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An Overview of Fundamentals to Prospective of Immunometabolism in New Therapy

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Abstract- Mitochondria represent a unique quality control cellular system, wherein they coordinate multiple functional activities. They are highly dynamic organelles and can easily modify their morphology by fusion or fission to adapt to cellular responses to various challenges. The intercommunication among mitochondria and other cellular organelles is responsible for the assembly and maintenance of the cell. The current overview followed PRISMA guidelines and used the PubMed/Medline databases to explore and summarize the entire literature generated and dedicated during the past few years to applications of mitochondrial biology in biomedicine. Analysis of the data demonstrated that mitochondria have a dual cellular function the "Immunometabolism"; in addition to their well-recognized bioenergetic function, they are key members of the innate immune system. Further, it is found that many disorders are associated with insufficient mitochondrial quality control. It is concluded that understanding the molecular mechanisms of mitochondrial function and dysfunction is a new exciting field. Outstanding candidate potential therapeutic roles of mitochondria are emerging. This research topic may take humanity to a new era of secure and efficient diagnosis, prevention, or therapy of human diseases.

Keywords - Mitochondria; Mitoimmunity; Mitophagy; Mitochondrial DNA; Mitochondrial dynamics; mitochondrial therapy.

I. INTRODUCTION

Mitochondria are dynamic cellular organelles that are continuously changing in their morphology and/or number according to cellular needs, and pathological conditions [1]. They can rapidly switch from their primarily well-known roles as bioenergetic organelles; that generate ATP to anabolic organelles that synthesize both ATP and utilize the tricarboxylic acid (TCA) cycle for the building of macromolecules [2]. Apart from their bioenergetic functions, mitochondria appear to function as strategic members of the innate immune system [3].

The link between the metabolic function of mitochondria and the immune cell function is now a well-known field called 'immunometabolism' [4]. A key feature of mitochondria is that they can participate in crucial decisions regarding proliferation, differentiation, death, and senescence of immune cells [4], [5]-[8].

Mitochondria represent a quality control system that includes mitochondrial fusion, fission, and mitophagy [9]. The system is controlled by several genes, transcriptional factors, proteins, and reactive oxygen species (ROS). Furthermore, mitochondria are capable of interacting with other organelles through the principal interface of the outer mitochondria membrane (OMM) with the endoplasmic reticulum (ER), lysosomes, and lipid droplets [10]. Mitochondrial mechanisms such as metabolic pathways, amino acid metabolism, antioxidant systems, mitochondrial dynamics, mtDNA, mitophagy, and mitochondrial ROS (mROS) are critical for immune functions. Specific actin-binding proteins (ABPs), such as Gelsolin, have also been found to be engaged in the pathophysiology of mitochondrial oxidative phosphorylation disorders [11].

Damage-associated molecules may originate from cellular components like the nucleus, plasma membrane, and intracellular proteins. Following the damage to the cell integrity and intracellular inclusions may be released into the extracellular regions. The contents can act as stress signals called damage-associated alarmins [12]. In the last few years, several epidemiological studies have suggested substantial links between leukocyte mtDNA copy number (mtDNAcn), scarcity of immune system cells, vor deficiency in the differentiation of confirmed that mitochondrial dysfunction can trigger signals in a particular way in response to stress, a feature that may be useful to search for new therapeutic strategies [13].

Immune cells and risk a range of diseases and health conditions. This includes glioma [14]. Various pathological conditions [15], [16], cardiovascular diseases [17], as well as Parkinson's disease, and Fulminating Hepatitis [18]. Immunological analysis in these reports demonstrated that patients with high mtDNA content had significantly less incidence of natural killer (NK) cells in peripheral blood mononuclear cells (PBMCs) and higher plasma concentrations of cytokines known to damage

mitochondria; IL-2 and TNF-α. These findings suggested that an immune suppression-related mechanism is involved in mtDNA-mediated prognosis. \

The current review aims at examining the potential role of mitochondria as biomarkers of early diagnosis of human diseases. Another goal is addressing mitochondrial dysfunction and stress signaling as promising targets for novel drug development. We introduce pivotal immunometabolism concepts and discuss the role of metabolites with important immunomodulatory effects (immunometabolites). This constitutes the basic background for a more in-depth analysis of the emerging pathophysiological functions of mitochondria.

It concludes that disruption of the mitochondrial life cycle, quality control processes, and their non-energetic functions have been associated with a range of inherited and acquired human diseases. The bright side of this is that targeting these processes may have clinical therapeutic applications. A more detailed discussion of the included topics can be found in specialized reviews referenced in each section.

