



# A new paradigm in intracellular immunology: Mitochondria emerging as leading immune organelles

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## ARTICLE INFO

### Keywords:

mitochondria  
Immune responses  
Intracellular immunity  
Inflammation  
Organelle crosstalk  
Immunometabolism

## ABSTRACT

Mitochondria, traditionally recognized as cellular ‘powerhouses’ due to their pivotal role in energy production, have emerged as multifunctional organelles at the intersection of bioenergetics, metabolic signaling, and immunity. However, the understanding of their exact contributions to immunity and inflammation is still developing. This review first introduces the innovative concept of intracellular immunity, emphasizing how mitochondria serve as critical immune signaling hubs. They are instrumental in recognizing and responding to pathogen and danger signals, and in modulating immune responses. We also propose mitochondria as the leading immune organelles, drawing parallels with the broader immune system in their functions of antigen presentation, immune regulation, and immune response. Our comprehensive review explores mitochondrial immune signaling pathways, their therapeutic potential in managing inflammation and chronic diseases, and discusses cutting-edge methodologies for mitochondrial research. Targeting a broad readership of both experts in mitochondrial functions and newcomers to the field, this review sets forth new directions that could transform our understanding of intracellular immunity and the integrated immune functions of intracellular organelles.

## 1. Introduction

Inflammation is a natural and protective immune response that is triggered by the detection of potential threats, such as pathogen-associated molecular patterns (PAMPs) and endogenous metabolites-derived conditional danger-associated molecular patterns (DAMPs) [1–4]. Recent research shows that stressed organelles, particularly mitochondria, significantly contribute to inflammation and immune responses. Mitochondria are not just energy suppliers; they play critical roles in maintaining ion homeostasis, facilitating protein transport, signaling immune and inflammatory responses, adapting respiratory processes, and orchestrating cell death [5,6] in response to various stimuli. However, the comprehensive understanding of mitochondria’s involvement in immune regulation and inflammation is still in its early stages, with many aspects remaining non-integrated and inconclusive.

Given that mitochondria are intracellular organelles, a key question arises: how do they contribute to the activation of the immune system

within cells, defining a distinct form of intracellular immunity? During the activation of immune system, immune cells undergo a significant metabolic shift from relative bioenergetic quiescence to a highly active state [7]. This shift underscores the critical role mitochondria play in supporting immune cell proliferation through ATP production and their broader function as a nexus for metabolic and immune signaling than that of the quiescence state [8,9]. Mitochondria are integral to intracellular immune processes in several ways. They sense intracellular and extracellular danger signals, akin to the detection of DAMPs and PAMPs in the immune system. Additionally, mitochondria modulate the release of immune metabolites and cytokines, underscoring their roles in immune response regulation. They also influence cell survival and death, paralleling the cytotoxicity and immunogenic cell death observed in immune reactions. Given these diverse functions, mitochondria can be considered immune organelles due to their involvement in both metabolic and immune-related activities.

In this review, our goal is to elucidate the multifaceted roles of

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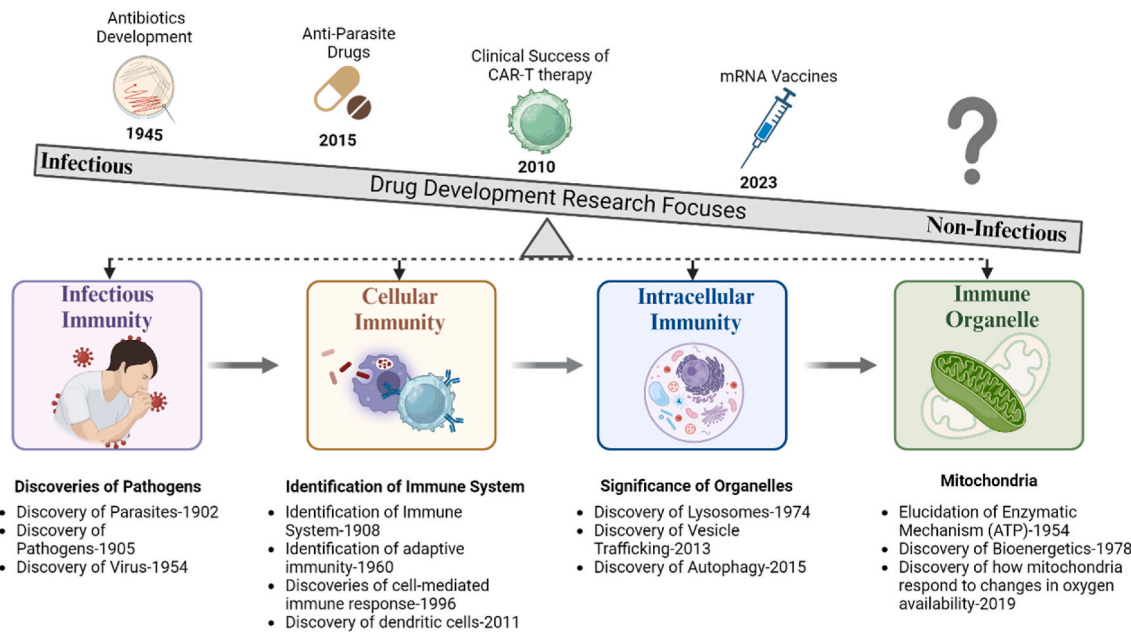
<https://doi.org/10.1016/j.redox.2024.103331>

Received 19 July 2024; Received in revised form 23 August 2024; Accepted 27 August 2024

Available online 29 August 2024

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## Milestones in Immunotherapy: Cellular to Intracellular Advances



**Fig. 1. Milestones in Immunotherapy: From Cellular to Intracellular Advances.** This figure highlights the major focal points of immunology over the past century, showcasing key milestones in the field, particularly those recognized by the Nobel Prize. It begins with the discovery of pathogens and the identification of the innate immune system, which led early treatments such as antibiotics. As our understanding expanded, the focus shifted to cellular immunity, marked by the development of vaccines and CAR-T therapy. In more recent years, attention has increasingly turned to intracellular mechanisms, revealing the critical roles of organelles like lysosomes and mitochondria in immune regulation. This progression underscores the shift from combating external pathogens to exploring and leveraging intracellular processes for advanced therapeutic strategies.

mitochondria from seven major perspectives: 1) introducing the new concept and significance of intracellular immunity by tracing the history of immunology and mitochondrial research over the past century; 2) discussing the specific roles that different mitochondrial structures play in inflammation and immune responses; 3) explaining why mitochondria function as signaling hubs by categorizing the signals they release as risk factors for immune activation and the danger signals they receive across various structures; 4) exploring the connections between mitochondria and cancer development; 5) examining the pleiotropic functions of mitochondria in age-related inflammation; 6) investigating the role of mitochondrial dysfunction in COVID-19 infection, long COVID, and COVID-induced energy disruptions; and 7) summarizing the experimental approaches used in mitochondrial research. Through this comprehensive review, we aim to bridge gaps in our understanding of intracellular organelle immunological functions and present new research directions that could reshape our perspective on intracellular immunity.

### 2. Mitochondria in intracellular immunology: exploring immune responses within cells

Historically, the study of the immune system has primarily focused on understanding how it combats infectious diseases, particularly in recognizing and responding to extracellular pathogens like viruses, bacteria, fungi, and parasites. This focus began almost simultaneously with the discovery of pathogens, leading to the identification of the innate immune system, which is crucial for recognizing these external threats [10]. During this period, research predominantly emphasized

the interactions between the microbiota and immune cells, with the development of anti-pathogenic treatments being a major focus. For example, the discovery and creation of the first antibiotics in 1945 marked a significant milestone [11]. As scientific techniques advanced, our understanding of immunity deepened. Researchers began to delve deeper into cellular immunity, exploring how the adaptive immune system processes antigens to generate specific T cells and antibodies [12]. This marked the beginning of the “cellular immunity era,” characterized by the development of inactivated or live-attenuated vaccines to prevent natural infections, and the advent of chimeric antigen receptors of T cell (CAR-T) therapy for treating relapsed and/or refractory B cell lymphomas, B cell acute lymphoblastic leukemia and multiple myeloma [13].

In the past 50 years, the focus has expanded from cellular to intracellular mechanisms (Fig. 1) [14]. This shift has paved the way for more advanced immunotherapies, such as mRNA vaccines, which have proven to be powerful tools not only against natural infections but also against certain genetic diseases. Consequently, intracellular immunoregulation has emerged as a major research area. However, the question remains: What is the next frontier in intracellular research? Reflecting on the history of immunology, it's evident that the immune system is a dynamic and adaptive entity. Recent research has identified a complex array of intracellular immune sensors [15], including DAMPs/PAMPs sensors (e.g., Toll-like receptors, NLRs/inflammasomes [16]), nucleic acid sensors (e.g., cyclic GMP-AMP synthase (cGAS), stimulator of interferon response CGAMP interactor 1 (STING), RNA Sensor RIG-I (RIG-I)), and others that detect viral components (e.g. tethering for budding virus) or antibodies (e.g. MX1, TRIM5a, and TRIM21) [17].

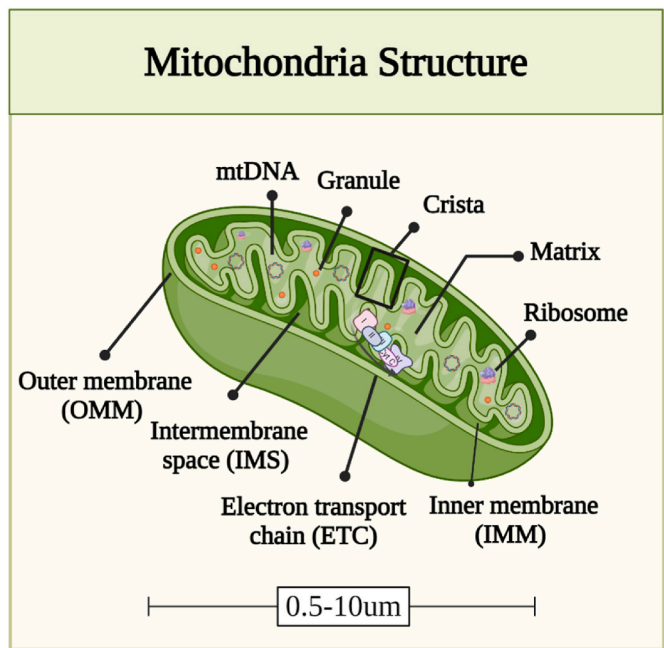


Fig. 2. The general structure of mitochondria.

