Common methods in mitochondrial research (Review)

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Abstract. Mitochondrial abnormalities are primarily seen in morphology, structure and function. They can cause damage to organs, including the heart, brain and muscle, by various mechanisms, such as oxidative stress, abnormal energy metabolism, or genetic mutations. Identifying and detecting pathophysiological alterations in mitochondria is the principal means of studying mitochondrial abnormalities. The present study reviewed methods in mitochondrial research and focused on three aspects: Mitochondrial extraction and purification, morphology and structure and function. In addition to classical methods, such as electron microscopy and mitochondrial membrane potential monitoring, newly developed methods, such as mitochondrial ultrastructural determination, mtDNA mutation assays, metabolomics and analyses of regulatory mechanisms, have also been utilized in recent years. These approaches enable the accurate detection of mitochondrial abnormalities and provide guidance for the diagnosis and treatment of related diseases.

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1. Introduction

Mitochondria are semi-autonomous organelles found in most eukaryotic cells with a bilayered structure consisting of an outer membrane, an intermembrane space and an inner membrane. They serve key roles in a variety of cellular processes, including cell metabolism, signal transduction and the regulation of cell death. Mitochondria have numerous biological functions, including the production of ATP for cellular energy, regulation of the dynamic balance of intracellular Ca²⁺, production of reactive oxygen species (ROS), the release of cytochrome c and regulation of intracellular environmental homeostasis. As an important signaling hub in cells, the mitochondrion serves a key role in diseases such as aging and obesity. Mitochondrial biogenesis and mitochondrial homeostasis require the expression of nuclear genes and mitochondria-nuclear signaling pathways to be regulated (1). On the one hand, it depends on the regulatory pathways of nuclear gene transcription and anterograde signaling. Mitochondria, on the other hand, pass intracellular signaling molecules, such as Ca²⁺, mitochondrial DNA (mtDNA), reactive oxygen species (ROS), adenosine triphosphate (ATP), coenzyme Q (CoQ) and nicotinamide adenine dinucleotide (NAD) and then present mitochondrial abnormalities and cellular metabolic change signals to the nucleus (retrograde signaling). This triggers the nucleus to activate important signaling pathways by mobilizing a series of nuclear transcription factors (2-5), mitochondrial transcription and mitochondrial biosynthesis. Among them, the activation of signaling pathways is closely related to inflammation and tumorigenesis (6). During cellular stress and virus infection, mtDNA and ROS are released from abnormal mitochondria and retrogradely presented to the nucleus as danger signals. The nucleus can promote the expression of PTEN-induced kinase 1 (PINK1) and then upregulate mitophagy to clear abnormal mitochondria and maintain a stable intracellular environment. When too many abnormal mitochondria cannot be completely removed, mtDNA can activate Toll-like receptor 9 (TLR9) and its downstream inflammatory pathways and lead to inflammation. Excessive ROS can cause DNA damage by oxidizing nucleic acid bases, which is closely related to tumorigenesis. Abnormalities in mitochondrial structure and function can lead to a variety of intracellular signaling cascades, oxidative stress and the initiation of programmed cell death, thereby contributing to the development and progression of nearly all diseases. Therefore, the detection of mitochondrial abnormalities is crucial and various mitochondrial assays (Fig. 1) developed...
in the last century have contributed substantially to the differential diagnosis of mitochondrial diseases. The present study reviewed common experimental methods (Table I) in mitochondrial research. In particular, it discussed a wide range of imaging and detection techniques for (i) extraction and purification, (ii) analyses of morphology and structure and (iii) analyses of function, with a focus on the clinical implications for disease detection and treatment.

2. Extraction and purification of mitochondria

A suitable method is needed to extract purified mitochondria from various tissues and cells (7). The basic extraction method mainly relies on differential centrifugation, while purification mainly depends on density gradient centrifugation. The specificity of tissue cells determines the details of the method (8-10).

Extraction of mitochondria. When extracting mitochondria, because the homogenization process can heat the sample locally, resulting in protein denaturation and aggregation, the equipment must be pre-cooled and the temperature kept low throughout the process (11). Tissue or cell homogenization is followed by continuous differential centrifugation. Unlysed cells, cell debris and nuclei are first removed by low-speed centrifugation (600 x g or 1,000 x g) (12-15). As mitochondria can remain in flaky precipitates generated by low-speed centrifugation, resuspending the pellet and centrifuging it again at low speed increases mitochondrial yield. The supernatant obtained by two low-speed centrifugation steps is collected for high-speed centrifugation, resulting in a coarse-lifted mitochondrial supernatant. The pellets obtained by two low-speed centrifugations are mixed with anti-mitochondrial outer membrane protein (22) (TOM22) magnetic beads to separate Arabidopsis mitochondria. After the tissues are lysed, they are mixed with anti-mitochondrial outer membrane protein 22 (TOM22) magnetic beads and the mixed samples placed in the sorting column. Only mitochondria remain on the sorting column after washing, followed by elution, isolating the complete mitochondria in less than 30 min with a success rate, purity and integrity significantly higher than the density gradient centrifugation (43-47). Therefore, the magnetic bead method can be used to extract mitochondria in tissues with fewer mitochondria. As such, this approach will probably become increasingly common in mitochondrial extraction and purification (48-50). In conclusion, among the current mitochondrial extraction and purification methods, the magnetic bead method has the best effect on eliminating impurities such as microsomes and peroxisomes and the mitochondrial purity obtained by the differential centrifugation method is the lowest and the effect on eliminating these impurities is the worst.

3. Determination of mitochondrial morphology and structure

Mitochondria are organelles with a complex bi-membrane structure that regulate the entry and output of proteins, lipids, solutes and metabolite products and protect the cytoplasm from harmful mitochondrial products (51-53). Mitochondria can engulf abnormal mitochondria and remove excess harmful mitochondrial products to protect the body. This process is called mitophagy (54-56). Most mitochondria are spherical, rod-shaped, or tubular; however, mitochondrial morphology varies widely among tissues and cells depending on the energy
requirements of cells and the location of mitochondria within the cell (53,57). For example, mitochondria are spherical at synaptic terminals, whereas they appear as highly elongated rods in axons. In senescent and functionally impaired cells, mitochondrial morphology is significantly different from that in normal cells and they can be irregularly shaped (53,58,59). Therefore, morphological changes can be used in the initial assessment of mitochondrial function.

