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Educational review

Once we were bacteria… mitochondria to infinity and beyond

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Abstract

Mitochondria, cytoplasmic organelles originated from endosymbiotic bacteria, can be metaphorically described using "Janus bifrons" image, due to their involvement in life, providing cellular energy and resulting essential even for stem cells, but playing a key role also in cell death. Mitochondria own a maternally inherited genome and are the site of aerobic respiration; they can produce proteins, nucleotides, lipids, steroids and heme and result involved in iron homeostasis. Moreover, mitochondria can generate free radicals, break down waste products and represent the primary source of cellular heat. The size and shape of mitochondria depend on the intracellular metabolic status, from tubular presentation to a blob form in case of irreversible damage. Each mitochondrion carries different sets of DNA; when one set accumulates mutations, it can be replaced by another. It has been widely demonstrated that mitochondrial disorders are involved in many pathologies, including autism, multiple endocrinopathies, diabetes, Alzheimer's disease, ataxia, Barth's syndrome, myopathy, and even aging and cancer. Human population is characterized by different mitochondrial DNA haplogroups reflecting the mutations accumulated and useful to characterize genetic diversity. The mitochondrial role also results relevant in pregnancy, providing information about maternal-fetal dyad in physiological and in pathological conditions. Recent evidence suggests that an intriguing bidirectional inter-talk exists between microbiota and mitochondria, influencing cellular homeostasis and metabolism. A recently demonstrated mitochondrial property is the possibility to be transferred from a donor cell to a recipient cell, through a system of tunneling nanotubes. Recently, a promising integrated approach involving omics sophisticate technologies has been applied in mitochondrial pathophysiology. This is still at an early stage, and further studies will clarify such complex genotype-phenotype relationships. In conclusion, mitochondria are not simple energetic organelles but represent dynamic structures communicating with the cell nucleus and even with other cells, influencing metabolism and their targets' functions. More detailed knowledge of their involvement in disease, even though a combined omics approach, could represent a chance for new therapies.

Keywords

Mitochondria, microbiota, mitobiota, stem cells, tunneling nanotubes.

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Mitochondria: the way we were

The first report of mitochondria dates back to 1840 [1]; subsequently Altmann, in 1890, defined them "bioblasts" [2] and finally Benda, in 1898, coined the name "mitochondria" [3]. This word derives from the Greek terms "mitos" (thread) and "chondros" (granule). Siekevitz, in 1957, named them "the powerhouse of the cell" [4] and Nass, in 1963, discovered that mitochondria contain some DNA sequences [5]. Mitochondria can resemble a "Janus bifrons" image, due to their involvement in life, providing the cellular need for energy and therefore resulting essential even for stem cells (SCs). However, on the other side, they play a key role in two types of cell death: caspase-dependent apoptosis and caspase-independent intrinsic apoptosis [6].

A schematization of mitochondrial involvement in several physiological functions but also their involvement in various human diseases is provided in **Fig. 1**.

The "powerhouse of the cell" is multitasking: in fact, mitochondria are the site of aerobic respiration and supply the cells with the energetic coin Adenosine Triphosphate (ATP); moreover, they can produce chemical substances indispensable to the whole organism, including proteins, nucleotides, lipids, steroids and participate in the biosynthesis of heme too. These organelles result also involved in iron homeostasis and can generate free radicals, which are essential for physiological mechanisms but may also result deleterious,