II. METHODS

1. Data Sources and Search Strategy:

In this review, a systematic search of the literature was carried out using the PubMed/Medline databases from 2015 up to July 2022. The keywords used as search terms included "Immunometabolism; Mitochondrial DNA; Mitochondrial dynamics, Mitochondrial genome, Mitoimmunity, and Mitophagy. In addition, the snowball method was also used to extract other publications. Refined searching identified a few hundred publications, of which 100 were relevant according to their title and abstract and chosen for further analysis (Fig 1). The 'Blind' collection/analysis of the data was the main criterion that was used to eliminate 'bias' and to ensure the quality of studies.

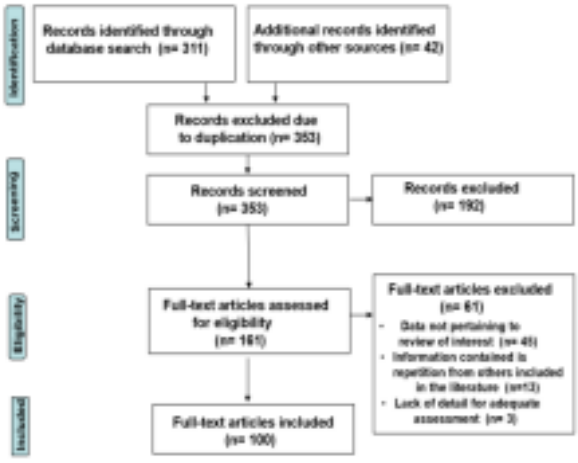

Fig 1. PRISMA flow diagram.

Presentation of the procedure of literature searching and selection with numbers of articles at each stage.

III. RESULTS AND DISCUSSION

1. Mitochondrial Genome:

Historically, mitochondria were first recognized as distinct cellular organelles in 1888 by Walther Flemming. Later, they were observed by Richard Altman in 1894. In 1898, the term mitochondrion from the Greek roots meaning 'thread' and 'grain' was coined by Altman and Karl Benda. In humans, there can be up to 1000 mitochondria per cell and all the mitochondria of one cell are called chondroma [19]. The human mitochondrial genome is small representing about 1% of total cellular DNA (about 16,568 bp per cell). Each mitochondrion can possess 2–10 copies of its DNA (mtDNA), a circular double stranded DNA (dsDNA) molecule [20]. The two strands can be physically separated into a heavy strand rich in purines (G and A) and a light strand rich in pyrimidines (C and T).

The human mtDNA contains 37 genes encoding 13 important protein subunits forming the apparatus of the mitochondrial electron transport system (METS) and the ATP synthase complex, 22 for mitochondrial TRNAs and 2 for rRNAs (*20*). More than 200 mutations in mtDNA have been reported [19]. Similar to that of its prokaryotic ancestors, mtDNA is made of a circular loop and includes a significant number of unmethylated DNA as CpG islands [3].

MitoCarta (http://www.broadinstitute.org/mitocarta) is comprised of 1136 human genes which encode proteins with information related to localizations of mitochondria, submitochondrial compartments, and pathway annotations [21]. The Integrated Mitochondrial Protein Index (IMPI) contains 1330 proteins located on mitochondria, 328 proteins whicthatect mitochondrial function, morphology, and dynamics, as well as 511 proteins with strong evidence for mitochondrial localizations [9].

1.1 Mitochondria in Immunometabolism:

Existing literature shows that the crucial role of mitochondria as signaling organelles in coordinating and regulating immune efficiency has become greatly acknowledged by researchers. This newly emerging field of studies, which links metabolism and immunological state is referred to as 'immunometabolism'. Accumulating evidence suggests that impaired immunometabolism contributes to infectious and inflammatory diseases. For example, the mitochondrial enzyme aconitate decarboxylase 1 (ACOD1, which is best known as immune responsive gene 1 [IRG1]) is overregulated under different inflammatory states [22]. This enzyme functions as a central controller of immunometabolism implicated in itaconate production from isocitrate, macrophage polarization, inflammasome activation, and oxidative

stress [22]. The specific mechanisms associating inflammation and metabolic reprogramming, which represent two important hallmarks of many pathobiological processes, have been detailed in several recent publications [6], [8[, [15], [16], [18], [23]-[29].