After over 50 years since its development, electron microscopy (EM) has become the central tool for observing organelles in eukaryotic cells and is the gold standard for observing mitochondrial structure (60). It can reveal mitochondrial swelling, rupture and other abnormalities of damaged mitochondria. However, it cannot clearly distinguish mitochondria from other membranous structures and is occasionally confusing. In the 1980s, atomic force microscopy, as an emerging observation method, could study the surface structure and properties of substances by detecting the extremely weak interatomic interaction between the surface of the sample to be tested and a miniature force-sensitive element. Due to the characteristics of resolution and real-time imaging, changes such as the formation of mitochondrial swelling can also be observed under liquid conditions but are significantly affected by the probe; thus, the application range is small (61-64).

The recently developed AiryScan microscope (Zeiss AG) can acquire images at high speed with high sensitivity to effectively observe the kinetic processes of mitochondrial fission, fusion and autophagy (65-67). In addition, both wide-field fluorescence microscopy and high-resolution confocal laser scanning microscopy can be used for imaging analyses of morphological changes in mitochondria with higher specificity than that of EM, but the dynamic changes of the mitochondria cannot be observed (68-76).

In most cases, microscopy can be used to observe and analyze two-dimensional mitochondrial morphologies and quantities. However, although this method is suitable for analyzing adherent cells with flat morphology, it is not suitable for thicker cells (77-83). Three-dimensional confocal microscopy can be used to observe mitochondrial morphology by observing specifically labeled mitochondrial proteins at the 3D level (84-87). In addition, after labeling mitochondria with specific dyes, mitochondrial morphology can be visualized using a combination of immunofluorescent staining and computer images (58,88,89).

4. Determination of mitochondrial function

Determination of mitochondrial membrane potential. Mitochondrial membrane potential (MMP) refers to the negative potential difference between the two sides of the inner mitochondrial membrane. It is a sensitive indicator for evaluating mitochondrial function (90-93). It is closely associated with cellular homeostasis and is most commonly used to determine the metabolic state of mitochondria (93-98).

Fluorescent dye probes used for flow cytometry are now commonly used in MMP assays. For example, rhodamine 123, a specific stain developed in the 1980s, is widely used in flow cytometry and MMP assays. In normal cells, rhodamine 123 can selectively enter the mitochondrial matrix depending on MMP and can emit bright yellow-green fluorescence; when cells undergo apoptosis or necrosis, the mitochondrial membrane permeability transition pore (mPTP) is abnormally opened and
Table I. Summary of mitochondrial research methods.