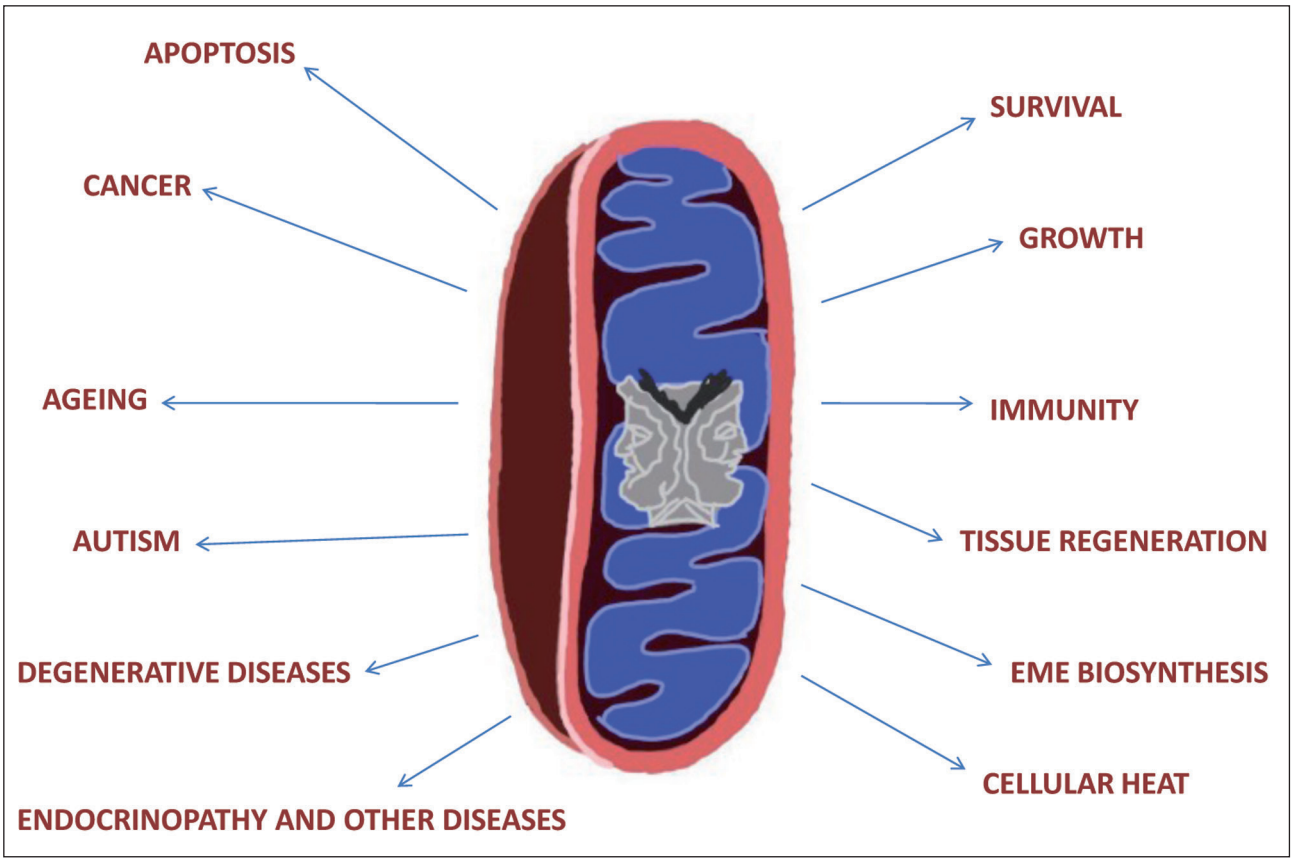

Figure 1. Schematization of mitochondrial involvement in several physiological functions and in diseases, since mitochondria can resemble a "Janus bifrons" image.

since their overproduction, if not inactivated by endogenous enzymes, can lead to increased oxidative stress. In addition, mitochondria break down waste products and recycle some of them to save energy [7].

Mitochondrial ATP, reactive oxygen species (ROS) and reactive nitrogen species production is even crucial during intense exercise; therefore, several physiological modifications occur to face the increasing energy needs, such as new mitochondria biogenesis, ROS increased production and acute inflammatory responses [8].

Furthermore, mitochondria are the main source of cellular heat. According to the recent literature, they can act like thermostatic radiators, operating at temperature reaching 50 degrees Celsius, in order to maintain our body in a defined temperature range [9].

The pivotal role of mitochondrial proteins in energy production also makes them an ideal system for a rapid adaptation to a new climate or different dietary habits [10].

Mitochondrial disorders are involved in autism, multiple endocrinopathies, diabetes, dementia, Alzheimer's disease, ataxia, Barth's syndrome, myopathy and even in aging [11-14].

In 1967, Lynn Margulis (previously Lynn Sagan), as first, proposed that mitochondria originate from endosymbiotic bacteria [15]. This hypothesis had been previously formulated by others but was considered controversial by the scientific community, until modern phylogenomic analyses confirmed that their genome sequences were closely related to alphaproteobacteria, a class that encompasses Rickettsiales [16].

However, to highlight the great complexity of this debate and the still open issues, we also report a very recent study demonstrating that mitochondrial origins may be more ancient than those of alphaproteobacteria, since they could evolve from a proteobacterial lineage [17].

The symbiont bringing on mitochondria was probably a flagellate that, after having lost its autonomy, was retained in the cell because of its capacity to generate ATP. The proto-eukaryote cell managed the growth and division of the symbiont by sending proteins synthesized by cytosolic ribosomes and lipids transferred from the endoplasmic reticulum. In addition, the proto-eukaryote had to segregate the mitochondria into daughter cells during cellular division. In eukaryotic lineages, mitochondrial genome is reduced if compared to the ancestors' alphaproteobacteria genome, since a thousand of genes were lost during the evolution.

Mitochondria have their own genome, maternally inherited. Mitochondrial DNA (mtDNA) is circular, double-stranded and composed by 15,000-17,000 base pairs; it encodes 37 genes, of which 13 are for proteins, 22 are for tRNA, 2 are for rRNA. However, most of the proteins present in the mitochondria (approximately 1,500 in mammals) are coded by nuclear DNA [18].