Mitochondria have several mechanisms that enable them to trigger cytosolic signaling pathways. For example, they can change metabolic routes; AMP/ATP ratio, and release ROS [2]. Furthermore, mitochondria can induce transcriptional alterations that result in totally different consequences in immune cells. The precise integration of the cellular signaling pathways with a metabolism that drives active cells provides directions for deciding cell fate (Fig 2).

The finding that the released components of mitochondria into the cytosol of the stressed cell are identified by the cellular intrinsic defense mechanisms as foreign, fits nicely with the bacterial evolutionary history of these organelles [30]-[33]. Several mechanisms for signal transduction between mitochondria and the rest of the cell are known. The first is anterograde signaling and involves signal transduction from the cytosol to mitochondria. The best instance of this mechanism is the prompt sequestration of Ca^{2+} into the mitochondrial matrix in response to increases in cytosolic Ca ions [33].

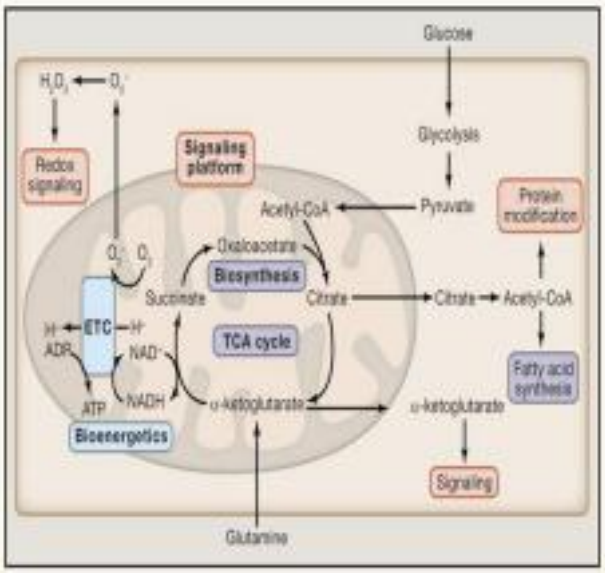

Fig 2. Schematic diagram showing the critical interplay between metabolic and roles of mitochondria. ETS: Electron Transport Chain; TCA: Tri Carboxylic Acid. Source: [2[.

The second is retrograde signaling which is signal transduction from mitochondria to the cytosol. An example of this is the production of mitochondrial reactive ROS controlling the activation of the transcription factor hypoxia-inducible factor 1 (HIF-1). The high AMP/ATP ratio initiates activation of AMP activated protein kinase (AMPK) that lowers the mammalian target of rapamycin (mTOR) activity to reduce anabolic reactions. This decreases ATP need and stimulates autophagy to increase metabolic supply by offering nutrients to mitochondria the for production of ATP [34]. The activation of AMPK also stimulates fatty acid oxidation while putting down fatty acid synthesis. Lastly, the OMM is known to act as a signaling podium to line up multiple proteins to permit synchronized interaction and consequent signaling [9].

The results of Collins's research group demonstrated that cell-intrinsic post-transcriptional control is a major driver of circadian output in macrophages [35]. This control of metabolic pathways plays an eminent role in determining cell response to immune stimuli. For instance, M1 macrophages, a subset of innate immune cells that sense and respond to the pathogen threat, show a broken TCA cycle and have a pro-inflammatory function. In contrast, M2 macrophages undertake β-oxidation to generate anti inflammatory responses.

Reprogramming a classically activated M1 macrophage towards a more anti inflammatory M2 macrophage suggests the induction of the repair or regeneration in a dynamic condition [36]-[39].

Additionally, the metabolism of certain amino acids, specifically arginine, glutamine, serine, glycine, and tryptophan, is crucial for T cell differentiation and macrophage polarization [41], [41]. In neutrophils, although earlier studies reported that they utilize glucose as the main source for their metabolic processes, emerging evidence demonstrates that these cells can also use other nutrients such as amino acids, carbohydrates, proteins, and lipids for energy production [42].

Neutrophils are often subjected to different immunological environments where nutrients are inadequate, therefore, they have to adapt to diverse metabolic pathways for diverse functions in response to the required immunological needs. In addition, recent research suggests that neutrophils undertake metabolic acclimatization under broad disease conditions. More investigations using techniques, like metabolic flux assay and metabolomics, might be fruitful for a deep understanding of neutrophil metabolism [43].