| Area of research                          | Methods                          | Scope of application | Advantages                              | Drawbacks                              |
|-------------------------------------------|----------------------------------|----------------------|------------------------------------------|-----------------------------------------|
| Extraction and purification of mitochondria | Differential centrifugation extraction | Tissues and cells | Detect mitochondrial morphological structure | Low mitochondrial purity               |
|                                            | Density gradient centrifugation | Sucrose              | Low cost and wide application            | Poor mitochondrial morphological integrity |
|                                            | purification (different media)   | Peroll               | Isolate platelet mitochondria            | Higher cost compared to sucrose         |
|                                            |                                  | Nycodenz             | Compared to sucrose, higher density and lower viscosity without affecting osmotic pressure |                                         |
|                                            | Optiprep                         |                      | Automatic gradients can be formed in a short time |                                         |
| Microbial morphology                      | Magnetic bead method             | Tissues and cells    | Mitochondrial purity and integrity superior to other methods | Not yet widely used                     |
| Mitochondrial morphology                  | Electron microscope              |                      | Gold standard                            | Cannot clearly distinguish mitochondria from other membranous structures |
|                                            | AiryScan microscope              |                      | Observable mitochondrial dynamics       | Not yet widely used                     |
|                                            | Atomic force microscope          |                      | Observation of mitochondrial swelling and mitochondrial dynamics |                                         |
| Mitochondrial function                    | 3D Confocal Microscopy           | Thicker cells        | For thicker cells                        | High cytotoxicity                       |
|                                            | Mitochondrial membrane potential | Rhodamine 123, JC-1  | Intuitively reflect changes in MMP       |                                         |
|                                            |                                  | TMRR, TMRE           | Low cytotoxicity for quantitative analysis of MMPs | Not yet widely used                     |
|                                            |                                  | TRR-CY               | Extremely sensitive to detect minute changes in MMP |                                         |
|                                            |                                  | FRET                 | Monitoring dynamic changes of MMP       |                                         |
|                                            | Mitochondrial oxygen consumption | Oxygen electrode     | Low cost, detection of respiratory control rate | Poor specificity                       |
|                                            |                                  | polarography         | Comprehensive analysis of mitochondria by measuring oxygen consumption rate | Can be affected by chemicals such as phenol red |
|                                            |                                  | Hippocampus analyzer |                                                      |                                         |
|                                            | Mitochondrial Ca²⁺ Detection     | Electrochemical analysis | Suitable for experiments with low sensitivity, unable to distinguish mitochondrial Ca²⁺ from total Ca²⁺ | Poor specificity                       |
| Area of research                        | Methods                                      | Scope of application | Advantages                                                                 | Drawbacks                                                                 |
|----------------------------------------|----------------------------------------------|----------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Calcium‑ Rhodamine 123                 | Tissues and cells                            | High specificity, suitable for the detection of mitochondrial Ca\(^{2+}\) in various living cells | Inability to distinguish between different cellular sources of Ca\(^{2+}\) |                                                                             |
| Fluo-3                                 |                                              |                      | Distinguish mitochondrial Ca\(^{2+}\) from Ca\(^{2+}\) in other intracellular organelles |                                                                             |
| Mitochondrial membrane permeability transition pore | Fully automatic patch clamp | Tissues and cells | Can be used for detection of suspension cells | Small scope of application |
| Fluo-3                                 | Suspension cells                            |                      | Strong specificity, can reflect the opening degree of mPTP in real time    | Easy to be quenched, timely observation is required |
| Mitochondrial ATP                      | High pressure liquid chromatography          | Tissues and cells    | Can detect differences in cellular energy substances in different states | Requires a larger sample size |
| Enzymatic analysis                     |                                              |                      | It is greatly affected by the absorbance of the tested sample              | Susceptible to redox reactions |
| Fluorescence analysis                  |                                              |                      | The amount of luminescence is proportional to ATP                          | Easy to quench |
| Mito-Rh                                |                                              |                      | Can specifically recognize ATP in mitochondria                            |                                                                           |
| Mitochondrial ATP                      | Spectrophotometry                            | Tissues and cells    | Wide range of applications, but less accurate                              | Vulnerable to external biochemical interference |
| Mitochondrial respiratory chain complex| NIR spectroscopy non-invasive measurements  | Tissues and cells    | Less affected by the outside world, high accuracy                          | Requires a very large sample size |
| ROS                                    | Chemical reaction selective electrode method | Tissues and cells    | High sensitivity, cheap and easy to operate, but poor specificity and unstable results | Poor specificity and unstable results |
| ROS                                    | Spectrophotometry                            | Tissues and cells    | High sensitivity and specificity, but cannot perform localization analysis of oxygen free radicals | Unable to perform localization analysis of oxygen free radicals |
| ROS                                    | Reagent test kit                             | Tissues and cells    | Strong specificity, easy operation, low background, large detection range, easy quenching | Easy to quench |
MMP is unbalanced. Rhodamine 123 is released from mitochondria, resulting in a significant decrease in the yellow-green fluorescence intensity in mitochondria, which reflects the changes in MMP (50,99-102). 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) has higher sensitivity than that of rhodamine 123. At low MMP levels, JC-1 exists as a monomer and produces green fluorescence; at high MMP levels, JC-1 aggregates in the mitochondrial matrix and forms polymeric JC-1. This can be used for qualitative and quantitative analyses of MMP by fluorescence microscopy or flow cytometry (50,96,101,103-108). Tetramethyl rhodamine methyl ester (TMRM) and tetramethyl rhodamine ethyl ester (TMRE), like JC-1, are specific dyes that have recently become common tools for measuring MMP (109-112). TMRM can be excited at 488 nm, showing red-orange fluorescence and its fluorescence intensity has a linear relationship with MMP. Compared to rhodamine 123 and JC-1, these two dyes are very soluble, have short loading times (15-20 min) and have extremely low cytotoxicity, requiring micromolar inhibition of mitochondrial function. With staining concentrations in the range of 0.5-30 nM (the concentration of JC-1 needs to be >0.1 µM), the accumulation in mitochondria is limited to the change of membrane potential and the sensitivity is extremely high; this is markedly suitable for quantitative analysis of mitochondrial membrane potential and quantitative flow cytometry (113-118). However, in quantitative flow cytometry studies, the data must be corrected for the signal of MitoTracker Green FM, a dye that is not dependent on mitochondrial membrane potential. It is worth noting that the above fluorescent probes for measuring MMP are applicable to most tissues and cells, including plant cells and bacteria.

Fluorescence resonance energy transfer (FRET) is a non-radiative energy transition that transfers energy from the excited state of the donor to the excited state of the acceptor through intermolecular electric dipole interactions (119,120). This process does not involve photons, so it is non-radiative. This analytical method has the advantages of rapidity, sensitivity and simplicity. Fluorescence resonance energy transfer molecular pairs (FRET Pairs) have been designed and synthesized to monitor MMPs (121). The FRET donor molecule (FixD) is constructed by attaching a benzyl chloride group to a fluorophore with green fluorescence emission. FixD can be attached to and fixed in mitochondria by sulfhydril groups of mitochondrial proteins. The FRET acceptor (LA) is a mitochondrial membrane potential-dependent probe with green absorption and deep red fluorescence emission. When MMP is at a normal level, both FixD and LA target mitochondria. When FixD has an excitation wavelength of 405 nm, FRET occurs between FixD and LA, allowing green fluorescence to be detected but not deep red LA fluorescence emissions. When MMP is gradually reduced, LA will gradually fall off from mitochondria. While FixD is still fixed in mitochondria, the distance between the molecules gradually blocks the occurrence of FRET between FixD and LA molecules, allowing deep red fluorescence emission to be detected gradually. The decrease and the gradual increase of green fluorescence emission can be used to monitor the dynamic changes of MMPs (122), providing new ideas for the development of novel MMP fluorescent probes and real-time in situ studies of MMPs in living organisms, tissues and cells (123,124).
MMP varies greatly among sites on the mitochondrial membrane; therefore, accurate measurement of MMP requires further study (125). In recent years, low concentrations of a hemicyanine derivative (TPP-cY) have been used to monitor trace changes in MMP at the subcellular level during apoptosis with very high sensitivity (125). This approach is a potentially useful tool for evaluating cell health.

**Determination of mitochondrial oxygen consumption.** Among organelles, mitochondria consume the most oxygen in cells and this oxygen consumption often reflects mitochondrial function (126-128). In the heart, mitochondrial oxygen consumption can be measured to determine cardiac mitochondrial function, providing an indicator of cardiac function (129-131). In children, mitochondrial dysfunction causes mitochondrial heart disease with hypertrophic myocardial infarction as the primary symptom; however, the exact mechanism and etiology remain to be investigated (129,132).