The mitochondrial proteome is composed by 1,100-1,900 distinct proteins, mostly encoded by the nuclear genome. Only 13 proteins are encoded by mitochondrial genome; they are involved in the respiratory chain. The majority of the proteins in human mitochondria, belonging to the "core mitochondrial proteins", are stably present in the cell. One-third of mitochondrial proteins have a dual localization being present both inside and outside mitochondria. Mitochondrial proteins, such as cytochrome-c (which is involved in the caspase-dependent apoptosis), Caspase-independent Apoptosis Inducing Factor and NADPH oxidase are usually released into the cytosol. Interestingly, the metabolic enzyme complex pyruvate dehydrogenase may be localized both in mitochondria and nucleus [19].

Some of these proteins also take part in critically important metabolic pathways, such as protein synthesis, amino acid and nucleotide metabolism, apoptosis [20].

Show me your shape…

Mitochondria are pleomorphic organelles whose size and shape change depending on the intracellular metabolic status. It is possible to recognize a healthy cell by the shape of its mitochondria: in physiological conditions, mitochondria look tubular; in stressing but still reversible conditions they acquire a donut form, reaching a blob form when the occurred damage is irreversible [21].

They are constituted by two membranes, the inner one and the outer one, that include the matrix and the inter-membrane space, respectively. The mitochondrial matrix contains enzymes belonging to the fatty acid beta-oxidation and the Kreb's cycle. Mitochondria are able to use molecules derived from nutrient uptakes, such as pyruvate and fatty acids, converting them to acetyl coenzyme A (CoA), which is involved in the citric acid cycle.

MtDNA replication does not strictly depend on the cellular cycle [22].

Each mitochondrium carries numerous and different sets of DNA; this expression refers to the co-existing presence of several copies of mtDNA [23]. Each of them is predisposed to the production of the same proteins and therefore, when one set accumulates mutations, it is eliminated and replaced by another, compensating its loss. MtDNA is more susceptible to mutations and damage, due to the lack of the protection conferred by histones [24] and other reasons, such as high levels of ROS and a reduced DNA repair ability, somatic mtDNA mutations (primarily in the displacement loop, a non-coding sequence also called D-loop). Therefore, a high number of mutations occur in each individual mtDNA over time. It is also known that the majority of these variants are benign and related to single nucleotide polymorphisms (SNPs) in the nuclear genome. The frequencies of these variants and the differences showed differ among populations and have been used to perform haplogrouping studies [10].

In fact, it has been postulated that the human population can be characterized by different mtDNA haplogroups, according to SNPs belonging to the mitochondrial genome. This would reflect the mutations accumulated via maternal lineage. For their ancient origin, mtDNA variants represent ancestral roots from tens of thousands of years ago. They can help to understand the migrations occurred, the accumulation and transmission of characteristic sets over generations and the growing genetic diversity. In conclusion, in this perspective, haplogroups may be useful to evaluate genomic ancestry, can be considered ancestral tags and additional links with other nuclear DNA markers can also be found [10].

There is a continuous cross-talk between mtDNA and nuclear DNA. MtDNA transcription is controlled by the mitochondrial transcription factors A and B which are nuclear encoded. In addition, mtDNA transcription and replication are regulated by the mitochondrial D-loop [25].

Human mtDNA is composed of heavy and light strands. MtDNA copy number for each mitochondrion varies from one to more copies. All the copies of mitochondrial genome are similar; however, both mutations and cytosine methylation may occur. The term "heteroplasmy" refers to the co-existence of the wild-type and mutant mtDNA, determining the presence of multiple mtDNA copies in each cell. It has recently been reported that epigenetic modifications can occur not only in nuclear DNA but also in mtDNA [26].

MtDNA resulted methylated at the D-loop. Both 5-methylcytosine and 5-hydroxymethylcytosine altered content have been reported in disease-related studies. Indeed, the methylation of mtDNA itself, in addition to nuclear DNA methylation, has been recently shown to regulate mitochondrial gene expression, potentially impacting mitochondrial function and leading to diseases [27].

Janssen et al. in 2015 found a positive association between mtDNA methylation and airborne particulate matter exposure in the early life development. In addition, newborns who showed a lower mtDNA content in placental tissue presented higher levels of mtDNA methylation. According to the authors, the lower mtDNA content might be related to an increased mitophagy and mitochondrial death [28].

The different sets of DNA exist and can vary according to biogenesis processes; these copies are identical in the first phases. During aging, mutations become more numerous and, with epigenetics mechanisms, can also be substituted or deleted.

Not all the cells of our body contain the same number of mitochondria and of mtDNA copies, which also differs among people; thus some people may have a reduced mitochondrial efficiency [29]. In this perspective, it has been shown that old women who have a reduced number of mtDNA copies show impaired cognitive abilities [30].