2. Regulation of Mitochondrial DNA:

The mtDNA gene coding regions are characterized by close connection and partial overlapping. The 13 mtDNA code for proteins forms the main mitochondrial electron transport chain (ETC) complexes. The 22 tRNAs and 2 mitochondrial rRNAs are important components in the mitochondrial translational complex [20]. Many proteins either directly bind or indirectly control the mtDNA transcription (*1*). Of these proteins, among others, mitochondrial transcription factors A, and the extracellular-controlled protein kinases (*9*). As reported

by Li and his colleagues [9], the reduced activity of cyclic AMP (cAMP) response element-binding protein, which promotes mtDNA gene expression, results in mitochondrial respiration dysfunction by downregulating the expression of many mitochondrial genes.

Environmental factors or cellular endogenous stresses lead to mitophagy, an important process in the mitochondria-associated activities, which facilitates the removal of injured mitochondria and preserves mitochondrial function and homeostasis [9]. Mitophagy triggers mtDNA release in the cytoplasm and the extracellular spaces circulating as small DNA fragments or encapsulated in vesicles [44].

This stimulates inflammatory signaling pathways and thus causes immune cell dysfunction and death [45], [46]. In the cytosol, mtDNA promotes the pathway of cyclic GMP–AMP synthase activator of interferon genes and affects cell proliferation, senescence, and inflammation [47]. In addition, mtDNA released into extracellular compartments can induce the generation of neutrophil extracellular traps and enhance type I interferon response (IFN I) [48].

In pathological circumstances, mtDNA can pass through created pores in the OMM on the basis of voltage-dependent anion channels [49]. The release of cell-free mDNA (cf-mtDNA from dysfunctional mitochondria is believed to stimulate multiple pathways associated with inflammation such as oxidative stress, and DNA damage [50], [51].

This includes the development of a number of human diseases [52], for example, diabetes [53], rheumatoid arthritis [54] as well as different cardiovascular problems [55], lung diseases [9], autoimmune neurodegenerative disorders [51], and immune mediated inflammatory skin diseases [56].

The antimicrobial innate immune system acts as an essential task in the mammalian immune response [2], [57]. mtDNA as a proinflammatory or inflammasome agonist affected immune responses and inflammatory pathology. Accumulating evidence from recent research has confirmed that mtDNA activates inflammatory reactions including some innate immune pathways by three mechanisms (Fig 3).

The discovery of the molecular pathways activated by mtDNA following its identification by innate immune cells may provide scientists with new targets for the treatment of related diseases. In particular, the changes in the mtDNAcn relative to nuclear DNA (nDNA) have been found to be in advance valuable diagnostic biomarkers and/or therapeutic targets for a range of phenotypes and diseases [58]-[59].

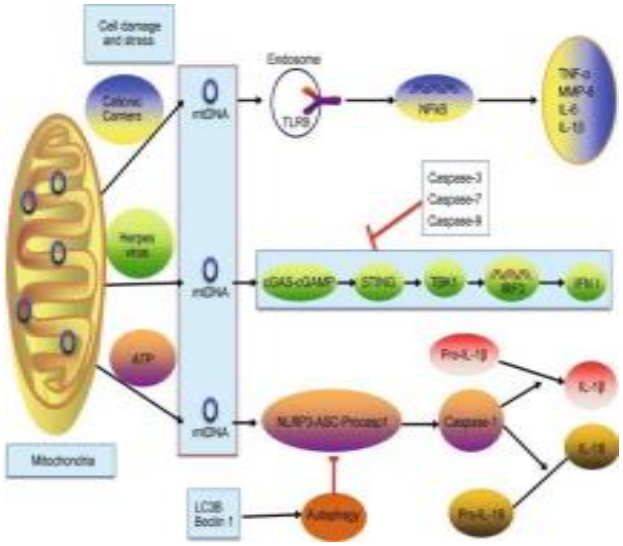

Fig 3. Various pathways for activation of innate immunity by mitochondrial DNA (mtDNA).

ASC: Apoptosis-associated Speck-like protein containing a Caspase recruitment domain [ASC], cGAMP: Cyclic GMP-AMP dinucleotide; cGAS: Cyclic GMP-AMP Synthase; IFN I: type I Interferon; IL: Interleukin; IRF3: Interferon Regulatory Factor 3; LC3B: Light Chain 3B; MMP-8: Matrix Metalloproteinase-8; NFκB: Nuclear Factor kappa B; NLR: Nod-like receptor; STING: Stimulator of Interferon Genes; TANK-Binding Kinase 1 (TBK1):TLR9: Toll-Like Receptor 9; TNF-α: Tumor Necrosis Factor-α. Source: [3].