Oxygen electrode polarography is a common method for determining mitochondrial oxygen consumption and refers to the incubation of mitochondria in an oxygen-consuming medium in a magnetically stirred incubator at 30°C. Briefly, rotenone is used to inhibit complex I in the electron transport chain, followed by the addition of succinate to measure mitochondrial state IV respiration (non-phosphorylating respiration). State III respiration is measured by incubating mitochondria in the presence of succinate and adenosine diphosphate. The respiratory control ratio (RcR) is the ratio of the state III respiration rate to state IV respiration rate, with a normal value of 3-10 (133-135). A low RCR indicates impaired mitochondrial ATP synthesis and mitochondrial dysfunction and a high RCR indicates vigorous cellular activity and accelerated metabolism (127,136,137).

In addition, the hippocampal analyzer can measure the changes in oxygen and pH levels through sensors and then automatically calculate the rate and detect the cellular oxygen consumption rate (OCR) and extracellular phosphorylation rate (ECAR) in real time to characterize the metabolic status of cells. Where OCR is caused by mitochondrial electron transfer, ECAR is derived from lactic acid fermentation (glycolytic acidification) and carbon dioxide produced by mitochondria (mitochondrial acidification) (138-140).

OCR is used to study mitochondrial oxidative phosphorylation function, with pMoles/min as the readout type (141). Generally, basal respiration in a normal state is measured first and then oligomycin is added to inhibit ATP synthase. This is a significant decrease in OCR, leaving only proton leakage (142). The oxygen consumption rate is caused by proton leakage and the reduced section is the oxygen consumption rate (ATP production) of oxidative phosphorylation. With the addition of the uncoupling agent FCCP, electron transport loses the constraints of the proton gradient and proceeds at a maximum rate (143). Therefore, the OCR increases sharply, reaching the maximum oxygen consumption (maximal respiration); the difference between this value and the basal respiration is termed the spare respiratory capacity. Finally, adding an electron transport inhibitor, such as antimycin A, completely inhibits electron transport and reduces the oxygen consumption rate to a minimum (144).

ECAR is often used to study metabolic conditions such as glycolysis, with mpH/min as the readout type (139,140,142). The basal value before adding glucose is non-catalytic acid production, such as mitochondrial acidification caused by carbon dioxide produced by mitochondrial respiration. Glucose is then added and the elevated value represents glycolysis. After the addition of oligomycin, the production of acid increases because oxidative phosphorylation is inhibited and the cells are forced to use lactic acid fermentation for energy. The value at this time is called glycolytic capacity and the difference from glycolysis is termed glycolytic reserve (140,142,143). Last added is 2-deoxyglucose, a competitive hexokinase inhibitor that can block glycolysis, so the curve should return to the basic value following its addition (144-146).

However, the direct measurement of glycolysis by ECAR is somewhat biased since the addition of glucose enhances glycolysis and oxidative phosphorylation. This will lead to increased mitochondrial acidification, causing the calculated amount of glycolysis to be high (147-149).

It is worth noting that during the measurement process of the hippocampal analyzer, the interference of phenol red should be avoided because it causes errors in the measurement results (141,150,151), but the specific reasons remain to be elucidated. In conclusion, the hippocampal analyzer can monitor OCR and ECAR to obtain multiple other parameters in a single analysis, including basal respiration, ATP-related respiration, maximal respiration, spare respiratory capacity and non-mitochondrial oxygen consumption, all of which can provide information on the mechanism of mitochondrial dysfunction (152,153).

**Determination of mitochondrial Ca²⁺.** Intracellular Ca²⁺ is primarily stored in organelles, such as the mitochondria and endoplasmic reticulum, and serves an important role in biological processes such as signal transduction, blood coagulation, transmembrane ion transport and cell division (154-156). Mitochondrial Ca²⁺ is a central regulator of oxidative phosphorylation and serves a key role in the control of ATP synthesis (157). A Ca²⁺ imbalance can cause abnormal mitochondrial function and even cell damage and death, leading to pathological changes and affecting organismal health (158,159). The accumulation of mitochondrial Ca²⁺ promotes ATP synthesis in mitochondria; conversely, decreased mitochondrial Ca²⁺ leads to a decrease in mitochondrial ATP. Impaired ATP synthesis further leads to a Ca²⁺ imbalance (157,159), which in turn leads to endocrine dysfunction and numerous diseases, such as mitochondrial diabetes mellitus (160-165).

Methods for the determination of mitochondrial Ca²⁺ include precipitation, electrochemical analysis, EDTA chelation titration, flame photometry and atomic absorption spectroscopy, among which electrochemical analysis is the most convenient (87,88,156,166-168). First developed in the 19th century, the electrochemical analysis applies electrochemical principles and techniques to a class of analytical methods that take advantage of the electrochemical properties of chemical cells in solution and their changes. It can be used for the detection of both organic and inorganic substances and is simple in operation. It can be both qualitative and quantitative, but is susceptible to interference by sodium,
potassium, phosphate and sulfate. It is suitable for real-time detection and experiments with low optical sensitivity requirements (132,169). In addition, FRET can also detect Ca\(^{2+}\); cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) are the most widely used FRET pairs in protein-protein interaction studies. The emission spectrum of CFP is similar to that of YFP. The absorption spectra of CFP overlap and when the distance between the two proteins is in the range of 5-10 nm, the fluorescence emitted by CFP can be absorbed by YFP and YFP is excited to emit yellow fluorescence. Whether the two proteins interact was determined by measuring the loss of CFP fluorescence intensity. The closer the two proteins are, the more fluorescence emitted by CFP is received by YFP and the less fluorescence is received by the detector. CFP and YFP are fused to calmodulin and calmodulin-binding peptide, respectively and expressed in the same cell (170-175). When the intracellular Ca\(^{2+}\) concentration is high, the combination of calmodulin and the calmodulin-binding peptide can induce FRET and the receptor protein YFP emits yellow fluorescence, so the cells appear yellow. When the intracellular Ca\(^{2+}\) concentration is low, FRET hardly occurs, so CFP is excited and emits green fluorescence during detection and the cells appear green (170,171,175). FRET can detect intracellular Ca\(^{2+}\), but cannot specifically detect mitochondrial Ca\(^{2+}\). A number of fluorescent probes have recently been used for the measurement of Ca\(^{2+}\) levels, including Quin-2AM, fluo-3AM, indo-1AM, Rhod-2, Fluo-4, Mag-fluo-4 and calcium-rhodamine 123 (rhodamine 123) (158,176-178). Quin-2AM, fluo-3AM, indo-1AM, Fluo-4 are cytosolic Ca\(^{2+}\) indicators. Mag-fluo-4 is an ER Ca\(^{2+}\) indicator. The rhodamine 123 complex assay is suitable for the determination of mitochondrial Ca\(^{2+}\) concentrations in various living cells owing to its simple operation and stable performance. It can be quantified by fluorescence spectrophotometry to detect aggregation in mitochondria and thereby to measure the Ca\(^{2+}\) content (179-184). At present, Fluo-3 is a widely used single-wavelength fluorescent indicator with an excitation wavelength in the visible light range (185,186). The maximum absorption peak and maximum emission wavelength are located at 506 and 526 nm, respectively. The fluorescence intensity of Fluo-3 combined with Ca\(^{2+}\) is ~40 times higher than that of free cells, thus avoiding the fluorescence interference of the cells themselves (185,187). As a long-wavelength indicator, Fluo-3 can be used in confocal laser imaging studies that can analyze the distribution of Ca\(^{2+}\) in individual intact living cells and distinguish mitochondrial Ca\(^{2+}\) from Ca\(^{2+}\) in other organelles within the cell; this method is suitable for mitochondrial Ca\(^{2+}\) in various living cells and is easy to operate, stable in performance and highly specific (155,187). However, the current mitochondrial Ca\(^{2+}\) fluorescent probes cannot distinguish mitochondrial Ca\(^{2+}\) from different cells.