Despite these advancements, the integration of these findings into the broader immunology framework has been limited.

One organelle that has garnered increasing attention in this context is the mitochondrion. Over the past century, mitochondria-related research has been recognized with five Nobel Prizes, underscoring their significant roles in cellular function and bioenergetics. Mitochondria are now recognized as key players in signaling regulation and are increasingly seen as integral to the immune system. Given their multifunctional nature, mitochondria could be considered as an “immune organelle.” To address the complexities of intracellular immunology, we propose a new concept: “intracellular immunology.” This emerging branch of immunology focuses on understanding how cellular and organelle-level mechanisms respond to intracellular threats, paving the way for the next generation of immunotherapies.

3. Mitochondrial structures and their functions in inflammation and immune responses

Understanding the intricate relationship between mitochondria, inflammation, and their contributions to intracellular immunity necessitates a foundational knowledge of mitochondrial structure and function. Mitochondria comprise six compartments: the outer mitochondrial membrane (OMM), intermembrane space (IMS), inner mitochondrial membrane (IMM), inner boundary membrane (IBM), mitochondrial cristae [18], and matrix (Fig. 2). OMM and IMM are constituted with a mosaic of proteins and phospholipids, which separate mitochondria from the rest of cytoplasm. In the middle of the OMM and IMM is intermembrane space (20 nm gap). The inner membrane forms the cristae, where OXPHOS complexes are localized) [18], by invaginating and curving in the formats of lamellar or tubular protrusions inward towards the mitochondrial matrix [19,20] (Table 1).

The OMM is smooth and porous, allowing ions such as sodium (Na+), potassium (K+), and chloride (Cl-) and small, uncharged molecules smaller than ~5000 Da, including nicotinamide adenine dinucleotide (NAD) and its reduced form NADH, nucleotides, adenosine triphosphate (ATP) and adenosine diphosphate (ADP), and intermediates of the Krebs cycle like glutamate, pyruvate, malate, and succinate, to pass freely through pore-forming membrane proteins called porins. The first mitochondrial porin was discovered in

Table 1  
Mitochondria Structure and Function and their relations with the inflammation and immunity. Five components are included in mitochondria: outer membrane, inner membrane (inner boundary membrane (IBM), IMM-mitochondrial cristae [18]), intermembrane space, and matrix. Key functions include energy production, reactive oxygen species generation, apoptosis regulation, and involvement in cellular signaling.

Mitochondria structure	Function	Relationship with Inflammation
Outer Mitochondrial membrane (OMM)	<b>Barrier protection:</b> Maintaining mitochondria integrity; Separate the contents of mitochondria from the cytoplasm <b>Selective Permeability:</b> Passive diffusion of small molecules (<5000 Da); Maintaining the exchange of molecules between mitochondria and cytoplasm <b>Import of Proteins and Enzymes:</b> Facilitating the import or translocation of proteins to mitochondria; Contains enzymes for metabolic pathways <b>Organelle Crosstalk:</b> A site for communication or exchange of metabolites, signals, and lipids between mitochondria and other cellular organelles <b>Membrane dynamic:</b> Fission and fusion; Maintaining the morphology of mitochondria and adapting mitochondrial function to cellular demands	<ul style="list-style-type: none"><li>• The dysfunction of the OMM could lead to an increase in MOMP, subsequently resulting in the release of mtDAMPs into the cytoplasm, which causes inflammation or promotes apoptosis.</li><li>• Mitochondrial GSDM pores could release mtDNA and activate the cGAS-STING pathway.</li><li>• Dysfunction of fission and fusion proteins increases mtROS generation.</li></ul>
Intermembrane space	<b>Metabolite and Signaling Exchange:</b> Facilitating the exchange of metabolites between the cytoplasm and mitochondria and participating in metabolic pathways or apoptosis regulation.	<ul style="list-style-type: none"><li>• IMS-stored proteins: Cytochrome C for apoptosis, Adenylate kinase and creatine kinase for energy metabolism.</li></ul>
Inner Mitochondrial membrane (IMM): Inner boundary membrane	<b>Barrier and Membrane Potential:</b> Providing impermeability to most ions and molecules; Generating and maintaining a proton gradient for ATP synthesis <b>Homeostasis of Redox Balance:</b> Preventing the leakage of protons or electron from the ETC and minimizing the diffusion of metabolites and the production of ROS. <b>Compartmentalization:</b> Dividing the inner mitochondrial space into distinct regions, facilitating the separation of specific metabolic processes.	<ul style="list-style-type: none"><li>• Disruption of the electrical and chemical transmembrane potential of the IMM.</li><li>• Harmful alteration in the function of the ETC.</li><li>• Lack of efficiency in metabolic process.</li><li>• Impaired functions in mitochondrial dynamics.</li></ul>
Inner Mitochondrial membrane (IMM): Cristae	<b>Surface Area Extension:</b> The cristae increase the area of the IMM, providing a larger area for the efficient functioning of the ETC and ATP synthase. <b>Bioenergetics:</b> ATP production and mitochondrial respiration <b>Dynamic Adaptation:</b> Undergoing remodeling, fusion, and fission processes in response to cellular energy demand or apoptosis <b>The Platform for Enzyme Organization:</b> Spatially arranging enzymes to optimize	

(continued on next page)

Table 1 (continued)

Mitochondria structure	Function	Relationship with Inflammation
Matrix	<p>the efficiency of metabolic process by bringing enzymes to close proximity.</p> <p><b>A Platform for Metabolic Process:</b> including the TCA cycle, fatty acid oxidation, pyruvate decarboxylation, and amino acid metabolism.</p> <p><b>DNA/RNA Replication:</b> Maintenance and expression of mitochondrial genes.</p> <p><b>Calcium Storage/Influx:</b> Regulation of calcium signaling within the mitochondria</p>	<ul style="list-style-type: none"><li>• mtDNA mutation leads to mitochondrial genetic diseases.</li><li>• mtDNA abnormalities related to metabolic disorders.</li></ul>

Paramecium tetraurelia, a model unicellular organism, by Schein et al., in 1976 [21]. This protein, named the voltage-dependent anion selective channel (VDAC), demonstrated its highest conductivity when the transmembrane potential was close to zero and exhibited a slight preference for anions over cations. Dysfunction in the VDAC family can lead to disruptions in energy production, calcium transportation, apoptosis, and mitochondrial oxidative stress [22]. The increased permeability of the OMM leads to the leakage of mitochondrial danger-associated molecular patterns (mtDAMPs) such as mtDNA, mitochondrial N-formyl peptides, ATP (1–10 mM) [23], mitochondrial transcription factor A (TFAM) [24], cytochrome c<sup>23</sup>, and other molecules into the cytosol. Due to permeability of OMM, mitochondrial disorders can result in the reception and release of danger signals, triggering inflammatory responses [25].

The OMM also plays a critical role in regulating mitochondrial dynamics, including fission, fusion, and mitophagy [26]. Fusion proteins like mitofusins (Mfn1 and Mfn2) located in the OMM modulate mitochondrial fusion and endoplasmic reticulum (ER)-mitochondria interactions [27]. In mouse macrophages, MFN2 has been shown to regulate NLR family pyrin domain containing 3 (NLRP3) inflammasome activation [28] and maintain mitochondrial respiratory function. Dysfunction of MFN2 can contribute to metabolic disorders, neurodegenerative diseases, and cardiovascular diseases (CVDs) [29]. An increase in fission proteins Dynamin 1 Like (Drp 1) and Fission, Mitochondrial 1 (Fis1) may lead to mitochondrial fragmentation, which is associated with heightened oxidative stress and inflammatory responses [30].

The IMM can be divided into two parts: the inner boundary membrane (IBM), which runs parallel to the OMM and is freely permeable to water, carbon dioxide, and oxygen; and the cristae, the primary site for oxidative phosphorylation (OXPHOS) [31]. Unlike the porous OMM, the IMM acts as a tight diffusion barrier for most molecules and ions. The ion selectivity of each transport protein results in the buildup of an electrochemical membrane potential of approximately 180 mV, mediated by the electron transport chain (ETC) complexes via pumping protons (H<sup>+</sup>) [32] from the matrix to the intermembrane space (IMS) [20]. This electrochemical gradient across the inner membrane facilitates ATP synthesis in the ETC complex V, heat generation (thermogenesis) [33] and mtROS generation [34–37]. Dysregulation of this gradient can impair mitochondrial functions such as energy production and the induction of apoptosis [38–40].

The cristae increase the surface area of the IMM and contain the fully assembled complexes of the ETC: Complex I (NADH/ubiquinone oxidoreductase) [41], Complex II (succinate dehydrogenase), Complex III (cytochrome c reductase), Complex IV (cytochrome c oxidase), and Complex V (mitochondrial ATP synthase) [32]. These complexes work together in OXPHOS and mitochondrial respiration [20]. The IMS houses various membrane protein complexes, and specific membrane transport proteins are required for species to cross the IMM. For

instance, cytochrome c, a key protein in the apoptosis process [40,42], interacts with apoptotic protease-activating factor 1 (APAF1) to form the apoptosome, which activates caspase 9 [25,43]. Additionally, adenylate kinase 4 has been reported to promote inflammatory gene expression through hypoxia-inducible factor 1 $\alpha$  (Hif1 $\alpha$ ) and AMP-activated protein kinase (AMPK) in macrophages [44].