In the first pathway (Fig 3, upper level**),** cell damage or stress such as cationic carriers induce the release of mtDNA from mitochondria. This pathway involves Toll-Like Receptor 9 (TLR9) signaling. The TLR9 in endosomes causes transcription of pro inflammatory cytokine genes and increases the release of pro-inflammatory cytokines, including Matrix Metalloproteinase-8 (MMP-8), Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), and IL-6 [3], [61].

In the second mechanism (Fig 3, middle level**),** herpes virus infection releases mtDNA that escaped into the cytoplasm. This event is sensed by the Cyclic GMP AMP Synthase- stimulator of interferon genes (STING) 3 (cGAS-cGAMP-STING) pathway. This results in TANK-Binding Kinase 1- Interferon Regulatory Factor 3 (TBK1-IRF3)-dependent expression of IFN I and inhibition of viral replication. The activation of IFN response can be prevented by the activation of caspases involved in the intrinsic pathway of apoptosis (caspase-3, caspase-7, and caspase-9).

The third axis of innate immune response (Fig 2, lower level) involves mtDNA-TLR9-NFκB, mtDNA NLRP3-caspase1 pathway, and mtDNA-STING-IRF3 [3], [62]. Stimulation of ATP causes dysfunction of mitochondria resulting in mtDNA liberation into the cytosol. This

cytosolic mtDNA as a signal regulates the transcription of immune cells. In the cytosol, mtDNA binds to and activates the Nod-like receptor; (NLR) and the NLR-family pyrin domain containing 3 (NLRP3) inflammasome. Interaction with the adaptor protein Asc and procaspase-1, the NLRP3 inflammasome allows the enrolment and activation of caspase-1.

This in turn cleaves pro-IL-1β and pro-IL 18 into their bioactive mature forms. On the other hand, microtubule-associated protein (MAP) 1 light chain 3B (LC3B)/Beclin 1-mediated autophagy is implicated in the clearance of mtDNA. Consequently, it negatively regulates the NLRP3 inflammasome activation. Also, mtDNA activates the NLRP3 inflammasome and mediates the secretion of IL-1β and IL-18, which might be associated with the induction of inflammatory disease [62].

3. Mitochondria as Quality Control System:

The loss of balance of mitochondrial dynamicity was reported to result in the increase of damaged mitochondria, leading to aging, cardiac dysfunction, or cancer [63]. Mitochondrial mass and mobility can be influenced by their fission and fusion (Fig 4). Mitochondrial fission involves extensive contact between the ER tubules and the OMM mediated (ABPs) which accomplish constriction of the mitochondria [10]. It is suggested that the ER tubules may play an active responsibility in defining the site of mitochondrial fission. The delicate balance between fusion and fission processes is beyond just defining the number of mitochondria in a cell. On one hand, fused mitochondria show more effective oxidative phosphorylation and exchange of metabolites and mtDNA [64]. On the other hand, the fission of mitochondria may enable their transport to different cellular sites. For instance, local signaling is needed for cell repair [65] and for clearing away impaired mitochondria by mitophagy [66].

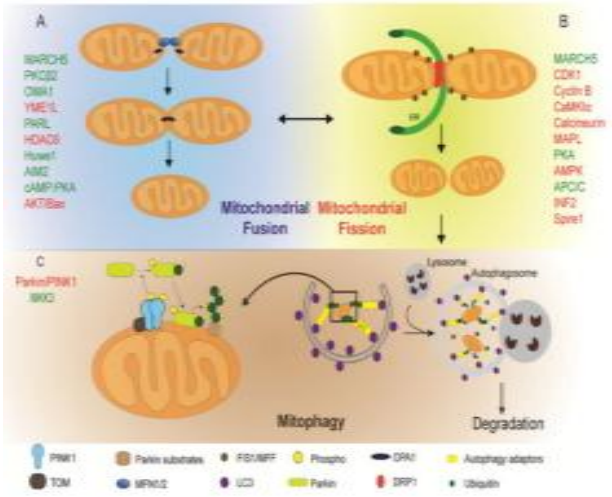

Fig 4. Schematic representation of the mitochondria quality control dynamic system that continuously adapts to different cellular and tissue metabolic requirements. A: Fusion. B: Fission. C: Autophagy (Mitophagy). The activities are controlled by several genes, transcriptional factors, proteins, and reactive oxygen species. Red and green marks indicate positive or green regulators, respectively. Source: [9].