In humans, the mtDNA copy number varies between 200 and 10,000 in the somatic cells to 100,000 and 200,000 in oocytes [26]. A human somatic cell contains on average about 1,000-2,000 mitochondria; however, this number can vary according to the cellular energetic metabolism and these organelles can be more abundant to face higher energetic needs. Therefore, metabolically active cells such as heart muscle cells or hepatic cells can reach the number of 5,000 and 2,000 mitochondria per cell, respectively [31].

Mitochondrial DNA: almost a Noah's Ark hypothesis?

The mtDNA allows us to reconstruct our genealogical trees, moving back to prehistory and acting as a black box of human evolution. In 2013, two studies published simultaneously showed that mtDNA of Eve and nuclear DNA of Adam were probably peers. Although Eve would take its name

from the Bible, she was not the only female of that period, but she was the only who would have produced an unbroken line of daughters.

The Y-chromosome is another example of non-recombinant DNA; a copy of it is inherited by the father and results similar to its copy. Therefore, it represents another important tool for the study of human history.

In the last years, two research groups studied the mutations'accumulation rate and discovered that the last common male ancestor would have lived about 125,000-200,000 years ago. Using the same technique to study mtDNA they calculated that mtDNA of Eve would have lived 99,000-148,000 years ago. MtDNA would act like the rings of a large tree able to show its age [32, 33].

According to a recent study, DNA would be a bar code since it perfectly defines the species to which it belongs. These findings would underline that about 90% of the species living on earth would have been born all together 100,000-200,000 years ago.

In conclusion, it can be stated that all the creatures from the primitive marsupials up to the evolved Homo Sapiens would be contemporaries [34].

Mitochondrial localization and morphology through human development

Each egg cell contains about 10,000 mitochondria; on the contrary, a sperm cell owns only a few dozens of them; other body cells contain an average of 300-400 mitochondria [35].

In oocytes, mitochondria are spherical with small cristae, and they are clustered around the nucleus [36]. After the fertilization, mitochondria split around the two pronuclei. The perinuclear position is probably necessary to face the increased energy needs [37].

Human embryonic SCs differ from mature cells because their metabolism depends on glycolysis. Since they own scarce mitochondria with underdeveloped cristae, their oxidative capacity is very restricted [38].

During the embryonic development, mitochondria become elongated, filamentous with many traverse cristae and move to a peripheric location. The mitochondrial networks increase as the human embryonic SCs differentiate, because their metabolism shifts toward the oxidative phosphorylation. It is possible to find a perinuclear localization of mitochondria in highly proliferative cells types, including other kinds of SCs and cancer cells [39].

This is why mitochondria are our destiny

Disproved the previous belief regarding the casual distribution of mitochondria into the daughter cell, at the moment of each cellular division, it has been demonstrated that this process can occur with well defined and codified rules. This can be observed especially in SCs' asymmetric division, where an asymmetric distribution of old and young mitochondria has been described. Extraordinarily, the most recently synthesized mitochondria are transferred to the SC-like daughter, which is destined to survive longer, maintaining stemness and undergoing numerous replications. On the contrary, old mitochondria become constituents of the tissue-progenitor daughters; these, containing older mitochondria, lose their stemness and undergo differentiation [40]. Finally, mitochondria may also influence SCs aging, since asymmetric division can generate a third kind of cell, which receives dysfunctional mitochondria and therefore undergoes a premature commitment [41].

Mitochondria among mother and son

The mitochondrial role has been recently evaluated also in the fetal period, finding interesting results which provide relevant information about maternal-fetal dyad, both in physiological and in pathological conditions. The content of mitochondria in the placenta also showed a key role in several conditions.

For example, many studies have shown that an adverse intrauterine environment characterized by oxygen and nutrient restriction, increased oxidative stress and inflammation, can induce and sustain mitochondrial biogenesis. Therefore, mitochondrial content results increased in fetal blood and placental tissue of intrauterine growth restriction (IUGR) and preeclamptic pregnancies. Altered mitochondrial content and function are also reported in gestational diabetes and maternal obesity [27, 42-47].

In the last years, many authors published similar results, reporting altered mitochondrial content, epigenetic modifications and impaired functions in the feto-placental unit and in mothers undergoing pathological pregnancies.

Whether this is a cause or an effect of the compromised intrauterine environment still remains unsolved, as this seems to represent a vicious circle linking mitochondrial alterations,

oxidative stress and inflammation within the impaired setting of these pregnancies.

Moreover, mitochondrial dysfunction has been related to impaired placentas, which can lead to fetal growth complications and long-term consequences for the future adult [48-50].

In fact, a poor and nutrient-restricted environment can damage developing organs through several ways, and this fetal programming can potentially affect the health of the individual longlife [51]. One among these mechanisms seems to be the reprogramming of mitochondrial function, to face the increasing energy needs and supply the uteroplacental insufficiency; therefore, a higher production of ROS and an increased oxidative stress occur, with systemic and local negative effects, especially on tissues such as the pancreatic β-cells, hepatocytes, myocytes, and placenta itself [51].

