induced cardiomyopathy.

Mitochondrial cardiomyopathy is characterized by structural or morphological abnormalities in the myocardium induced by defects in nuclear DNA or mtDNA genes, leading to hypertrophic cardiomyopathy, dilated cardiomyopathy, and cardiac conduction defects [158]. Mitochondrial transfer is a potential treatment for improving the prognosis of these diseases. As proposed by *Park* et al., mitochondrial transfer is a promising strategy for the treatment of various mitochondrial diseases, but its therapeutic efficacy requires verification in future studies [159].

Myocardial infarction and heart failure

Transplantation of mitochondria derived from human pluripotent adipocytes into CMs and ECs adjacent to the infarct zone increased the level of HO-1 and mitochondrial biogenesis [160]. The transfer of MSC-derived mitochondria provides a new approach for the treatment of myocardial infarction. A key pathological feature of heart failure is impaired myocardial mitochondrial function. Upon end-stage heart failure, the activity of citrate synthase complex I in the respiratory chain is inhibited by 28%; thus, mitochondrial transport has adequate potential for the treatment of heart failure. Autologous mitochondrial transfer from rat skeletal muscle cells to CMs rapidly restores cellular respiration and energy support. Transplantation of mitochondria from calf muscle cells to the right ventricular free wall in a porcine model of ventricular hypertrophy resulted in adaptation in the right ventricle under pressure overload and maintenance of contractile ability. M2-like macrophage transfer ameliorated cardiac fibrosis and apoptosis in doxorubicin-induced heart failure, potentially owing to mitochondrial transfer [161]. Moreover, mitochondrial transfer from macrophages to CMs repaired cardiac defects.

Pulmonary hypertension

Pulmonary hypertension, a lethal progressive vascular disease, is characterized by increased mean artery pulmonary artery pressure and right ventricular afterload, resulting in right ventricular hypertrophy and failure [162]. Recently, two studies revealed that mitochondrial transfer did not affect the survival rate of animals [149, 163]. Mitochondrial transfer in a pulmonary hypertension experimental model restricted the proliferation of pulmonary artery smooth muscle cells, pulmonary vasoconstriction, weakened pulmonary vascular remodeling, and restored right ventricular function [163]. Mitochondria transferred from rat femoral artery smooth muscle cells were intravenously delivered into rat pulmonary artery smooth muscle cells, resulting in suppressed pulmonary vasoconstriction and attenuated pulmonary vascular remodeling in a chronic hypoxia model. After mitochondrial injection into the immature rat soleus muscle, pulmonary hypertensive rats presented improved right ventricular mass and wall thickness and reduced serum B-type natriuretic peptide levels and ventricular diameter, indicating the ameliorative effects of mitochondrial transfer on respiratory activity, pulmonary artery remodeling, and right ventricle function [149, 163].

Outlook

This review delves into the critical functions of mitochondrial transfer in energy metabolism and signal transduction within the cardiovascular system, highlighting how disorders of mitochondrial transfer contribute to various cardiovascular diseases, including myocardial infarction, cardiomyopathies, and hypertension. We further examined the molecular mechanisms of mitochondrial transfer in CVD. This review emphasizes the potential of mitochondrial transfer as a therapeutic approach and discusses various mechanisms, therapeutic strategies, and potential challenges in implementing this novel treatment for CVD. We offer a comprehensive look into how advancing our understanding of mitochondrial transfer and its targeting could revolutionize the treatment of cardiovascular ailments.

Mitochondrial transfer helps treat myocardial infarction and heart failure by improving energy production and reducing oxidative stress. Transferred mitochondria restore ATP levels and balance ROS, protecting cells and promoting repair. These mechanisms support cell survival and reduce damage, making mitochondrial transfer an effective therapeutic approach. Further understanding these processes can aid in optimizing treatments.