The control of mitochondrial fission can bring back the young phenotype of endothelial progenitor, stimulate the collapse of cancer stem cells, and avoid tumor reoccurrence [67], [68]. Thus, the process of cellular metabolic reprogramming associated with mitochondrial dynamics can be an alternative way to identify and develop diagnostic biomarkers and therapeutic targets for diseases [69]. The clinical significance of the knowledge of mitochondrial dynamics can be exemplified by cancer therapy. Among the diverse cellular activities, tumor metastasis involves the capability of the extracellular matrix to control mitochondrial respiration coupled to ATP production, oxidative stress, mitochondrial fission, and morphology of cancer cells [69], [70].

In particular, the increases in mitochondrial fission change cellular motility and enhance the ability of malignant cells to undergo accelerated metastasis and acquire better drug resistance. In fact, the inhibition of one of the mitochondrial fission-related proteins (dynamin-related protein) was suggested as one of the strategies to destroy spread cells [71]. In addition, impairment of mitophagy has been linked to several pathologies [71]. Dysregulation of mitophagy processes has been demonstrated in cancer patients [34]. Regulation of mitochondrial dynamics including fission and mitophagy has proved fruitful in the alleviation of the inflammatory response in lung epithelial cells [72], and in increasing cardiac hypertrophy [73].

4. Intra and Intercellular Mitochondrial Communication:

There are communication circuits that underlie the mutual influence of systemic inflammation and metabolism. Figure (5) presents two levels of communication of systemic Immunometabolism. The existence of the exchange of information between neighboring mitochondria, where cristae ultrastructure becomes coordinated between adjacent organelles, has been demonstrated [74]. Moreover, there is distinct continuous intercommunication between mitochondria and other cellular organelles. The mutual interaction between mitochondria and subcellular regions (Fig 5A) is crucial in different activities of physiological and pathological processes, including metabolism, immunity, and apoptosis [75]-[78].

The signals that transmit information between mitochondria and the nucleus include ROS [79]. The generated epigenetic marks impact the expression of the nuclear genes by affecting the epigenetic landscape responsible for switching on and off genes across the

genome (*74*). Mitochondria are able of contacting other organelles by the principal interface of the OMM with ER, lysosomes, endosomes, and lipid droplets (LDs) [80]. Mitochondria-associated ER Membranes (MAMs) are the cellular structures that link the ER and mitochondria and are implicated in calcium signaling, lipid transfer, mitochondrial dynamic, and mitophagy [81].

In the innate immune cells, mitochondria are positioned close to the ER. That is to say; mitochondria-ER contacts are key drivers of rapid metabolic and functional reprogramming in memory $CD8^+$ T cells [82]. Hence, mitochondria and ER joint signaling can also impact immune cell metabolism. Disturbance of the ER-mitochondria connection sites influences mitochondrial dynamics, prevents the occurrence of autophagy, and reduces fatty acid oxidation and oxidative phosphorylation [83].

Furthermore, peridroplet mitochondria anchored to the LDs slow the motility and the control of lipid metabolism via bidirectional signaling [84]. The LDs mitochondria interaction maintains the function and stability of controls among mitochondrial dynamics and lipids, cytoplasmic homeostasis, as well as cell structure and function. The intercommunication among cellular organelles is responsible for the assembly of mitochondria-driven lipids, which allows converse maintenance of the interaction between mitochondria and other organelles. The LDs are significant in the control process of mitochondria dynamics-associated lipid metabolism when mitochondria and organelles signal each other [9], [85].

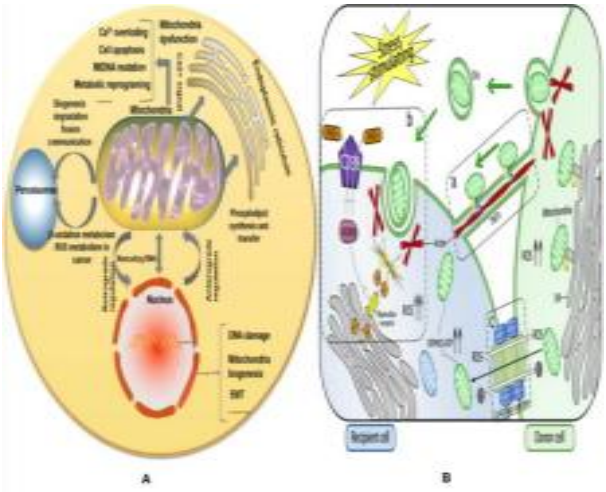

Fig 5. A. Intracellular communication, where mitochondria interact with the endoplasmic reticulum, peroxisomes, and nucleus by signal transduction, vesicle transport, and membrane contact positions to control energy metabolism, biosynthesis, immune response, and cell turnover. B. Intercellular transfer of the mitochondria.