As the structure progresses, the innermost space of mitochondria is called the matrix. The mitochondrial matrix is the site for biosynthetic reactions, including mitochondrial DNA replication, transcription, TCA cycle, fatty acid beta-oxidation (FAO), enzyme reactions, and protein synthesis [45]. MtDNA is a compact, double-stranded, circular genome comprising 16,569 base pairs. Unlike nuclear DNA, mtDNA is streamlined, lacking introns, and possessing only one significant noncoding region known as the displacement or D-loop. Within its structure, there are 37 genes, which encode 22 transfer RNAs (tRNAs), 2 ribosomal RNAs, and 13 polypeptide subunits that contribute to the formation of the OXPHOS system. The mutation rate of mtDNA is estimated to be 10 to 20 times higher than that of nuclear DNA due to several factors, including the absence of protective histones, the generation of mtROS in the inner mitochondrial membrane, and the presence of limited repair mechanisms [46]. Mutations in more than 350 genes in both mitochondrial and nuclear genomes are now recorded to cause primary mitochondrial diseases (PMD) following an inheritance pattern [47]. In the last decades, mutations causing mitochondrial energy generation disorders have been identified in 289 genes [48], but many patients still remain without a molecular diagnosis [48]. The Mitochondrial Disease Sequence Data Resource is a comprehensive database that organizes both nuclear and mtDNA variants in all known and candidate genes for PMD [47]. Mitochondria genetic mutations could fundamentally alter the mitochondrial functions and lead to diseases, which are mostly via maternal transmission [49].

4. Mitochondria are immune signaling hubs

The functioning of mitochondria strikingly parallels the operational phases of the innate immune system [50] (Fig. 3A), underscoring their ability to sense environmental changes and adapt cellular metabolism accordingly. Mitochondria facilitate signal transduction through three principal pathways: indirect signaling, direct signaling, and intercellular mitochondria transfer (Fig. 3B).

Indirect signaling is one of the most common and widely spread mitochondrial regulated signaling pathways. This pathway primarily involves the reception or release of soluble substrates, encompassing a wide spectrum of elements, including gases, ions, small molecules, metabolites, proteins, lipids, DNA, vesicles, and variations in temperature. Due to the characteristic of soluble substrates, mitochondria can remotely sense or modulate the signal, which can be either intra- [51] or intercellular [52]. Indirect signaling regulation is one-to-many and nonspecific, with well-established and common substrates including mtROS, metabolites, mtDNA [53], cardiolipin [51], and peptides, mitochondrial unfolded protein response (mtUPR), ATP, transcription factor A mitochondria (TFAM), cytochrome c, mitochondrial Ca<sup>2+</sup> and iron [54].

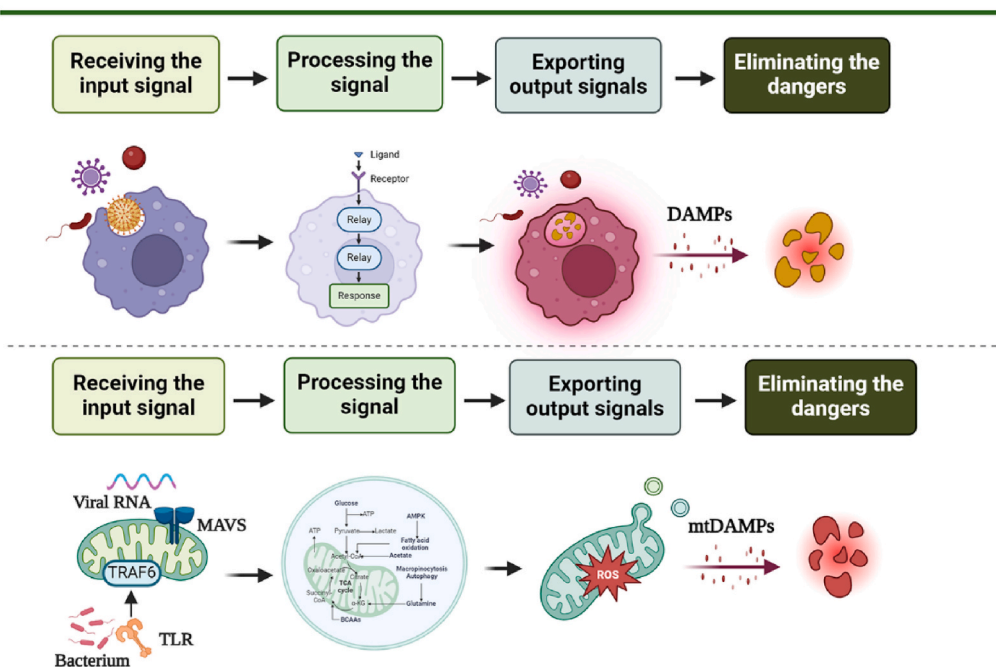
In contrast, mitochondrial regulated direct signaling is more specific and involves short-term regulation. This is primarily characterized by six types of organelle crosstalk, including mitochondria-ER crosstalk [55], which mediates innate immune responses [56], as well as mitochondria-sarcoplasmic reticulum crosstalk, mitochondria-nucleus crosstalk, mitochondria-Golgi crosstalk, mitochondria-plasma membrane crosstalk, and mitochondria-lysosome crosstalk [27].

Additionally, whole mitochondria can act as intercellular signals, facilitating tissue homeostasis, repair, immunoregulation, and even influencing tumor progression through cell-to-cell transfer [57]. This multifaceted signaling capacity highlights mitochondria’s critical roles in cellular communication and response mechanisms, as well as their integral functions within the intracellular immunity.

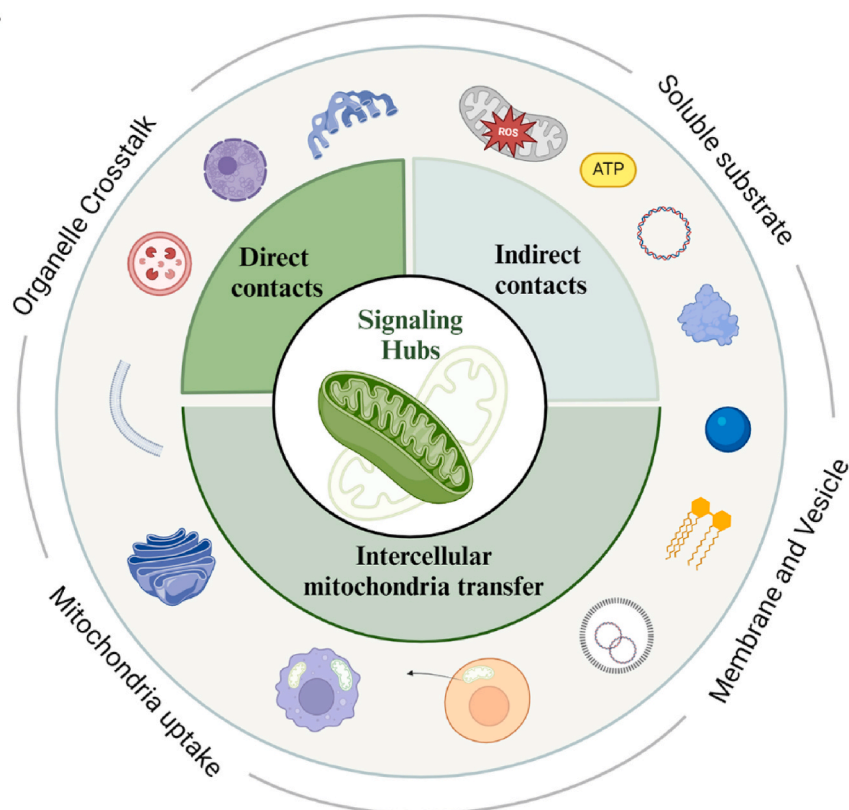


A.

## Similar framework between immune cells and immune organelle



B.



**Fig. 3. Mitochondria as Immune Organelles.** A. Mitochondria share a similar framework as innate immunity. This similarity underscores a sophisticated process: receiving input signals, processing these signals, generating responses and output signals, and ultimately neutralizing threats. B. Mitochondria are signaling hubs and facilitate signaling transduction through indirect contacts, direct contacts, and intercellular mitochondria transfer.

**Table 2**

**A.** Input signals that can be detected by mitochondria for mitochondrial-regulated indirect signaling include various molecular cues such as metabolites, ATP levels, ions, hormones, lipids, and others. **B.** Output signals generated or stored in mitochondria can be released into the cytosol for signaling regulation.