Detection of mitochondrial permeability transition pores. mPTP is a class of protein complexes between the inner and outer mitochondrial membranes that permit the passage of substances with a molecular weight of <1.5 kDa and serve as the structural basis for transitions in mitochondrial permeability (188-191). Additionally, mPTP is very sensitive to changes in intracellular and extracellular ion concentrations and serves an important role in signal transduction systems.

It is currently hypothesized that the abnormal opening of mPTP is closely associated with abnormal changes in Ca\(^{2+}\) concentrations, oxidative stress and mitochondrial DNA (mtDNA) mutations (154,188,189,192,193). By contrast, MMP and mitochondrial Ca\(^{2+}\) concentrations are the principal drivers of mPTP opening, resulting in the release of cytochrome c and other substances associated with cell death into the cytosol (191,192,194-197). This leads to mitochondrial swelling and reduced mitochondrial respiratory chain activity, which can cause various diseases, such as neurodegenerative diseases and cancers (190,198-200). Furthermore, studies have shown that PINK1 can inhibit mPTP opening by downregulating intracellular ROS levels, suggesting that mitochondrial autophagy serves a regulatory role in mPTP opening (191-193). Various methods have been developed for detecting mPTP, such as the patch-clamp, spectrophotometric and active substance labeling methods. The patch-clamp method is the earliest, originating in 1976 and can reflect ion channel activity by recording ion channel currents to evaluate mitochondrial function (188,189,201). As the magnification of AFM is as high as 1 billion times, the opening of mPTP can be directly observed, which can serve a guiding role in the abnormal opening of mPTP (202-205). Fully automated patch-clamp techniques have recently emerged; these are simple in operation and have greatly improved efficiency but are only applicable to the detection of cells in suspension. Compared to the active substance labeling and patch-clamp methods, spectrophotometry is simpler and more commonly used.

The calcein-cobalt fluorescent probe technique is an emerging technique for the detection of mPTP and is simple in operation and highly sensitive (Fig. 2). Calcein-AM (190,194,198,206,207), in which the acetyl methoxy methyl ester (AM) group enhances the hydrophobicity of the stain for easy penetration of the living cell membrane, is used to fluorescently label living cells. Next, calcein-AM is cleaved by intracellular esterases to yield highly fluorescent and polar calcein (208-210). When cells are incubated with calcein and Co\(^{2+}\), both enter the cytoplasm; however, calcein is further captured by mitochondria (211,212). Calcein that accumulates in the mitochondria exhibits fluorescent staining, whereas calcein remaining in the cytoplasm or released from the mitochondria into the cytoplasm is rapidly quenched by Co\(^{2+}\) (213-219). Under normal physiological conditions, mPTP opens transiently and calcein that enters the cytoplasm from the mitochondria is rapidly captured by mitochondria (211,212). Calcein that accumulates in the mitochondria exhibits fluorescent staining, whereas calcein remaining in the cytoplasm or released from the mitochondria into the cytoplasm is rapidly quenched by Co\(^{2+}\) (213-219). Under normal physiological conditions, mPTP opens transiently and calcein that enters the cytoplasm from the mitochondria is rapidly quenched. In pathological states, such as calcium overload and oxidative stress, mPTP can appear to be continuously open and Co\(^{2+}\) in the cytoplasm can enter the mitochondria to quench the calcein fluorescence, resulting in a gradual decrease in fluorescence intensity in the mitochondria, thus indicating the degree of mPTP opening (195,196,220-222).

Determination of mitochondrial ATP. ATP is often considered the primary energy currency of cells and is primarily derived from the mitochondria (137,223-228). It serves major roles in material transport, energy conversion and information transfer. Mitochondria are sensitive to external environmental stimuli, such as hypoxia, oxidative stress, toxic substances and high glucose. Once mitochondria are damaged, ATP production decreases and free radical production increases, which
affects a number of cellular processes and contributes to the
development of a number of diseases, such as Parkinson's
disease, cancer, cardiovascular disease and endocrine dysfunc-
tion (224-227). Therefore, ATP levels are a key indicator of
the status of cellular energy metabolism and mitochondrial
function.