Currently, pharmacological treatments, such as beta-blockers, calcium channel inhibitors, nitrates, acetylsalicylic acid, and clopidogrel, which effectively slow the progression of CVDs, are widely used. Antiplatelet agents, such as acetylsalicylic acid or clopidogrel, are capable of lowering the risk of major vascular events [164]. However, these drugs are insufficient to reverse cardiomyocyte loss. Owing to the limited regenerative capacity of the adult human heart, stem cell therapies have shed light on the repair or replacement of damaged hearts. The beneficial effects of incorporating MSCs and iPSC-derived cardiomyocytes, as well as stem

cell-derived extracellular vesicles, in CVD treatment have been demonstrated in multiple studies. Although some of these studies have been validated in clinical studies, the modest efficacy of these therapies has been presented, limiting the wide usage of these treatments. The possible reasons might be the different bioactivities of the administered MSCs and the delivery methods used. Compared with pharmacological treatments and conventional stem cell therapy, the potential of mitochondrial transfer as a treatment for cardiovascular diseases is notable, particularly because of its role in repairing cardiac cell damage, optimizing energy metabolism, and reducing inflammatory responses. This innovative approach involves transferring healthy mitochondria to damaged cardiac cells, aiming to restore cellular function and enhance the overall efficiency of the heart [158]. Furthermore, mitochondrial transfer has the potential to regulate immune responses within the heart, reduce inflammation and potentially aid in myocardial regeneration. Despite its promise, this technique is still in the research phase, and challenges such as enhancing transfer efficiency, achieving precise targeting, understanding long-term effects, and mitigating potential side effects are areas of active investigation.

In the context of targeting, the process of mitochondrial transfer requires the precise identification of damaged cells and the development of effective delivery systems, such as liposomes or nanoparticles, for mitochondrial transport. For example, when the lipophilic stearoyl tail was used in addition to the cationic peptide-based dendritic head, arginine-rich second-generation dendritic lipopeptide-based liposomes resulted in the best mitochondrial targeting efficiency, with almost 6-fold greater mitochondrial targeting efficiency than classic triphenylphosphonium (TPP)-decorated liposomes. NPs, such as iron oxide nanoparticles (IONPs), selectively increase intercellular mitochondrial transfer from human mesenchymal stem cells (hMSCs) to injured cells by enhancing the formation of connexin 43-containing gap junctional channels triggered by ionized IONPs. IONP-engineered hMSC therapy effectively mitigates fibrotic progression by increasing intercellular mitochondrial transfer, but no serious safety issues have been identified in a mouse model of pulmonary fibrosis [165].

Ensuring the safe passage of mitochondria across cellular membranes and their subsequent functional integration into target cells is crucial. Additionally, maintaining mitochondrial activity during transfer and minimizing immune reactions are critical to successful treatment [72]. Clinical trials play a vital role in refining these approaches, and advancements in related fields such as cell biology, material science, drug delivery, and immunology are likely to significantly increase the efficacy of this treatment.

One of the major challenges associated with mitochondrial transfer is the potential for immune responses, as the recipient's immune system may recognize transferred mitochondria as foreign entities, leading to immune rejection [166]. Additionally, the long-term viability of transferred mitochondria remains uncertain, as mitochondria may degrade or fail to integrate properly into the host cell's mitochondrial network. This could compromise the therapeutic effect over time. Technical challenges also exist, particularly in delivering mitochondria to target cells with precision and efficiency. Improper mitochondrial integration can disrupt cellular functions or trigger inflammatory responses, further complicating therapeutic success. These challenges underscore the need for continued research to optimize mitochondrial transfer techniques and minimize potential side effects, ensuring both safety and efficacy in clinical applications.

Potential side effects of mitochondrial transfer include immune responses due to the recognition of transferred mitochondria as foreign entities, disruptions in cellular function from improper mitochondrial integration, localized or systemic inflammatory responses, and tissue damage during the transfer process [156, 157]. The unknown long-term effects and current technical limitations of this therapy also present challenges. It is essential to carefully consider these risks in treatment planning, ensuring that the benefits of mitochondrial transfer outweigh its potential drawbacks.