A.					
Category	Input signal	Sensing receptor/localization	Relationship with inflammation	PMID	
Metabolites	Pyruvate, Glutamine, leucine, Isoleucine, Valine, Arginine, Serine, Glycine, Tryptophan	Mitochondrial Carrier System/IMM	T cell differentiation and macrophage polarization	[66]	
Hormones	Thyroid hormones, sex hormones (estrogen and androgen), glucocorticoids	DNA-binding receptor/IMM, Matrix	Apoptosis, stress	[67]	
	Angiotensin II, melatonin, endocannabinoids, purines	GPCRs/IMM, OMM	mtROS production, OXPHOS, apoptosis	[58]	
Ions	Calcium (Ca <sup>2+</sup> )	Mitochondrial Na <sup>+</sup> /Ca <sup>2+</sup> exchanger (NCLX)/IMM	Pro-inflammatory cytokine production (TNF $\alpha$ , IL1 $\beta$ , IL6)	[87]	
	Magnesium (Mn), inorganic phosphate (Pi), Chloride (Cl), iron (Fe), lithium (Li)	–	Hydroxyl radical-mediated damage, proinflammatory cytokine production	[88]	
Gases	Nitric oxide (NO)	Complex I& IV	Inhibition of cytochrome c oxidase (low concentration); Inhibition of respiratory chain, uncoupling, cell death (High concentration)	[89]	
	Oxygen (O <sub>2</sub> )	Complex I& III	Decreases in oxygen levels increase the ADP/ATP ratio	[90]	
Energetics	mtROS	Complex I& III	Tissue damage, Activation of NLRP3 inflammasome	[91]	
	ATP/ADP	ADP/ATP carrier protein or Adenine nucleotide transporter (ANT)/IMM	Chemotaxis releasing, ROS generation, immune cell activation	[82]	
Anti-virus	Mitochondrial antiviral-signaling protein (MAVS)	OMM	Detect viral RNA and produce interferons (IFNs)	[68]	
Lipid	Cholesterol	CYP450scc/IMM	Steroidogenesis	[58]	
Others	Temperature	Matrix	Mitochondrial uncoupling	[92]	
B.					
Category	Output signals	Localization before releasing	Translocation to	Reactions	PMID
Protein	Cytochrome c	IMS	Cytoplasm	Apoptosis	[93]
	SMAC/DIABLO	IMS	Cytoplasm	Apoptosis, Immunomodulatory effect on T cell	[93]
	Cyclophilin D (CYPD)	Matrix	OMM/VDAC	Necrosis/Opening of mPTP	[94, 95]
	Mitochondria transcription factor A (TFAM)	Matrix	Extracellular	Pro-inflammatory cytokine secretion	[82]
DNA and RNA	mtRNA	Matrix	Cytosol	RNA sensor RIG-I (RIG-1) signaling and cytokine secretion	[68]
	mtDNA	Matrix	Cytosol/ Extracellular	Absent in melanoma 2 (AIM2), NLRP3 inflammasome activation, Cyclic GMP-AMP Synthase (cGAS)/STING pathway, Toll-like receptor 9 (TLR9) signaling	[82]
ATP	ATP	IMM/IMS	Extracellular	Recognized by purinergic receptor P2X 2 (P2XR)/P2YR, Activation of mitogen-activated protein kinase (MAPK), NLRP3 inflammasome	
Gas	Mitochondrial ROS (mtROS)	IMM	Cytosol/ Extracellular	Activate the immune system via retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), inflammasome, and MAPK	[96]
Metabolite	Heme	Matrix	Extracellular	Activate TLR4 and advanced glycosylation end-product specific receptor (AGER)	[68]
	Succinate	Matrix	Extracellular	Antigen-specific activation of helper T cells	[97, 98]
Lipid	Cardiolipin	IMM	OMM/Cell surface	Activation of macrophage	[99]
	Pregnaolone	Matrix	ER	Generate progesterone and steroid intermediates	[58]
Small peptide	Mitochondrial N-formyl peptides	Matrix	Cytoplasm	Inflammation/Attracting leukocytes and polymorphonuclear leukocyte	[100]

#### 4.1. Mitochondrial regulated indirect signaling

Indirect signaling in mitochondria is a pervasive mechanism that involves the exchange of soluble substrates, enabling these organelles to detect or exert influence over cellular signals. This process is facilitated by various sensors and receptors located within and on the mitochondrial membranes [58], allowing mitochondria to receive input signals ranging from metabolic changes to stress indicators (Table 2A). Barnett and Ting's review pointed out that stimulation by Gram-bacteria endotoxin lipopolysaccharide (LPS) [59] causes the cleavage of N-terminal Gasdermin D (GSDMD), the formation of GSDMD N-terminal pores could be transferred to the OMM [60]. This, in turn, leads to the release of mitochondrial DNA (mtDNA) into cytosol via mitochondrial permeability transition (MPT) pores and mitochondrial exosome vesicles [61] as mitochondrial SOS [62,63] and promotes inflammation [64], CVDs [61], and Alzheimer's disease [62]. Moreover, the metabolism of amino acids like glutamine, arginine, glycine, serine, and tryptophan is pivotal for T cell differentiation [65] and macrophage polarization [66]. Glucocorticoids are known to induce apoptosis [67],

while the beta-oxidation of fatty acids (FAO) supports the survival of memory CD8<sup>+</sup> T cells [66]. Additionally, an overexpression of mtROS can result in tissue damage and trigger the activation of the NLRP3 inflammasome [68]. Furthermore, mitochondria are key regulators of different types of cell death, including apoptosis [69], necrosis, pyroptosis, mitochondrial permeability transition pore (mPTP) [70–72]-mediated death, caspase-independent cell death (CICD, parthanatos [73]), necroptosis, ferroptosis (GPX4 controlled) [71,74–76], cuproptosis (copper induced cell death) [77,78], mitophagy-related cell death [79], and neutrophil extracellular trap cell death (NETosis) [80, 81]. Those processes dramatically alter mitochondrial permeability and disrupt mitochondrial compartmentalization [68].

In response to internal disturbances or external threats, mitochondria can generate mitochondrial danger-associated molecular patterns (mtDAMPs) as output signals. These mtDAMPs, including ATP, mtDNA, mtROS, and others, can then translocate outside the mitochondria [82] (Table 2B). Mitochondria mediated apoptotic cell death triggered by intrinsic stimuli such as growth factor withdrawal or cell receptor ligands via cell death receptors-tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) receptor

(TNFR1)-associated death domain protein (TRADD)/Fas-associating protein with death domain (FADD) [83]-caspase-8- BH3 interacting domain death agonist (Bid) pathway [84]. Upon induction of mitochondrial apoptosis [42,85], mitochondrial permeability transition pore (mPTP) decides a cell to die. Apoptotic signaling downstream of MOMP involves cytochrome c release from mitochondria and subsequent caspase activation. Therefore, MOMP can be therapeutically targeted to manipulate cell death holds tremendous therapeutic potential across different diseases, including neurodegenerative diseases, autoimmune diseases, and cancers. Recent reports showed that in addition to eliciting caspase activation, MOMP also plays various pro-inflammatory signaling roles. The roles of MOMP in inflammation suggests that mitochondria-derived signaling downstream of pro-apoptotic cues may also have non-lethal functions. The mitochondrial ATP synthase (ETC complex V) is a negative regulator of the mPTP [86].

Collectively, mitochondria provide a distinct platform for the redistribution of mtDAMPs, regulation of pattern recognition receptor (PRR, DAMP/PAMP receptors) signaling, inflammation, and ultimately, the activation of the immune systems.

#### 4.1.1. Reactive oxygen species (ROS)

Mitochondrial ROS (mtROS) are primary mtDAMP outputs when discussing the interplay between mitochondria and inflammation. The generation of mtROS arises due to the unintended escape of electrons, forming superoxide at complex I and complex III during the process of OXPHOS and ETC uncoupling [35,36]. However, whether mtROS [35, 96,101] in cells trigger inflammation depends on their concentrations. In hypoxic conditions, where oxygen levels are reduced, mitochondria adapt by generating low levels of mtROS as part of a broad metabolic adjustment. These low doses of mtROS play a pivotal role in physiological processes, particularly in activating human aortic endothelial cells [34,35,96]. This activation is crucial for the recruitment of immune surveillance cells into tissues and arteries, facilitating the early detection and response to microbial infections, the emergence of tumorigenesis, and the presence of DAMPs/PAMPs [35]. Moderate levels of mtROS are often induced by danger signals, such as bacterial endotoxin LPS, which play a crucial role in regulating the inflammatory response. High levels of mtROS activation can initiate apoptosis and autophagy cell death pathways [71], potentially resulting in cellular damage [96,102]. Therefore, mitochondrial ROS serve as double-edged sword in host defense and pathological inflammation during infection [91].

A recent review from our laboratory has categorized 11 types of ROS into seven functional groups, delineating their roles in processes ranging from metabolic stress sensing to the initiation of inflammatory responses. A notable finding is the significant role of increased nuclear ROS in promoting cell death [103], establishing nuclear ROS systems [103]. This balance between ROS levels is indicative of either physiological homeostasis or a marker of pathological stress across various cellular compartments. Oxidative stress contributes significantly to the pathologies of cancer, CVDs, diabetes, neurological disorders (Alzheimer's disease, Parkinson's disease, and Down syndrome) [104], psychiatric diseases (depression, schizophrenia, and bipolar disorder), renal disease, lung disease (chronic pulmonary obstruction and lung cancer), and aging [105].

#### 4.1.2. Adenosine triphosphate (ATP)

ATP was initially perceived as an intracellular energy currency; however, more recent discoveries have revealed its significance as a vital extracellular signaling molecule. Under pathological conditions, such as cellular stress or damage, the opening of the mitochondrial permeability transition pore or the formation of mitochondrial-derived vesicles can facilitate the leakage of ATP from the mitochondria into the cytoplasm and extracellular space [106]. Subsequently, extracellular ATP serves as DAMP [106], capable of recognizing and activating purinergic membrane receptor subtypes, namely P2X receptors (P2XR) or P2Y receptors (P2YR) [106]. This activation can elicit inflammatory pathways such as

p38 mitogen-activated protein kinase (MAPK) pathway [59,107] or the NLRP3 inflammasome [82,106].