(a)TNTs join the cytoplasm of two cells, whose major framework is F-actin. Mitochondria move from one cell to another via attaching them to the actin skeleton by a specific transport complex. (b) EV endocytosis is facilitated via the NAD+/CD38/cADPR/Ca^{2+} pathway: Intracellular NAD+ increases and transfers to the extracellular environment under the influence of stress conditions. The cADPR acts on Ryanodine receptors (RyRs) on the endoplasmic reticulum, leading to an elevation in the intracellular Ca^{2+} concentration, following the changing of the actin cytoskeleton and the invagination of the plasma membrane, thus completing the endocytosis of EVs. (c) Cx43 GJs GJCs also take part in the transcellular transfer of mitochondria though there are three possible trails: Ca^{2+} or ROS exchange via Cx43 GJCs to modify the formation of channels transferring mitochondria or the direct transfer of mitochondria. Sources: A. From [86] and B from [90]. Abbreviations: cADPR: Cyclic ADP-ribose; EVs: Extracellular vesicles; GJs: Cx43 gap junctions; OXPHOS: Oxidative phosphorylation; TNTs: Tunneling nanotubes.

In addition, the lysosome-mitochondria interaction modulates the process of mitochondrial dynamics and enables the transfer of metabolites or ions. These events control the cytoplasmic concentration of Ca^{2+} and impact the production of ROS [33], [86]-[88]. Mitochondria-lysosome contact points control mitochondrial calcium dynamics and transport Ca^{2+} to mitochondria through the lysosomal Ca^{2+} efflux channel and specialized transient receptors (*33*). At mitochondria-lysosome contacts, mitochondrial calcium uptake is modulated by OMM and inner mitochondrial membrane (IMM) situated channels, as well as voltage-dependent anion channel, and mitochondrial Ca^{2+}uniporte [9]. At contact sites, mitochondria are recruited to interact with ER and endosomes. Through this interaction, mitochondria regulate phospholipid transport and endosomal maturation [89].

Recently, researchers have emphasized the central role of noncoding RNA in the communication between the nucleus and mitochondria, as the noncoding RNA almost covers the entire process of gene transcription and expression. The theory of noncoding RNA as a new regulator in nuclear and mitochondrial communication is starting to emerge. Most investigations have concentrated on long noncoding RNA, as regulators of nuclear and mitochondria communication. It has been reported that nuclear-encoded lncRNA are transmitted into the mitochondria and coordinate mitochondria-induced apoptosis.

The 'intercellular mitochondrial transfer' (Fig 5B) – That is the movement of the entire mitochondria from one cell to another, has been described. The transfer of mitochondria across cells plays a vital role during physiological and pathological states, such as rescuing recipient cells from a bioenergetic deficit and tumorigenesis [75]. Important functions for stem cell

behavior and functional interactions between cancer and stromal cells have been also recognized [75]. Mitochondrial communication across organs has been reported in inflammatory and infectious diseases [6]. It has been reported [90] that functional mitochondrial movement between cells was first demonstrated from mesenchymal stem cells (MSCs) to mammalian cells *via* tunneling nanotubes (TNTs). Transcellular mobility of mitochondria enables the incorporation of the donated mitochondria into the endogenous network of recipient cells, resulting in changes in the bioenergetic profile and other functional characteristics of the recipient cell. This process plays a crucial role in different pathological situations including repair of tissue injury, inflammatory regulation, oncogenesis, and tumor drug resistance, as well as in physical conditions maintaining tissue homeostasis [90]. Studies have shown that transcellular transfer of mitochondria involves multiple mechanisms, such as the formation of TNTs, extracellular vesicles (EVs), gap junctions, endocytosis and exocytosis of naked mitochondria, cytoplasmic fusion, as well as other metastasis modes (*90*). Additionally, the transfer of mitochondria can also be utilized as a cure for mitochondrial dysfunction diseases, including organ degeneration and cancer.