Analyzing ATP levels requires freshly extracted mito-
chondria, as mitochondria must remain intact and in a coupled
state (229). Several techniques are available to measure
mitochondrial ATP levels, including chromatography, electro-
phoresis, high-performance liquid chromatography (HPLC)
and enzymatic analysis (225,227,229-232). Chromatography
and electrophoresis are chemical methods that were developed
in the 18 and 19th centuries and have gradually improved.
Classic liquid chromatography uses a large-diameter glass tube
column and a difference in liquid levels at room temperature
and atmospheric pressure to force the mobile phase (231,232).
However, this technique has low column efficiency and is very
time-consuming (often requiring several hours). HPLC was
developed based on classic liquid chromatography following the
introduction of gas chromatography theory in the late 1960s.
The differences between HPLC and classic liquid chromatog‑
raphy include a faster analysis speed, smaller and more uniform
particles as packing material and high column efficiency of
the small particles. However, this causes high resistance and
requires high pressure to force the mobile phase; therefore,
this technique is also known as high-speed liquid chromatog‑
raphy (233-235). HPLC can be used to determine differences in
cellular energy substances in different states, is easy to operate
and has high sensitivity (233-236). The enzymatic method is
based on spectrophotometry, where ADP production is assessed
by measuring the absorbance of NAD+ in phosphoenolpyru‑
vate (237-240). Fluorescence analysis techniques have been
improved in recent years and are commonly used to determine
mitochondrial ATP synthesis activity (241-244). For example,
in the luciferin-luciferase luminescence method, luciferin is
rapidly oxidized under the action of luciferase, producing
green fluorescence and the amount of luminescence is linearly
related with the level of ATP (245,246). This is a fast and
accurate method; however, fluorescein is an amphiphilic
molecule whose carboxyl group is charged at physiological pH
and thus does not easily cross the cell membrane (244-246). A
novel synthetic fluorescent probe called Mito-Rh can specifi-
cally identify ATP in mitochondria with high sensitivity and a
detection range of 0.1-10 mM. In another method, the level of
ATP can be determined directly by measuring the amount of
inorganic phosphate based on the principle that ATP gives rise
to ADP and inorganic phosphate (225). In addition, FRET can
also be used to detect the level of ATP synthesis after labeling
the ATP synthase subunit. When CFP and YFP are labeled
on ATP synthase subunits, when the ATP synthase activity is
enhanced, the interaction between the subunits is enhanced, the
shortened distance between the subunits brings CFP and YFP
closer to each other and FRET occurs and CFP excites YFP
to emit yellow fluorescence. The lower the green fluorescence
intensity received by the detector, the higher the ATP synthase
activity and the higher the ATP level. When ATP synthase
activity is low, the interaction between subunits is weakened,
FRET hardly occurs and CFP is excited at this time and the cell
emits green light.

In addition, a multi-color ATP indicator has appeared in
recent years. Different from the previous indicators that can
only specifically detect intracellular ATP, the multi-color ATP
indicator is based on a single fluorescent protein indicator
with red, green and blue colors (247-249). Alternatively, it can
simultaneously detect ATP in different organelles in the same
cell and simultaneously detect ATP dynamics in the mito‑
chondria of mammalian, plant and even worm cells and will
have an assured role in promoting energy metabolism research
in the future (225,226).
Detection of mitochondrial respiratory chain complexes. The mitochondrial respiratory chain, with functions in energy production, the regulation of cell death and calcium metabolism (183,250-253), is located on the inner mitochondrial membrane and consists of five complexes. Mitochondrial respiratory chain complex I (NADH oxidase) and mitochondrial respiratory chain complex II (succinate dehydrogenase) are the major elements for electrons entering the mitochondrial electron transport chain (ETC). Complex I oxidizes NADH and transfers electrons to coenzyme Q (254-257). Complex II transfers electrons from succinate to coenzyme Q, a process that does not involve proton transport (258-260). Mitochondrial respiratory chain complex III (cytochrome c reductase) is an essential protein for mitochondrial oxidative phosphorylation, the gatekeeper of the mitochondrial respiratory chain and a major source of third reactive oxygen species. Complex III transfers electrons from coenzyme Q to cytochrome c while using the released energy to pump protons into the intermembrane space. The mitochondrial respiratory chain complex IV (cytochrome c oxidase) is the terminal electron acceptor of the mitochondrial electron transport chain. Complex IV transfers electrons from cytochrome c to oxygen, half the number of protons is synthesized into water and the other half is pumped into the intermembrane space. Mitochondrial respiratory chain complex V and the above four complexes constitute the oxidative phosphorylation to generate ATP, which is called ATP synthase, also known as F1F0-ATPase (254,260-265). The energy released by complex V through the electron transport chain during respiration or photosynthesis is first converted into a transmembrane proton (H+) gradient and the proton then flows along the proton gradient and passes through ATP synthase to enable ADP+Pi to synthesize ATP (266-269). It is also hypothesized that abnormalities in mitochondrial complexes are closely associated with mitochondrial encephalopathy, mitochondrial liver disease and mitochondrial nephropathy (265). It should be noted that the mitochondrial respiratory chain complex is closely related to the occurrence of tumors (251,270-272). Therefore, mitochondrial complex inhibitors may be used as a new treatment for tumors (252,253,260,273). Therefore, the accurate detection of mitochondrial complexes is essential and spectrophotometric assays remain the first-line technique for detecting the activity of mitochondrial respiratory chain complexes I-V (266,274,275).