#### 4.1.3. Metabolites

Mitochondria serve as critical hubs for metabolic waste management, with certain metabolites synthesized or stored within these organelles acting as sources of mtDAMPs and regulators of the immune response [41,108,109]. Examples include heme (porphyrins), which is recognized as an agonist of TLR4 with various immunomodulatory effects [68]. Succinate, when released from cells, acts as an extracellular mediator that triggers the production of proinflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ) [41,110] and macrophage activation [41] through G protein-coupled receptor 91 (GPR91, succinate receptor 1, SUCNR1 [41]). In immunometabolism, substances like succinate and itaconate within mitochondria illustrate their roles in immune regulation. Succinate accumulation promotes inflammation through various mechanisms, including the inhibition of prolyl hydroxylases (PDH) by mitochondria-released mtROS and further inhibits hypoxia inducible factor 1 subunit  $\alpha$  (HIF-1 $\alpha$ ), which promotes aerobic glycolysis and increases IL-1 $\beta$  [41]. Additionally, accumulation of succinate can enhance antigen-specific activation of helper T (Th) cells in both humans and mice [98]. Sirtuins, anti-inflammatory nicotinamide adenine dinucleotide (NAD) + -dependent protein deacetylases [111], are influenced by reduction of OXPHOS, leading to increases in NAD + [112] and Sirtuin 1 and subsequent modulation of inflammatory pathways [113]. Acetyl coenzyme A (acetyl-CoA) [114] serves as a key signaling convergent points for trained immunity (innate immune memory) [112,115,116], where it can be converted into malonyl-CoA and make glyceraldehyde 3-phosphate dehydrogenase (GAPDH) malonylation to induce proinflammatory cytokine TNF $\alpha$  [41]. Itaconate in mitochondria becomes itaconyl-CoA and inhibit methylmalonyl-CoA mutase (MUT) and ETC complex II [41]. Itaconate, after transported from mitochondria into cytosol via oxoglutarate carrier (OGC) [41], it displays anti-inflammatory and antioxidant [117] properties, in part because of its ability to activate the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which has antioxidant and anti-inflammatory activity [118–120], and transcription factor ATF3, while inhibiting IL-6 [41] (also see an outstanding review by Picard M, Shirihai OS [58]).

#### 4.2. Mitochondria regulated direct signaling

The output signals produced by mitochondria are not only recognizable by neighboring cells but also capable of being perceived and integrated by other intracellular organelles [121,122]. Mitochondrial-associated membrane and contact sites (MSCs) with other organelles are the major mechanism of mitochondrial-regulated direct signaling [123].

In 2021, our lab conducted a comprehensive -Omics data mining analysis focusing on the expressions of 260 organelle crosstalk regulators (OCRGs) across 16 functional groups [27]. Significant patterns were uncovered in the context of 23 diseases and 28 tumor types. The findings revealed relationships between OCRG expression and disease states, shedding light on the complex interplay between organelle function and pathological conditions. The key findings include: (1) the ratio of upregulated to downregulated OCRGs varies by disease type, indicating specific organelle crosstalk patterns associated with acute inflammations, metabolic diseases, autoimmune diseases, and organ failures. (2) In conditions like sepsis and trauma, certain OCRG groups (vesicle, mitochondrial fission, and mitophagy) are upregulated, termed cell crisis-handling OCRGs. (3) The suppression of autophagosome-lysosome fusion, particularly in endothelial and epithelial cells, is crucial for viral replication, highlighting these downregulated OCRGs as vital for viral proliferation. (4) CD4<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells (Tregs) [42,124–129] show a higher expression of OCRGs compared to CD4<sup>+</sup>CD25<sup>+</sup> T effector controls, suggesting a role in immune regulation. This research highlights the potential of

**Table 3**  
Mitochondria-endoplasmic reticulum contact sites and potential functions.

Mito-ER	Contact sites on mitochondria	Contact sites on ER	Functions	PMID
	ATAD3A, CISD2, DRP1, FATE1, Fis1, FKBP8, FUNDC1, MFN1, MFN2, Miga 2, MITOL/MARCH5, Mul 1 (MAPL), RHOT1/2, RMDN3, SLC25A46, Spire1c, SYNJ2BP, Tom 70, VDAC1, VDAC2	BAP31, CKAP4, EMC, EMD, GRP75, INF2, IP3R, PDZD8, PS2, Reep1, VAPB, TMX1, PERK	Steroidogenesis, Cholesterol homeostasis, mitochondrial fission/fusion, phospholipid exchange, Ca2+ transport, Autophagy	[147]

manipulating organelle crosstalk mechanisms as a novel strategy for treating inflammations, sepsis, trauma, organ failures, autoimmune diseases [130], metabolic CVDs, and cancers [27,131].

4.2.1. Mitochondria and endoplasmic reticulum (ER) crosstalk

The ER is a vital organelle within cells, functioning in material synthesis and information exchange. It plays roles in multiple cellular functions [104], including synthesis, folding, lipid biogenesis, ER-associated degradation (ERAD), lipid homeostasis [132], post-translational modification (PTM), calcium metabolism, and transport of proteins to appropriate cellular destination [133]. The distance between the OMM of mitochondria and the ER can range from 10 to 100 nm. The close spatial proximity between mitochondria and the ER facilitates frequent communication through Mito-ER (mitochondria-ER) contact sites, allowing for the coordination of cellular responses. Mitochondria establish more contact points with the smooth ER compared to the rough ER, primarily because the smooth ER lacks ribosomes, making it more accommodating for membrane interactions [134]. Mitochondria establish physical contact with the ER via cholesterol-rich microdomains known as mitochondria-associated membranes (MAMs), serving as a pivotal interface for the communication and exchange of molecules [135] (Table 3).

Mito-ER contacts play a critical role in regulating significant pathophysiological processes, including calcium and lipid homeostasis, mitochondrial dynamics, autophagy, and inflammation. For instance, the inositol 1,4,5-trisphosphate receptor (IP3R) is a calcium ion channel primarily located on the ER membrane. On the OMM, voltage-dependent anion channel 1 (VDAC1), a porin protein, has the capacity to establish a connection with type 1 IP3R through G protein-coupled receptor 75 (GPR75). Consequently, the IP3R-GPR75-VDAC1 complex facilitates the translocation of calcium ions from the ER to the mitochondria. Moreover, the distance between the Mito-ER contact sites can impact the efficiency of calcium transport, with greater distances, approximately 15 nm, leading to enhanced calcium transportation, and conversely, shorter distances, around 5 nm, limiting this process [133]. Hence, both the spatial distance between organelles and Mito-ER contacts plays crucial roles in preserving calcium-signaling homeostasis [34]. Disruptions in calcium homeostasis can have significant implications for various diseases, particularly neurological disorders [136].

Inflammation is significantly influenced by Mito-ER contacts, which can activate signal transduction pathways of PRRs and enhance proinflammatory cytokine release. A notable example of this regulation is the NLRP3 inflammasome [16,106,137]. Studies have reported that the activation of NLRP3 is mediated by the calcium-sensing receptor, resulting in an elevation of intracellular calcium levels [138]. Sustained release of calcium from the ER can trigger mitochondrial destabilization, leading to an increase of mtROS production and the opening of the mitochondrial permeability transition pore (MPTP) [139]. In addition to elevation of oxidative stress, NLRP3 could activate NFkB [140] and induce the caspase1-dependent pathway to produce proinflammatory cytokines such as IL1β and IL18 [141]. In addition, the ER stress kinase

PERK (eukaryotic translation initiation factor 2 alpha) [104,142], required at Mito-ER contact sites, mediates apoptosis following ROS-induced ER stress [143]. Additionally, substances like the chronic kidney disease-related gut microbiota generated uremic toxin trimethylamine N-oxide (TMAO) [144] can enhance inflammation and trained immunity in endothelial cells through pathways involving PERK [145], mtROS, and glycolysis [104,142]. ER stress has been implicated in augmenting innate immune memory and influencing disease susceptibility, such as in aneurysm development, by mediating interactions between the ER and mitochondria [146]. These results suggest that ER-mitochondrial contact sites have synergy in sensing DAMPs and promote inflammation and trained immunity [112].

4.2.2. Mitochondria and nucleus crosstalk

The mitochondrial proteome is primarily controlled by nuclear DNA, with 99 % of mitochondrial proteins encoded therein, and only 13 essential proteins produced by mtDNA. The mitoCarta3.0 database, curated by the Broad Institute at MIT [148], catalogs 1136 human genes that influence mitochondrial function, with variations in expression across different diseases [146,149]. Notably, about 48 % of the mitochondrial proteome is found in other cellular compartments such as the nucleoplasm, nucleoli, and cytosol, and 30 % in mitochondria exhibit variation at the single-cell level [150], underscoring the interconnected nature of intracellular compartments. Research has also shown that the nuclear genome plays a crucial role in encoding mitochondrial proteins, with changes in nucleal genomic DNA encoded mitoCarta genes and 1121 mitochondrial genes in the Human Protein Atlas database observed in various conditions such as Alzheimer's disease [151] and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [119]. This highlights the dynamic interplay between the nuclear [152] and mitochondrial genomes in responding to disease states and external stimuli.

Quiros et al. presented a great outline to include nucleus-to-mitochondria (anterograde) and mitochondria-to-nucleus (retrograde) communication, mito-nuclear feedback signaling, and proteostasis regulation, the integrated stress response, and non-cell-autonomous communication [153]. For example, the chemotherapy agent Doxorubicin and ionizing radiation (IR) provide good examples of mito-nuclear feedback signaling. These agents trigger significant metabolic alterations within cells, including NAD depletion and mitochondrial stunning—a reversible state of mitochondrial dysfunction that occurs without cell death, even under conditions of ATP depletion [154]. This is driven by the upregulation of ribosomal protein S6 kinase A1 (p90RSK), leading to mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase 5 (ERK5) phosphorylation and activation of poly (ADP-ribose) polymerase (PARP), creating a nucleus-mitochondria feedback loop, inducing a senescence-associated secretory phenotype (SASP) [155] in myeloid cells, increasing their sensitivity to ROS. Transiently inhibiting PARP activity [156] during IR can reverse mitochondrial stunning and its associated effects, highlighting the crucial role of the p90RSK-ERK5 module in mitochondrial function and inflammation. The interaction of mitochondrial and nuclear PARP during oxidant-induced cell death [103,157], at least in some cell types – works “behind the curtains” as an orchestrator of many important cellular functions [158].