Collectively, challenges include inconsistent integration and retention of transplanted mitochondria, potential immune responses, and risks of improper integration disrupting cell function. Additionally, technical issues with preparing and delivering viable mitochondria need to be addressed. Further research is essential to improve targeted delivery and ensure sustained mitochondrial function in clinical use.

The ethical implications of mitochondrial transfer must be carefully considered, particularly with respect to patient safety and the potential long-term genetic effects on future generations. As mitochondria carry their own DNA, any unintended alterations could have lasting impacts. Additionally, clinical applications should ensure rigorous oversight to prevent unforeseen consequences, ensuring that mitochondrial therapies are both safe and ethically sound. Ethical frameworks will be necessary to guide the clinical translation of this novel therapeutic approach.

Future research should focus on improving mitochondrial delivery methods, such as nanoparticle carriers or engineered extracellular vesicles, to increase targeting and internalization. Minimizing immune responses is also crucial and may be achieved through immune modulation or the use of autologous mitochondria to reduce rejection risk. Enhancing mitochondrial survival

post-transplantation will require optimizing storage conditions and promoting integration into host cells. Also, personalized mitochondrial therapies based on individual patient profiles should be explored. Tailoring treatments to a patient's genetic background, mitochondrial function, and disease state could improve outcomes and reduce side effects, advancing precision medicine.

Abbreviations

ATP	Adenosine triphosphate
CVD	Cardiovascular Disease
MI	Myocardial infection
HF	Heart failure
MSC	Mesenchymal Stem Cell
iPSC	Induced pluripotent stem cell
EV	Extracellular vesicle
TNT	Tunnelling Nanotube
HMT	Horizontal mitochondrial transfer
MFN2	Mitofusin 2
MPT	Mitochondrial permeability transition
PPARγ	Peroxisome proliferator-activated receptor gamma
ROS	Reactive oxygen species
mtDNA	Mitochondrial DNA
TNF-α	Tumor necrosis factor alpha
RYR2	Ryanodine receptor 2
SERCA	Sarcoplasmic/endoplasmic reticulum calcium ATPase
MCU	Mitochondrial Calcium Uniporter
SLC8B1	Solute carrier family 8 member B1
HO-1	Heme oxygenase-1
CD38	Cluster of differentiation 38
MAM	Mitochondria-associated membrane
MFN1	Mitofusin 1
OPA1	Optic Atrophy 1
DNM1L	Dynamin-1-Like Protein
FIS1	Mitochondrial fission 1 protein
MFF	Mitochondrial fission factor
TLR9	Toll-like receptor 9
DAMP	Damage-associated molecular pattern
CGAS	Cyclic GMP-AMP Synthase

Acknowledgements
We thank Home for Researchers (www.home-for-researchers.com) for their language modification service.

Author contributions
Baile Zuo, Xiaoyan Li, Yang Yang, Yi Luan, and Bi Zhang conceptualized and wrote the manuscript and created the figures. Dawei Xu, Liping Zhao, Yang Yang, Yi Luan, and Bi Zhang contributed to the writing of the manuscript. Baile Zuo, Xiaoyan Li, Dawei Xu, Liping Zhao, Yang Yang, Yi Luan, and Bi Zhang reviewed and modified the manuscript. All authors approved the final version of the manuscript.

Funding
This work was supported by the Key Scientific Research Projects of Higher Education Institutions in Henan Province (22A180027) and the Youth Research Project of Shanxi Natural Science Foundation (202303021212349).

Data availability
All the data generated or analyzed in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors have approved this manuscript for publication.

Competing interests
The authors declare that they have no competing interests.

Received: 29 September 2024 / Accepted: 13 December 2024
Published online: 31 December 2024

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