Samples are generally selected from purified mitochondria and 4-40 µg of mitochondrial protein is required per respiratory chain complex assay (257,269,276-279). To compare the activity of mitochondrial respiratory chain complexes in different cells or tissues, the activity of citrate synthase in the Krebs cycle is measured simultaneously as a control and the reaction system is carried out at 30°C in a volume of 200 µl or 1 ml. The activity of complexes I and V is directly proportional to, and can be determined by measuring, the oxidation rate of NADH, which is measured as the decrease in absorbance at 340 nm (280). In the oxidation of succinate catalyzed by complex II, 2,6-dichlorophenolindophenol (DCPIP) is used as a dye and absorbance at 600 nm decreases as DCPIP decreases (259,281,282), which is used to measure the activity of complex II (283-287). The activity of complexes III and IV can be determined by measuring cytochrome activity (absorbance at 550 nm) (268,288-294). However, the spectrophotometric method is susceptible to external biochemical interference that can lead to changes in enzyme kinetics (chemicals in the liquid or gas phase react with the sample resulting in a change in the absorbance of the sample), which can have serious effects on the sensitivity and accuracy of the assay (255,258,280,295-298). In addition, western blotting can directly reflect the expression level of respiratory chain complexes I-V in the band by using the specific antibody reaction of the complex, which has been widely used in experiments related to mitochondrial research (274,296,297). However, the protein expression level and protein activity are occasionally not correlated and spectrophotometry is still the preferred method for detecting mitochondrial respiratory chain complexes. In recent years, great progress has been made in the non-invasive measurement of mitochondrial complexes using near-infrared spectroscopy. This method is similar to spectrophotometry in principle but is less affected by the external environment (265-268). The fundamental reason why near-infrared light can achieve non-invasive optical measurement is that in the near-infrared light region of 600-900 nm, biological tissue is relatively transparent because the absorption of water and hemoglobin in this wavelength region is very small. As an ‘optical window’, some studies have used it to detect the activity of complex IV to judge the severity of depression. Myoglobin is essential for oxygen metabolism in muscle tissue, including a group of blood cells similar to hemoglobin. The most important of which is complex IV, which has been used to detect the activity of complex IV to judge the severity of depression (299,300). However, due to the large amount of samples required for near-infrared spectroscopy and different instrument models, it has severe limitations and has not been widely used (183,250-253).

Mitochondrial respiratory chain function can also be determined by RCR, which reflects both mitochondrial integrity and mitochondrial oxidative respiratory chain function (256,265,267,301).

Measurement of ROS. As the central organelle for cellular oxidative phosphorylation, mitochondria are the principal site of ROS production (3,302-305). Under physiological conditions, the intracellular antioxidant defense system is in equilibrium with oxygen radicals. The levels of intracellular ROS, including superoxide radicals, hydrogen peroxide and its downstream products (peroxides and hydroxyl radicals), are maintained at low physiological ranges. Under pathological conditions, the balance between the intracellular antioxidant system and oxygen radicals is disrupted. When intracellular ROS levels are too high, mitochondrial structure and function are impaired and cytochrome c is released through mPTP, resulting in damage to mitochondrial enzymes, lipids and nucleic acids as well as oxidative stress (303,306-310). ROS can also attack mitochondrial DNA (mtDNA) to produce oxidative damage, resulting in reduced mitochondrial ATP synthesis and MMP damage. Therefore, the functional status of mitochondria can be determined by measuring ROS levels (311-313).

Common methods for detecting ROS include the chemical reaction method, selective electrode method, spectrophotometry and direct detection by kits. ROS shows high reactivity and...
can react with different compounds to produce various products, which can be analyzed quantitatively or qualitatively. The chemical reaction method is characterized by high sensitivity, low cost and simple operation; however, it has poor specificity and measurement results are easily affected by some redox reactions or enzyme-catalyzed reactions. Tetranitromethane, nitrotetrazolium blue chloride (NBT), cytochrome c, epinephrine and reduced coenzyme I are commonly used for spectrophotometric methods; these react with superoxide anion radicals to produce ferrous cytochromes with a specific absorbance (detectable at a wavelength of 550 nm), which can be used to directly measure ROS levels (307,314-317). The NBT assay is highly sensitive and is commonly used for the histochemical localization of oxygen radicals; however, it is difficult to measure dynamic changes in oxygen radicals in cells or aqueous systems. Cytochrome c has oxidative activity and can be used to detect the production of oxygen radicals. However, cytochrome c is easily reduced by other reducing agents and is therefore limited for the accurate localization of oxygen radicals. In the last decade, a number of ROS kits have been developed to detect intracellular or mitochondrial ROS (mtROS) levels directly. Intracellular ROS are usually measured using the fluorescent probe DCFH-DA, which is non-fluorescent and can freely cross the cell membrane. After DCFH-DA enters cells, it is hydrolyzed by intracellular esterases to generate DCFH, which cannot enter or exit the cell membrane, thus allowing the probe to easily label the cell. In the presence of ROS, DCFH is oxidized to produce the fluorescent substance DCF, whose fluorescence intensity is directly proportional to intracellular ROS levels. mtROS is usually measured using the fluorescent probe MitoSOX, which is highly specific to mitochondrial ROS and is characterized by simple operation, low background signals, wide linear range and high detection efficiency; however, it requires the immediate imaging of assay results and protection from light to prevent fluorescence quenching. Prior to the widespread use of kits, ROS levels were indirectly measured by detecting products of oxidative damage. Levels of malondialdehyde (MDA) reflect the degree of lipid peroxidation in the body and can be measured using the thiobarbituric acid (TBA) chemical colorimetric method. Condensation under acidic conditions generates the MDA-TBA complex, a red product with a maximum absorption peak at 535 nm, which can be used to indirectly determine the MDA content by spectrophotometry, indicating ROS levels. However, this technique has poor sensitivity and is prone to contamination. Fluorescent protein-based ROS detection methods are designed by combining fluorescent proteins and prokaryotic redox-sensitive proteins (318,319). The recombinant proteins are introduced into cells via plasmids or adenoviruses and target organelles to detect intracellular redox status (320,321). Redox-dependent fluorescence spectral changes of recombinant proteins are achieved through structural changes of disulfide bonds and part of the backbone under oxidative conditions (319,321).