Furthermore, the identification of a nuclear mitochondrial-related genes (NMRGs) signature from MITOMAP suggests the potential for predicting patient responses to treatments like sorafenib or transcatheter arterial chemoembolization in hepatocellular carcinoma [159]. Integrated studies combining RNA-sequencing and mass spectrometry have elucidated the cellular and mitochondrial responses to OXPHOS dysfunction, revealing significant post-transcriptional regulation of the mitoproteome and adaptations in metabolic pathways in response to mitochondrial stress. For instance, ATF4 [146] and Myc transcription factors are increased in mouse hearts with severe mitochondrial dysfunction. Mitochondrial dysfunction causes strong



**Table 4**  
Metabolic inhibitors and mitochondria-promoted cancers.

Target	Metabolic inhibitors	Target functions	Related cancers
Indoleamine 2,3-dioxygenase 1 (IDO1)	Epacadostat, Linrodostat, KHK2455, HTI1090	T-cell activity	Metastatic prostate, bladder, glioblastoma, endometrial, hepatocellular, head and neck squamous cell carcinoma
Isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2)	Olutasidenib, LY3410738, DS-1001b, IDH305, Vorasidenib	Catalyze the oxidative decarboxylation of isocitrate to $\alpha$ -ketoglutarate ( $\alpha$ -KG) in an NADP-dependent manner.	Advanced solid tumor and gliomas with mutant IDH1
Glutaminase (GLS1)	Telaglenastat (CB-839), Sirpigenastat	Deamidation of glutamine to glutamate and participate in the TCA cycle	Cancers that exhibit glutamine dependence: Triple-negative breast, non-small cell lung cancer, Acute myeloid leukemia, renal cell carcinoma, and lymphoma
Monocarboxylate transporter 1 (MCT1)	AZD3965	Lactate efflux	Lymphoma, metastatic melanoma
Ketoglutarate dehydrogenase (KGDH) and pyruvate dehydrogenase (PDH)	Devimistat (CPI-613)	Mitochondrial metabolism	Pancreatic tumors, Acute myeloid leukemia
Mitochondrial complex I	IACS-010759, IM156	OXPHOS	Acute myeloid leukemia, melanoma brain metastases, lung metastases
Arginase	INCB001158 (CB-1158)	Arginine metabolism/Urea cycle	Advanced biliary cancers
Tyrosine	Racemetyrosine (SM-88)	Tyrosine pathway	Pancreatic, lung, breast, prostate, sarcoma cancers, lymphoma
Large neutral amino acid transporter 1 (LAT1)	JPH203	LAT1 plays a role in branched-chain amino acids and aromatic amino acids transportation	Colon cancers, cholangiocarcinoma, thyroid carcinoma
Methionine adenosyl transferase 2 alpha (MAT2A)	AG-270	Methionine metabolism	Methylthioadenosine phosphorylase-null tumor, advanced solid tumor
Fatty acid synthase (FASN)	TVB-2640	Biosynthesis of long-chain fatty acids	Astrocytoma, metastatic breast cancer
Sphingosine kinase-2 (SK2)	Opaganib (ABC294640)	Lipid metabolism	Cholangiocarcinoma, metastatic castration-resistant prostate cancer
Nicotinamide phosphoribosyl transferase (NAMPT)	KPT-9274	Nicotinamide adenine dinucleotide (NAD) salvage	Rhabdomyosarcoma xenograft, melanoma, glioblastoma
Thymidylate synthase (TYMS) and dihydrofolate reductase (DHFR)	Pemetrexed, 5-Fluorouracil, methotrexate	dUMP to dTMP conversion, folate to tetrahydrofolate (THF) conversion	Non-small-cell, colorectal cancer, gastric and breast cancer
Phosphoribosyl pyrophosphate amidotransferase (PPAT)	6-Mercaptopurine	Purine synthesis	lung cancer, non-Hodgkin lymphomas, head and neck squamous cell carcinoma
			Acute myeloid leukemia

post-transcriptional regulation of the mitoproteome. Mitochondrial 1C pathway enzymes are upregulated early in the progression of OXPHOS dysfunction. Increased proline synthesis from glutamate upon OXPHOS dysfunction. Secondary coenzyme Q deficiency develops in response to OXPHOS dysfunction [160]. To determine the functional interplays between mitochondrial genome and nuclear genome, using whole-genome genetic data, 64 nuclear loci associated with expression levels of 14 genes encoded in the mitochondrial genome are identified, including missense variants within genes involved in mitochondrial function (transforming growth factor beta (TGF- $\beta$ ) regulator 4 (TBRG4), mitochondrial poly(A) polymerase (MTPAP) and mitochondrial lon peptidase 1 (LONP1)), implicating genetic mechanisms that act in *trans* across the two genomes. The authors replicate ~21 % of associations with independent tissue-matched datasets and find genetic variants linked to these nuclear loci that are associated with cardio-metabolic phenotypes and Vitiligo, supporting a potential role for variable mitochondrial-encoded gene expression in complex disease [161].

#### 4.2.3. Mitochondrial unfolded protein response (UPR<sup>mt</sup>)

The UPR<sup>mt</sup> plays an essential role to safeguard mitochondria from proteotoxic damage by activating a transcriptional response in the nucleus to restore proteostasis [162]. Mitochondria misfolding stress (MMS) leads to the release of mtROS into the cytosol. Concurrently, MMS also causes defects in mitochondrial protein import, leading to the accumulation of mitochondrial protein precursors in the cytosol (c-mtProt). These two signals integrate to activate the UPR<sup>mt</sup>; released mtROS oxidize the cytosolic DnaJ heat shock protein family member B1 (HSP40 protein DNAJA1), which enhances the recruitment of cytosolic HSP70 to c-mtProt. Consequently, HSP70 releases heat shock transcription factor 1 (HSF1), which then translocates to the nucleus and activates the transcription of UPR<sup>mt</sup> genes [162]. Phosphatidylinositol 4-kinase beta (PIFK-1), the orthologue of the *Drosophila* PI 4-kinase four-wheel drive (FWD), is the only known factor essential for the

UPR of both mitochondria and the endoplasmic reticulum [163]. Morel J et al. reported an extensive strain-specific response in folate-induced acute kidney injury (AKI), ranging from complete resistance in the CAST/EiJ to high sensitivity in the C57BL/6J, DBA/2J, and PWK/PhJ strains. In susceptible AKI strains, severe early kidney injury is accompanied by the induction of mitochondrial stress response (MSR) genes and the attenuation of NAD + synthesis pathways. This is associated with delayed healing and a prolonged inflammatory and adaptive immune response six weeks after AKI insult, which may signal a transition to chronic kidney disease [164].

#### 4.3. Intercellular mitochondria transfer

Mitochondria not only mediate signaling transduction but also serve as a physical scaffold for the activation of certain PRRs. This is attributed to the nature of mitochondria, which are evolutionary remnants of ancestral Alphaproteobacteria and exhibit bacterial similarity features. Consequently, some components of mitochondria are considered to function as PRR ligands. The unique bilayer structure of mitochondria effectively segregates the mtDAMPs from their respective PRRs [68]. This property makes mitochondria important in signaling and regulating inflammation. Entire mitochondria can also be transferred between cells to provide energy supplementation or serve as DAMPs for inflammatory stimulation [165]. In 2006, Spees et al. demonstrated the transfer of normal and functional mitochondria from mesenchymal stem cells to mammalian cells with dysfunctional mitochondria, ultimately aiding these cells in restoring aerobic respiration [166]. Furthermore, mitochondria also play a role in cellular differentiation. Cells containing newly generated mitochondria are more likely to maintain their stem-like characteristics during early fate determination after division, whereas those with aged mitochondria tend to become specialized and stable [167]. This is one of the reasons why impaired cells can acquire mitochondria from stem cells to restore their functions, suggesting that

stem cell mitochondria have cell regenerative functions [168].

In addition to whole mitochondria transfer, fragments of mitochondria can also be transferred between cells. Neutrophil Extracellular Traps (NETs) [169], formed through a process called NETosis, represent a unique immune response mechanism wherein neutrophils release web-like structures composed of decondensed DNA, citrullinated histones (cit-H3), and various enzymes and proteins into the extracellular space [170]. This extrusion involves not only nuclear contents but also granular enzymes like neutrophil elastase, myeloperoxidase, and cathepsin G, among others. Recent research indicates that NETs play a role in regulating mitochondrial homeostasis in tumors, suggesting that mitochondria or their fragments can be released into the extracellular space through NETosis.

NETs have been implicated in exacerbating inflammatory, autoimmune, thrombotic, and CVDs due to their potent inflammatory potential. NETosis is marked by the activation of peptidylarginine deiminase 4 (PAD4) [72], an enzyme that citrullinates histones within the nucleus, leading to chromatin decondensation and the subsequent release of NETs. Further research has revealed that PAD4 deficiency in tumors leads to reduced mitochondrial density and ATP production, alongside decreased levels of mitochondrial biogenesis proteins such as peroxisome proliferator activated receptor gamma (PPARG) coactivator 1 alpha (PGC1α), TFAM, and nuclear respiratory factor 1 (NRF-1). These observations hint at a crucial role for NETs in maintaining mitochondrial homeostasis within tumors, potentially affecting anti-tumor immunity [171]. By influencing mitochondrial function and biogenesis, NETs may play an integral part in the metabolic adaptation of tumor cells, highlighting a novel aspect of the complex interplay between inflammation, immune responses, and cancer progression.

5. Mitochondria modulate trans-differentiation of cells and cancers

Epithelial-to-mesenchymal transition (EMT) allows epithelial cancer cells to assume mesenchymal features, conferring EMT cells with enhanced motility and invasiveness, thus promoting cancer metastasis. The induction of EMT is facilitated by EMT-inducing transcription factors that turn on the expression of “mesenchymal” genes and turn off the expression of “epithelial” genes. Mitochondrial dysfunction is a hallmark of cancer and has been associated with progression to a metastatic and drug-resistant phenotype [172]. Table 4 lists the cancers related mitochondrial and their metabolic inhibitors.