Different patterns of methylation have also been described in such pathological conditions. In particular, a decreased mtDNA methylated cytosine content has been found in fetal cord blood of pregnancies with IUGR and preeclampsia [27].

Inherited and acquired mitochondrial diseases

Inherited mitochondrial diseases characteristically do not affect fetal growth but become clinically evident after birth, showing a variable spectrum of symptoms mostly characterized by organ failure and lactic acidosis. This aspect suggests that glycolytic ATP production is the essential metabolic pathway during fetal development, whereas oxidative phosphorylation results more important after birth. Inherited and acquired mitochondrial dysfunctions can affect any tissue, but brain, liver and heart are the most susceptible organs [52].

Forever young

It is widely accepted that mitochondrial dysfunction in aging is related to the accumulation of mtDNA mutations and increased ROS production. However, the increase in ROS is not the primary trigger of aging. Currently, some scientists have found other mechanisms, since the ROS-dependent actions are dose-related and not always fundamental for the cell. Thus, the new idea of mitochondrial "Hormesis" has been proposed, introducing the concept that low doses of ROS may promote general health and lifespan, while higher levels may increase mortality [53]. Many other mechanisms, such as altered mitochondrial dynamics with an imbalance between fusion and fission, defective mitophagy, increased mtDNA mutations and reduced ATP levels may lead to mitochondrial dysfunction. Alzheimer's disease is the most common neurodegenerative disease; it has been demonstrated that the neurons of affected patients contain a lower percentage of normal mitochondria; moreover, many neural cells show fragmented, punctiform or elongated mitochondria with a net-like structure or broken cristae. Clear mtDNA dysfunction features could contribute to Alzheimer's disease. Mitochondrial dysfunction has also been implicated in the pathogenesis of Parkinson's disease. Hence, it has also been shown that there are direct or indirect interactions between mitochondria and proteins encoded by mutated genes even in Mendelian Parkinson's disease [54].

Mitochondria and autism

The current literature suggests a potential connection between mitochondrial dysfunction and autism and also provides clinical, biochemical and genetic evidence to support this association. Researchers demonstrated that children affected by autism can show three periods of abnormal brain development. Thus, their head size is generally normal at birth, but generally undergoes an overgrowth during infancy and a premature growth arrest in childhood; this is followed by a shrinking and a degeneration between adolescence and middle age, when the brain starts to decrease in size. A post-mortem study showed that autistic children had about 65% increase of neurons in the dorsolateral prefrontal cortex and 25% increase of neurons in the medial prefrontal cortex, if compared with brains from age-matched control patients. The authors hypothesized that a deficit in pruning, in association with the overabundance of damaged neurons, could cause subsequent faulty connection among the different regions of the brain [55]. Clinically, the occurrence of macrosomia and macrocephaly, occurring in about 20% of autistic patients, seems to be more frequent in those with underlying mitochondrial diseases. Regarding molecular findings, a subgroup of autistic patients showed increased levels of plasmatic lactate and pyruvate, in addition to organic acids in the urine, suggesting a possible defect in oxidative phosphorylation processes. Recent evidence,

obtained from post-mortem studies, points out an abnormal mitochondrial function. In the brain of autistic children aged between 4 and 10 years, a defect in ETC complexes and an increase in the mitochondrial copy number have been shown. In addition, mtDNA defects occurred in a substantial percentage of autistic patients presenting secondary mitochondrial dysfunction [56].

Microbiome-mitobiota axis

Recent evidence in metagenomics and clinical research suggest that an intriguing bidirectional inter-talk may exist between microbiota and mitochondria, being able to influence cellular homeostasis and metabolism. This connection is strengthened by the prokaryotic mitochondrial origin [8, 57, 58].

Mitochondria and microbiota share two important features, since they both have a maternal inheritance and a circular genome. In addition, it has been shown that patients with mitochondrial diseases are more susceptible to develop bacterial infections [59].

Recent research underlined the correlation between microbiota quality and diversity and mitochondrial function [57].

Zorov et al. in 2014 [60] coined the term "mitobiota" to highlight the combination of different phenotypic manifestations of mitochondria, which result related to variation in the expression of mtDNA. Similarly to what happens to the microbiota, biochemical and structural differences can be evidenced among mitochondria belonging to different cells, organs and even during different metabolic cellular states. Moreover, it is also possible to find mitochondria with different structures, as previously explained, coexisting in the same cell [60].

Microbiota could interact with the cells promoting the insertion of mtDNA in the nuclear DNA sequences [59]. In addition, the gut microbiota may produce metabolites that modulate mitochondria activity, such as short-chain fatty acids, that can activate AMP kinase and lead to mitochondrial genesis [58]. Furthermore, in worms, mitochondrial dynamics and aging could be influenced through the transfer of the bacterial polysaccharide colanic acid [61].