5. Limitations:

This overview is limited by the notion that, as is true for the PRISMA methodology, a database limits the number of keywords used. Consequently, the search had to be repeated several times to make sure that all search query keywords were included. In this overview, only articles written in English were included. However, the discussion could have been stronger if research articles in languages other than English had been consulted. Finally, the screening of records and the collection of data from articles were carried out by a single author. So, there is a possibility that some relevant research studies have been missed or certain errors made.

III. CONCLUSIONS AND FUTURE DIRECTIONS

This overview showed the existence of intercommunication networks between mitochondria and other cellular organelles. Understanding the nature of these relationships is a likely option for identifying and developing promising therapeutic targets. Increased mitochondrial fission has been found to play a fundamental role in the reprogramming of lipid metabolism in hepatocellular carcinoma cells [91].

This suggests a strong approach for the use of this process as a drug target in the treatment of this cancer. Currently, there are important emerging pathophysiological roles of mitochondria in the regulation of the immune response [8], [23], [24]. A growing school of scientists advocates the observation that mitochondrial cargo associated with mitophagy, for example, RNA, DNA, and proteins will probably get increased attention from researchers in the coming years [92].

Biomarkers of mitochondrial dysfunction have been correlated with inflammation and gene sets related to leukocyte activation. Nowadays, there is a possibility of accurate quantification of mtDNAcn in cells [78]. Quantification of this number can be carried out by different methods such as next-generation sequencing, microarrays, droplet digital PCR, and quantitative real time PCR (qPCR) (*93-95*) Gene adjustment and editing on mtDNA by CRISPR-Cas9 technology have been suggested to be likely therapeutic methods for mitochondrial diseases, mitochondria-associated tissue damage, and organ failure [85], [96], [97].

The maintenance of mitochondrial function through bioenergy-based regulations to improve cell sensitivities to challenges and drugs is another therapeutic strategy for diseases [9]. Still, mitochondrial transplantation into targeted tissues and cells has been considered as a further promising approach to returning to normal mitochondrial function [98]. Lately, innate immunometabolism was placed at the cornerstone of inflammaging (immune-metabolic viewpoint for age-related diseases), immunosenescence, and autoimmunity COVID-19 [28], [99]. Given that the mortality observed in COVID-19 is linked to extreme inflammation, a better perception of the immunological foundations of the distinctive responses seen in COVID-infected patients is necessary not only as potential biomarkers but also as therapeutic targets in HIV infection (*39*).

Direct measurement of mtDNA levels in peripheral blood mononuclear cells (PBMCs) proves to be a successful reliable prognostic marker and an indicator for the host defense of COVID-19 patients [100]. These workers reported that high levels of mt-DNA in PBMCs have been associated with COVID-19 and its decrease could be employed as a potential measure of the risk of severity and mortality of patients with COVID-19. However, while the integrity of mitochondria in mononuclear cells in the blood of COVID-19 patients continues to be under investigation, the utilization of mt-DNA as a marker of prognosis and severity is a promising field yet to be further explored. Success would be a great achievement in mitochondrial therapy. Challenges still exist, for example, sex differences in mitochondrial biology and immune functions. In addition, repeated measures in one individual demonstrated variation in PBMC type distributions as well as changes in the week-to-week mitochondrial activities. Moreover, these researchers reported potential age- and sex-related differences in mtDNAcn.

Innovation of mitophagy-targeted interferences and/or development of novel therapies may improve the chances

for treatment of other diseases including COVID-19 and cancer. A list of new regulators of mitochondrial function is continuously growing and some are being tested as candidates for drug discovery or supplementations for clinical trials. Here again, before reaching this goal, several critical confounding issues in the field of mitochondrial biomedicine are awaiting further clarification including underlying mechanisms, standardization of mitochondrial isolation and preservation methods, as well as maintenance of long-term therapeutic efficacy.

Larger studies are required to validate and mechanistically extend these findings. These mitochondrial phenotyping data build upon established immunometabolic differences among leukocyte subpopulations and provide foundational quantitative knowledge to develop interpretable blood based assays of mitochondrial health.

IV. STATEMENTS AND DECLARATIONS

ETHICS APPROVAL

This is an observational study. The Institutional Research Ethics Committee has confirmed that no ethical approval is required.

- **Consent to Participate:** Not applicable
- **Consent to Publish:** Not applicable
- **Authors Contribution:** Not applicable. This is a single-authored work.
- **Funding:** The author declares that no funds, grants, or other support were received during the preparation of this manuscript.
- **Competing Interests:** The author has no relevant financial or non-financial interests to disclose
- **Availability of data and materials:** Not applicable

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