6. Pleiotropic functions of mitochondria in inflammaging (age-dependent low-grade inflammation)

Recent progress indicates that mitochondrial stress pathways play multiple roles in cellular and systemic homeostasis, which can influence protective or detrimental responses during aging [173]. Defective mitophagy is a key contributor to age-dependent low-grade inflammation, termed inflammaging. For instance, Parkin knockout mice challenged with low-dose lipopolysaccharide (LPS) develop Parkinson’s disease-like symptoms with loss of dopaminergic neurons and motor defects [174], a condition also observed in aged Pink 1–/– mice infected with Gram-negative bacteria [175]. Mitophagy prevents inflammation and restrains innate immune pathways by promoting the clearance of mtDNA from damaged mitochondria and preventing cytosolic mtDNA release and STING1 activation [176]. However, during acute inflammation, such as sepsis, pharmacological inhibition of mitophagy enhances macrophage activation for bacterial clearance, leading to a higher survival rate, whereas mitophagy promotion can lead to immunoparalysis, a secondary immune suppression in sepsis [177]. Circulating mtDNA levels gradually increase with aging, correlating with elevated serum inflammatory markers [178].

On the other hand, genetic ablation of ETC impairs histone acetylation [179]. Acetyl-CoA appears to be an essential mitochondrial signal

Table 5 Experimental approaches for mitochondrial research.			
Research Aims	Approaches	Technical characteristics	Related protocol PMID
Mitochondrial morphology study	Electron microscope	Gold standard for mitochondria morphology study	[203]
	Electron cryotomography	Three-dimensional (3D) visualization in situ	[204]
	Confocal microscope	High Resolution and live cell imaging	[190]
Mitochondrial dynamics	Imaging approaches	High resolution and time-lapse imaging of specific mitochondrial markers	
	Western and polymerase chain reaction (PCR)	Fission/fusion-related gene expressions in protein or molecular levels	[205]
	Histology	Fission/fusion-related gene expressions in specific cell types/ tissues	
Mitochondria bioenergetics and metabolism study	Agilent Seahorse Analytics	Supplementary Table 1	<a href="http://www.agilent.com">http://www.agilent.com</a>
Mitochondrial membrane potential	Patch clamp	Gold standard for quantitatively analyzing electrogenic ion exchange across membranes	[206]
	Flow cytometry	JC-1: lipophilic, showing red with hyperpolarization but exhibiting green in depolarization TMRE: Exhibit red with hyperpolarization	[192]
mtDAMPs (Mitochondrial ROS)	Flow cytometry/ Confocal	MitoSOX: Dihydroethidium (DHE) derivative that emits red fluorescence when oxidized by superoxide	[207]
		MitoNeoD: An extended method for the detection of mtROS, which can be used both in vivo and in vitro.	[208]
		The quantity of ATP from cells is determined by counting the photons emitted when firefly luciferase breaks down ATP and luciferin	[209]
mtDAMPs (Mitochondrial DNA)	Mito-Rh-S is a ratiometric near-infrared (NIR) fluorescent probe (Mito-Rh) single-molecule mtFIBER fluorescence in situ hybridization (FISH) Sequencing	A fluorescent probe for ATP recognition	[210]
		Fluorescence in situ hybridization to study nuclear mitochondrial DNA	[211]
		Next-generation sequencing (NGS) methods for analyzing mutations associated with mitochondrial	[212]

(continued on next page)

Table 5 (continued)

Research Aims	Approaches	Technical characteristics	Related protocol PMID
ETC complex study	Cytochrome C test	diseases include whole-exome sequencing and targeted gene sequencing.	[199]
		An additional experiment to the Mitostress test to evaluate whether cytochrome C loss of function/release has occurred	
		An additional experiment to the Mitostress test to evaluate the functionality of complexes I and II	
	Complex I/II test	An additional experiment to the Mitostress test to evaluate whether the respiratory deficit lies at complex I or somewhere upstream	[213]
	Complex I-IV linked activity assay	An additional experiment to the Mitostress test to measure complex IV function	
	Complex IV test	Enzymatic activity of the individual OXPHOS complexes I–V	
Mitochondrial Calcium imaging	Patch clamp	ETC complex-related gene expressions in protein or molecular levels	[214]
		Investigating mitochondrial calcium transport	
	Fluorescent Microscopy	Investigating mitochondrial calcium transport	[215]
Isotope tracing method	Isotope tracing followed by mass spectrometer analysis	Fluorescent Ca2+ indicator Fluo-4, AM	[216]
		A specific and sensitive method for dynamically investigating metabolic pathways and functions within mitochondria	
Mitochondrial unfolded protein response (UPRmt)	Volcano plot of the DNAJA1 interaction proteomics	A specific and sensitive method for dynamically investigating metabolic pathways and functions within mitochondria	[202]
Mitochondrial unfolded protein response (UPRmt)	Volcano plot of the DNAJA1 interaction proteomics	To study the mechanism of mitochondria maintenance.	[162]

regulating the rate of aging in *C. elegans* [180]. The DNA and histone-methylation status are regulated by 2-oxoglutarate-dependent dioxygenases (2-OGDO), which use  $\alpha$ -ketoglutarate ( $\alpha$ -KG) as a substrate. Replenishment of  $\alpha$ -KG, an endogenous metabolite, attenuates several age-related diseases [173]; administration of  $\alpha$ -KG extends lifespan in *C. elegans* and *Drosophila* [181,182], and increasing  $\alpha$ -KG levels reduces inflammation [183].  $\alpha$ -KG extends lifespan and compresses morbidity in aging mice [183]. Additionally, the administration of fumarate and malate to worms extends lifespan by inducing the glyoxylate shunt, an extra-mitochondrial pathway of energy production, mild mitochondrial uncoupling, and expression of longevity regulators DAF-16 and SIR-2.1 [184,185]. Conversely, mutations in succinate dehydrogenase (SDH) leading to succinate accumulation are associated with increased ROS levels and accelerated aging [186,187]. Therefore, changes of nutritional pathways and epigenetic states are crucial to

aging and are regulated by TCA metabolites.

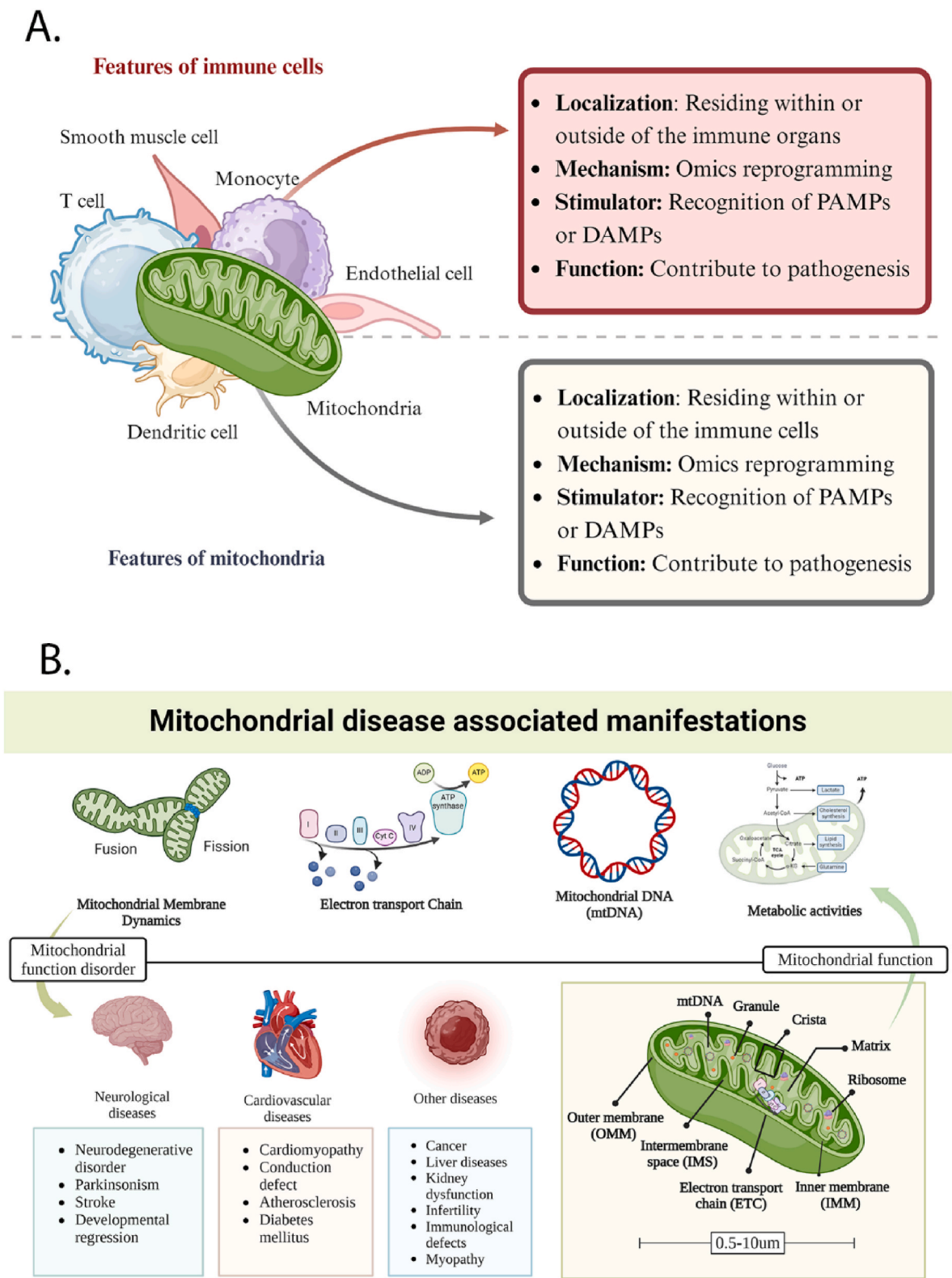
7. Mitochondrial dysfunction, lipids metabolism, amino acid biosynthesis, and COVID-19