Another mechanism applied by gut microbiota to influence mitochondrial activity is to modify key transcriptional co-activators synthesis, transcription factors and enzymes involved in mitochondrial biogenesis, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), sirtuin 1 (SIRT1) and adenosine monophosphate-activated protein kinase (AMPK) genes.

Moreover, the gut microbiota and its metabolites, such as short-chain fatty acids, bile acids, pyrroloquinoline quinone and fermentation gases, contribute to host energy production, ROS and inflammation modulation [8, 62]. Mitochondrial genetic variants, thus, have a crucial role in regulating the gut microbiota composition and activity, mostly influencing intestinal barrier function and mucosal immune responses [8].

According to these data, the microbiota targets mitochondria influencing their interaction with the host and the deregulation of these mechanisms could lead to several pathologies [57].

Since it is well known that dietary habits highly affect the composition of the gut microbiota and that microbial communities have a prominent role in the extraction of calories from otherwise indigestible food, studies evaluating gut microbiota and possible dietary modifications could be useful to influence mitochondria-related ROS production, pro-inflammatory signals and metabolic patterns improving gut health and even endurance in athletes [8].

In fact, due to the possibility that bacterial species can trigger insertion of bacterial DNA or mtDNA in the host genome and induce mutation of the somatic cell, the microbiota becomes an interesting target to affect intestinal ROS, inflammation and other metabolic pathways. Up to now, probiotics administration, diet and fecal transplantation are promising strategies to shape the host microbiota [57].

Evidence suggests that the host genomic ancestry may modulate the microbiome. Ma et al., taking into account the high mutation rate showed by mtDNA and mtSNPs in response to several factors (including adaptation to thermal conditions and natural selection) and the possible link of these sets with different human haplogroups, hypothesized that such polymorphisms may be associated with variations in the human microbiome taxonomic abundance, carriage patterns and functional profiling [10]. This mechanism has not been fully clarified yet, but mtDNA variations could influence microbiome through a potential selective and different inflammation response to different levels of ROS activity [10]. In their study, Ma

and colleagues showed significant association within several haplogroups between mtDNA sets and specific microbiota community. Hirose et al. also demonstrated similar findings in mice [63].

It is important to underline that these associations may potentially have also functional and clinical implications. In conclusion, mitochondrial genome can shape the microbiome of the host and metagenomics represents a very interesting technique to explore such interactions [10].

Mitochondria, immunity and inflammation

Mitochondria play multiple and important roles in both innate and adaptive immunity. Firstly, mitochondrial ROS are able to digest phagocytosed pathogens. Secondly, they may activate the most versatile and clinically involved cytosolic molecular complex termed NOD-like receptor protein (NLRP3)-inflammasome, which regulates the production of the inflammatory cytokine IL1-β. Moreover, mtDNA is capable of amplifying the activation of NLRP3 and to activate many innate immune signaling pathways acting in the nucleus. Finally, succinate, fumarate and citrate (representing some intermediates of Krebs' cycle), are regulators of inflammation and result involved in antibacterial host defense [64].

Evidence suggests a reciprocal cross-talk between mitochondrial fission and fusion dynamics and the immune cell metabolism and function. Hence, several studies reported that the ablation of fission-fusion proteins may damage immune cells. Quiescent naive T cells become activated by the presence of antigens becoming T effector. Most of the T effector cells die, while a small number of T memory cells remains in a quiescent state. Despite the fact that T cells are metabolically characterized by high rates of aerobic glycolysis, such condition involves mitochondrial function because of the need of metabolites generated by the tricarboxylic acid cycle to build macromolecules and to change the epigenetic prospect. T effector cells are in an anabolic state and contain primarily fragmented mitochondria. On the contrary, T memory cells but also regulatory T cells and alternatively activated macrophages rely on catabolic metabolism involving pyruvate or fatty acid oxidative phosphorylation and present hyperfused mitochondria. Consequently, mitochondrial metabolic programs seem to dictate the function and the fate of immune cells and probably other cell types [65].

Mitochondria and cancer

Mitochondrial dysfunction seems to be actively involved in tumorigenesis and it has been reported in many types of cancers, even if the exact relationship still remains unclear. In breast, colon and hepatocellular carcinomas, astrocytomas and prostate cancers, a low copy number of mtDNA has been observed, whereas mutations in mtDNA are common in renal adenocarcinoma, thyroid tumors, head and neck cancer [66].

As a consequence, cancer cells show an impaired oxidative phosphorylation process and a less efficient ETC. MtDNA mutations, inducing a higher ROS production, activate several pathways which result critical for the tumor growth [67].

Both mitochondrial biogenesis and mitophagy are essential in cancer initiation, growth, survival and metastasization. The modified mitochondrial enzymes, due to mtDNA mutations, are involved in the production of oncometabolites that could trigger tumorigenesis. In addition, an imbalance between mitochondrial fission and fusion, together with a metabolic reprogramming and an increase in oxidative stress signaling are all processes that may promote tumor growth. Finally, an impaired apoptosis and an alteration in mitochondrial mass may even contribute to the survival of cancer cells [68, 69].