The metabolic changes induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are indicative of disease progression. Sanchez A et al. conducted a multiomics study using plasma from 103 patients with varying degrees of coronavirus disease-2019 (COVID-19) severity during the course of SARS-CoV-2 infection [188]. As the severity of COVID-19 increased, significant alterations were observed in circulating proteomic, metabolomic, and lipidomic profiles. At 4–8 weeks into the evolution of COVID-19 (recovery phase), of a total of 239 proteins, 78 metabolites, and 112 lipids analyzed, the Kruskal–Wallis test revealed significant differences in 31 proteins, 51 metabolites, and 31 lipids. Among these, seven were found across all three severity groups, 18 differed significantly between mild and severe cases, four between mild and critical cases, and six between severe and critical cases. Notably, severe and critical patients with high levels of histidine-rich glycoprotein (HRG) and cholesteryl ester (ChoE) (20:3), along with low levels of  $\alpha$ -ketoglutaric acid ( $\alpha$ -KG), were found to have a high likelihood of unfavorable disease progression (area under the curve (AUC) = 0.925). Consequently, patients with the worst prognosis exhibited alterations in the TCA cycle (mitochondrial dysfunction), lipid metabolism, amino acid biosynthesis, and coagulation pathways. These significant findings enhance our understanding of how SARS-CoV-2 infection impacts various metabolic pathways, induces mitochondrial dysfunction, and contribute to our comprehension of the future consequences of COVID-19 and mitochondrial immunology, helping to identify potential therapeutic targets [188].

8. Advanced tools for detecting mitochondria in inflammatory diseases

Cutting-edge research tools play a pivotal role in advancing various fields of science by serving as a bridge that connects theoretical knowledge with practical application (Table 5). The discovery of mitochondria dates back to the year 1857. Swiss anatomist and physiologist Albert von Kolliker discovered that muscle cells included granule-like structures. Two centuries have passed since the discovery of mitochondria, and scientists now better understand their structure and functions with the aid of cutting-edged technologies. The architecture of mitochondrial membranes and their macromolecular components is being revealed in increasing detail. Single-particle cryo-electron microscopy (cryo-EM) demonstrates membrane protein complexes' near-atomic resolution. Electron cryo-tomography (cryo-ET) illustrates mitochondrial macromolecular components in situ and obtains sub-nanometer resolution [20]. Under inflammatory, immune response, and disease conditions, mitochondria can induce both morphological and functional alterations. High-resolution three-dimensional (3D) imaging of mitochondria is invaluable, not only for gaining a comprehensive understanding of mitochondrial structure but also for analyzing their structural interactions with other organelles within the cell. With advancements in technology, co-focal and two/multi-photon [189] microscopy is now employed not just for discovering the overall morphology of mitochondria but could be used for further investigating the time-lapse imaging of mitochondrial dynamics. Furthermore, employing fluorescent tags to label fusion or fission proteins can enhance the precision and clarity of visualization [190].

In addition to the mitochondrial morphology studies, many of the other techniques can be applied to investigate mitochondrial biochemical functional studies. The Seahorse XF Cell Mito Stress Test is considered the gold standard assay [34] and is widely employed for measuring mitochondrial oxygen consumption functions in cells [142]. Therefore, comparing cells under normal conditions with cells subjected to stress or stimuli allows us to observe how mitochondria change in oxygen



**Fig. 4. Working model.** A. The features of mitochondria as leading immune organelles in immune and non-immune cells. B. Mitochondria functions and related innate immune diseases, inflammatory diseases, autoimmune diseases, neurodegenerative diseases, transplantation immunology and cancers.

consumption and ETC in response to stress during specific disease conditions in a particular cell type. Agilent Seahorse offers a range of kits designed for the study of mitochondrial bioenergetics and metabolism [7]. These include the glycolytic rate assay, glycolysis stress test [142], cell energy phenotype test, Mito fuel flex test, real-time ATP rate assay,

substrate oxidation stress test, Mito-toxicity assay, T cell metabolic profiling test, and T cell activation assay (Supplementary Table 1). The mitochondrial membrane potential serves as a valuable indicator of cell health and functional status. Fluorescent dyes are commonly employed to monitor mitochondrial membrane potential. An increase in



mitochondrial membrane potential indicates that the mitochondrial inner membrane potential ( $\Delta\Psi_m$ ) [34] becomes more polarized. This often reflects a heightened demand for cellular energy, enhancing the efficiency of OXPHOS. However, excessive hyperpolarization may result in an overproduction of ROS, potentially leading to mitochondrial damage. Conversely, a moderate decrease in  $\Delta\Psi_m$  is indicative of a normal apoptosis process, typically associated with mitochondrial transition pore opening. On the other hand, significant depolarization can serve as an indicator of mitochondrial dysfunction or stress, potentially leading to cell death. Currently, 5,5,6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) dye and Tetramethylrhodamine, Ethyl Ester, and Perchlorate (TMRE) are two common commercial fluorescent kits used for  $\Delta\Psi_m$  detection [191,192]. Dysfunction of mitochondrial permeability could lead to mtDAMPs releasing from the mitochondria, including mtROS, mtATP, and mtDNA. Fluorescent probes are good methods for mtDAMP detection.

Recently, increasing methods have been developed for profiling metabolomes and assaying metabolic fluxes, as we have reported [193,194]. These include glucose injections to examine glycolysis, palmitate additions to medium for fatty acid metabolism studies, and the use of specific inhibitors such as glucose-6-phosphate isomerase (GPI) inhibitor 2-deoxy-glucose (2-DG) to disrupt glycolysis [142], mitochondrial pyruvate carrier (MPC) inhibitor UK5099 [195] to block pyruvate import into mitochondria, and oligomycin A to inhibit ATP synthase and promote glycolysis. Tools like the Oroboros Oxygraph 2000 [196,197] and Seahorse mitochondrial test kits (Supplementary Table 1) have been employed to identify mitochondrial ROS generation and glycolysis in human aortic endothelial cells in response to stimuli from conditional DAMP lysophosphatidylcholine (lysoPC) and gut microbiota-generated chronic kidney disease (CKD)-related uremic toxin trimethylamine N-oxide (TMAO) [142,144,198].

Other methods, such as respirometry, are used to map mitochondrial respiratory chain deficiencies [199]. Cell respiratory capacities are compared by varying cell densities and uncoupler titration, adding cytochrome c, performing complex IV tests using complex IV-specific electron donor (TMPD) and ascorbate, and assessing complex I versus complex II-linked substrates in permeabilized cells. Additionally, single-cell metabolic profiling methods like a matchmaker using single-cell profiling (scMEP), a flow cytometry-based method capturing the metabolic state of immune cells by targeting key proteins and rate-limiting enzymes across multiple pathways (Met-Flow), and functionally profile metabolism in multiple cells in parallel by flow cytometry (SCENITH), which utilize flow cytometry to profile energy metabolism at the single-cell level, have also been developed [200,201]. Inspection of metabolic activities within distinct subcellular compartments has long been a major challenge to our understanding of eukaryotic cell metabolism. A novel method has been reported for inferring physiological metabolic fluxes and metabolite concentrations in mitochondria and the cytosol based on isotope tracing experiments conducted with intact cells. Through computational deconvolution of metabolite isotopic labeling patterns and concentrations into their cytosolic and mitochondrial counterparts, combined with metabolic and thermodynamic modelling, Stern A. et al. have significantly reduced the uncertainty in compartmentalized fluxes and concentrations by one and three orders of magnitude, respectively, compared to existing modelling approaches. The authors develop a quantitative view of mitochondrial and cytosolic metabolic activities in central carbon metabolism across cultured cell lines without the need for cell fractionation, revealing substantial variability in compartmentalized malate-aspartate shuttle fluxes. This new approach for inferring metabolism at subcellular resolution is expected to be instrumental in a variety of studies related to metabolic dysfunction in human diseases and mitochondrial functions in inflammation and immune responses [202].

## 9. Conclusion

In this review, we have explored the evolving role of mitochondria as immune organelles and their critical contribution to intracellular immunity. They are key players in inflammation and immune signaling. We highlighted mitochondria's ability to detect and release danger signals, regulate immune functions, and engage in intercellular cross-talk, reinforcing their unique position in cellular biology.

The insights from this review suggest that mitochondria's role in intracellular immunity extends beyond immune cells, impacting a broader spectrum of cellular functions. This new perspective opens innovative avenues for therapeutic interventions. For instance, mitochondrial transfer could be a novel approach for diagnosing, regulating, and treating diseases [217,218], where transferring healthy mitochondria may restore cellular function in damaged tissues. Mitochondrial metabolites could serve as therapeutic agents [219–222], similar to cytokine [223] and antibody therapies [224], and may act as epigenetic cues [225] in immunotherapies [226,227].

Mitochondrial-based therapies could benefit patients with cardiovascular conditions by enhancing heart cell function, while in neurological disorders [221], they could contribute to neural repair and recovery. In immunological diseases, targeting mitochondrial pathways could modulate immune responses to reduce inflammation or autoimmunity. Additionally, precision medicine in cancer treatment might leverage mitochondrial signaling to enhance the effectiveness of existing therapies or to develop new, more targeted approaches (Fig. 4).

## CRediT authorship contribution statement

**Keman Xu:** Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization. **Fatma Saaoud:** Writing – review & editing. **Ying Shao:** Conceptualization. **Yifan Lu:** Conceptualization. **Qiaoxi Yang:** Conceptualization. **Xiaohua Jiang:** Project administration. **Hong Wang:** Supervision. **Xiaofeng Yang:** Writing – review & editing, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Data availability

Data will be made available on request.

## Acknowledgements

This study was supported by research grants from National Institute of Health to XY (R01 HL163570-01A1 and 1R01HL147565-01), and American Heart Association Postdoctoral fellowship to KX (24POST1196349).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2024.103331>.

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