An interesting and innovative therapeutic approach has been proposed and evaluated by Lamb and colleagues, considering that cancer stem cells (CSCs) represent the tumor-initiating cells and result indispensable for tumor growth and metastasization, independently from the tissue giving origin to the tumor itself.

This group, recognizing the need of mitochondrial biogenesis as a weak point in the clonal expansion of CSCs, evaluated the efficacy of five class of FDA-approved antibiotics (i.e. erythromycin, tetracyclines, glycylcyclines, an anti-parasitic drug and chloramphenicol) known to inhibit mitochondrial biogenesis as a well-tolerated side-effect, in the treatment of cancer. A power in eradicating CSCs in 12 different cancer cell lines and across 8 different tumor types has been demonstrated, confirming that mitochondrial biogenesis is absolutely indispensable for the survival and propagation of CSCs. This would be an alternative and promising non-toxic anti-

cancer therapy, although further studies are still needed to clarify its implications [70].

To infinity and beyond

A very interesting and promising mitochondrial property is the recently demonstrated possibility that such organelles can be transferred from a donor cell to a recipient cell, providing to this recipient several proteins, nucleic acids, ions and other small molecules, through a system of tunneling nanotubes (TNTs). These are conical shaped extensions which can also connect a donor mitochondria to a receiver mitochondria [71].

Intercellular communication, among similar or different cell types, is a fundamental mechanism to promote the homeostasis of tissues and organs; it can occur through the sharing of diffusible factors, the secretion of microvesicles or the passage of various molecules through gap junctions. Moreover, in several cell types and even in some bacteria, a long-distance communication has also been described, based on a direct cell-to-cell contact via TNTs, tubular structures mostly composed by cytoskeleton, microtubules or actin and reaching a length of hundreds of microns [72, 73].

This mechanism can involve mitochondrial structures, which can be transferred from a cell to another, potentially improving the energetic metabolism of the targeted cells and modifying their functional properties. As reported in **Fig. 2**, in addition to mitochondria, TNTs also allow the transfer of Ca^{2+} ions, lysosomes, vesicles, bacteria, and microRNAs.

Up to now, the enormous and enigmatic potential of mitochondrial trafficking should be deeply defined, especially considering that it also seems to occur among cancer cells and even connecting these to normal cells or SCs, in particular mesenchymal SCs [72].

This could represent a key mechanism to provide energy to cancerous cells, promoting their survival.

Moreover, many therapeutic applications could take advantage of the transfer of mitochondria, potentially restoring and sustaining cellular metabolism after an ischemic damage. Finally, mitochondrial diseases, neurodegenerative and neuromuscular disorders, inflammatory damage

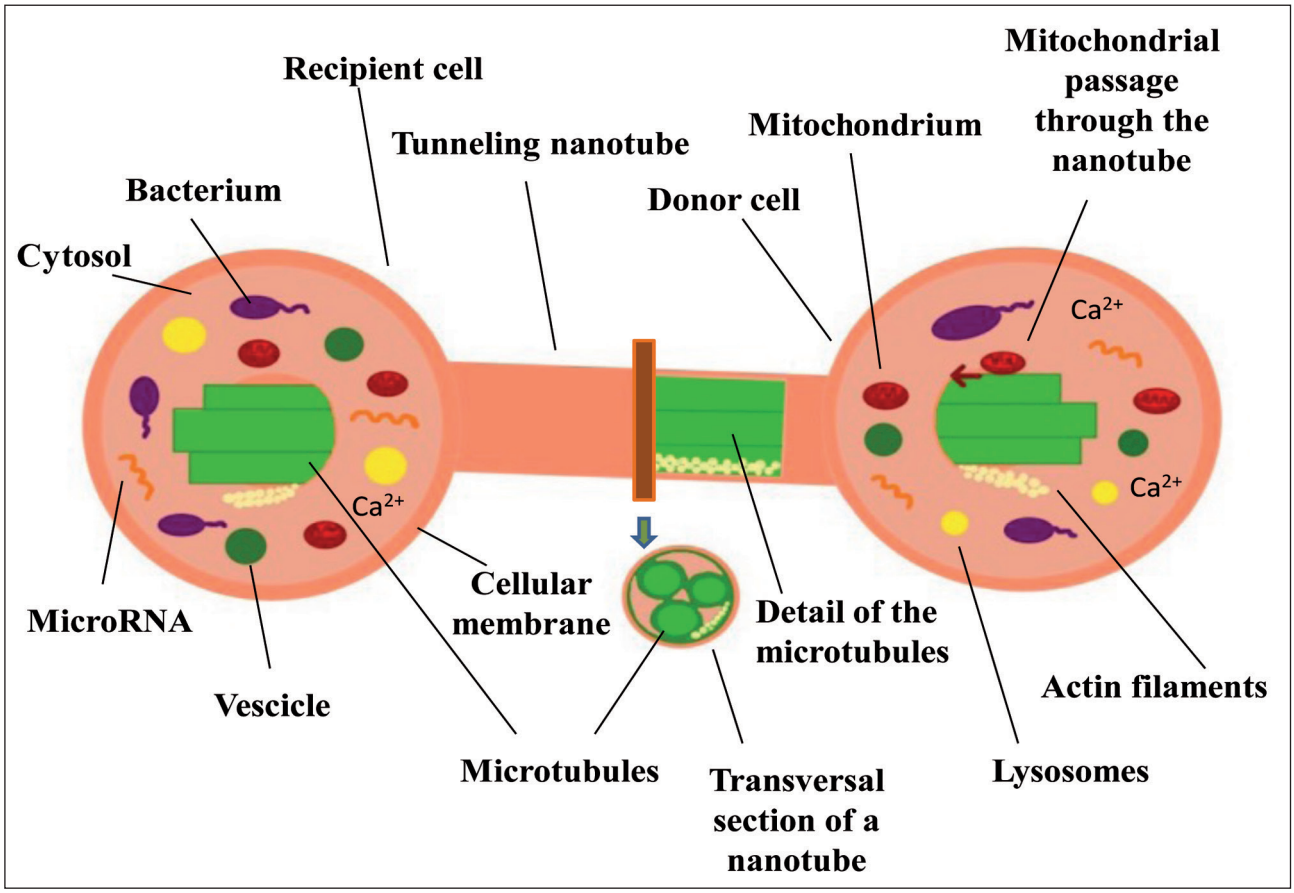

Figure 2. Intercellular trafficking through the tunneling nanotubes (TNTs).

and even aging are only a few of the fields in which future mitochondrial research could induce therapeutic steps forwards.

Mitochondrial involvement in the omics science revolution

Mitochondrial diseases, currently known as a heterogeneous spectrum of disorders characterized by variable phenotypic and biochemical presentation, may be determined by mutations involving almost 350 genes of mitochondrial or nuclear origin; several mechanisms have been related to their occurrence, such as an impaired oxidative phosphorylation, the modification of mitochondrial ultrastructure, the alteration of metabolic processes such as the synthesis of vitamins of cofactors [74].

Due to such variability and the incomplete knowledge of all the mitochondria-related processes and metabolic pathways, many issues about mitochondrial diseases' pathogenesis, manifestations and even reliable therapies are still under debate.

In the last years, the growing interest regarding omics sophisticate technologies helped to understand more in detail mitochondrial pathophysiology, how they may be involved in cellular health and disease, also providing new strategies for a more accurate diagnosis of mitochondrial diseases and new targets for targeted therapies [74].

For example, although oxidative phosphorylation structure and function were described several years ago, omics sciences recently characterized them more in detail; in addition, genome-wide Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) knock-out screens allowed the description of interesting mitochondrial functions and could reveal new therapeutic targets [74, 75].

A transcriptomics approach may suggest useful biomarkers of mitochondrial disease and quantitative proteomics can be used to investigate the deep interactions among mitochondrial proteins and their post-translational modifications, potentially helping in the identification of new genes responsible for mitochondrial disorders.

Moreover, systematic metabolomics studies can be useful to analyze on a global scale the metabolic consequences of mitochondrial dysfunctions, such as the oxidative stress, redox imbalance and energy deficiency, leading to the identification of biomarkers potentially predictive of the clinical outcome and therapeutic targets. Finally, through lipidomics, mitochondrial lipid trafficking and the diseases related to an impaired in membrane homeostasis can be analyzed and characterized.

This really promising integrated approach is still in an early stage and further studies will be necessary to fully understand the complex genotype-phenotype relationships, both regarding nuclear and mtDNA, in physiological conditions and even highlighting the modifications occurring in pathology [74].

Conclusions

Although studies on mitochondria started more than a hundred years ago, during the last decades a growing interest and the efforts of numerous researchers worldwide lead to an improved knowledge on this intriguing topic, which could constitute our closer and older link with prokaryotes and even a connecting bridge towards promising future perspectives.

It is now clear that mitochondria are not simple organelles providing to energetic metabolism but really earned the dignity of dynamic structures in constant communication with the cell nucleus itself and even, at short- or long-distances, with other cells. Thus, they seem to influence metabolism and functions of their targets [76].

Since mitochondrial involvement has been related to several pathologic conditions and degenerative processes, a deeper knowledge of their metabolism and of signaling pathways regulating mitochondrial transfer, even using a combined omics sciences approach, could represent a key passage to develop innovative and beneficial applications [77].

Declaration of interest

The Authors declare that there is no conflict of interest.

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