Electron spin resonance (ESR) technology has emerged in recent years. Also known as electron paramagnetic resonance (EPR), its principle is similar to nuclear magnetic resonance (322-325). The sample is controlled in a fixed frequency microwave and the applied magnetic field is then changed so that the electron energy level difference is the same as the microwave energy (326,327). Unpaired electrons can move between the two energy levels and the net absorption energy of the microwave can be measured to obtain the ESR spectrum. Due to the high reactivity and short lifespan of ROS, the ESR signal is not easy to detect directly. The combination of ESR and spin traps can make up for this defect. The spin-electron trapping agent reacts with free radicals to generate relatively stable free radical addition products that are easily detected by ESR, which is then determined by ESR technology. This powerful and reliable technique can unambiguously measure the presence of free radicals in biological samples. ROS is the most direct and effective method for detecting free radicals and is widely used in physics, chemistry and biomedicine (328-331).

Detection of mtDNA. Human mitochondria carry a small circular double-stranded genome of 16569 bp known as mtDNA, which encodes mitochondrial 16S and 12S ribosomal RNA, 22 mitochondrial tRNA molecules and 13 respiratory chain proteins. Each organism contains only one type of mtDNA and mutations such as the conversion, inversion, insertion, or deletion of one or several bases of mtDNA, resulting in more than one type of mtDNA within an individual, are referred to as mtDNA heterogeneity (332-335). Owing to the lack of protective histones and effective DNA repair systems, the mutation frequency of mtDNA is ~10 times higher than that of nuclear DNA (336-339). Moreover, mutated mtDNA gradually accumulates and can cause irreversible damage to the nervous, cardiovascular, respiratory and reproductive systems after reaching a certain threshold (60-80%). In addition to these diseases, studies have also shown that mtDNA mutations are closely associated with the development of infertility (308,339-342). mtDNA dysfunction can be both quantitative (e.g., mtDNA copy number variation and deletions) and qualitative (e.g., strand breaks, point mutations and oxidative damage) (343-345).

mtDNA can be released from the cell as circulating free mitochondrial DNA (CCF-mtDNA) via extracellular vesicles (EVs) (346,347). CCF-mtDNA can serve as a damage-associated molecular pattern leading to the activation of inflammatory pathways, a process closely associated with TLR9. Numerous reports have shown that elevated levels of CCF-mtDNA are associated with various TLR9-dependent pathologies, such as rheumatoid arthritis, atherosclerosis, hypertension, acute liver injury and nonalcoholic steatohepatitis (48,348).

mtDNA damage can be detected using PCR, fluorescence in situ hybridization (FISH), DNA sequencing technology and the probe method, among others. The principle of DNA sequencing is to use DNA polymerase to extend the primers bound to the template of the undetermined sequence until a chain-terminating nucleotide is incorporated. Termination of replication and detection with isotopic labeling is the gold standard for detecting heterogeneity, but speed is limited when working on large-scale projects. The speed of large-scale projects was not guaranteed until the advent of high-throughput sequencing. PCR, as a molecular biology technology that emerged in the 1980s, is a method for enzymatically synthesizing and amplifying specific nucleic acid fragments in vitro based on the semi-conservative replication mechanism of DNA. This can purposefully amplify target regions and
is especially suitable for enriching small-scale genomes such as mtDNA (349-353). However, mtDNA is present in primer-binding regions, but accuracy is not sufficient due to heterogeneity. Over time, reverse transcription-quantitative (RT-q) PCR is able to monitor the number of amplified DNA molecules in real time, facilitating the determination of mtDNA in individual cells, along with the copy number and other impairments (deletions) (350-352). As a contemporary product of PCR, FISH is also a classic specific detection method. It uses fluorescently labeled specific nucleic acid probes to hybridize with corresponding target DNA or RNA molecules in cells. Fluorescent signaling with relatively poor specificity and insufficient hybridization compared to PCR is not the method of choice for the detection of mtDNA (149,354-362). Moreover, after the mitochondria are separated from cells or tissues, the DNA in the remaining material is extracted (kits can be used) and the DNA of the sample can be sequenced. qPCR or chromatin immunoprecipitation (ChIP) experimental methods can be used to detect the level of CCF-mtDNA, among which ChIP is often used to verify the binding of mtDNA to downstream signaling pathways, such as TLR9 inflammatory pathway or cGAS signaling pathway (335,363-371). As a DNA sensor in the cytoplasm, cGAS can recognize CCF-mtDNA and then catalyze the formation of the second messenger cGAMP (2’3’-cGAMP) to activate the interferon-stimulated gene-dependent signaling pathway. In addition, CCF-mtDNA containing unmethylated DNA (CpG DNA) fragments can be recognized by TLR9, causing TLR9 dimerization and activation of MyD88-mediated inflammatory pathway.

Unrepaired depurinated/deprimidinated sites (AP sites) in mtDNA lead to the misbinding of nucleotides, which can have serious downstream effects (372-374). Therefore, the rapid and accurate quantification of AP sites in mtDNA is crucial for the real-time assessment of mtDNA oxidative damage. Researchers have used a specific fluorescent probe (BTBM-CN2) for the real-time detection of mtDNA (375-378). At ~20 sec after contact with AP sites, red fluorescence is detectable at 598 nm and after ~100 sec, green fluorescence is detectable at 480 nm. More AP sites result in green fluorescence with greater intensity and duration and the degree of mtDNA damage can be quantified based on the time of appearance and intensity of fluorescence at 480 nm. Doxorubicin (Dox), a common anticancer drug, not only causes damage to the nuclear DNA of cells but can also be rapidly inserted into the mtDNA of living cells, causing the aggregation of mtDNA nucleoids and changing the distribution of nuclear proteins (375-382). Therefore, after Dox induces mtDNA damage, morphological changes of mtDNA can be tracked in real time using the two-photon fluorescent probe CNQ, which emits red fluorescence and is localized to mtDNA. When incubated with Dox, dynamic changes in mtDNA can be observed, providing a new method for studying mtDNA damage in real time (383,384).

5. Treatment of mitochondrial diseases

In addition to primary mitochondrial disease caused by mtDNA damage, mitochondrial dysfunction occurs in a number of infectious and non-infectious diseases (262,385,386), such as inflammation, neurodegeneration, diabetes, obesity and cardiovascular disease and several therapies targeting mitochondria have been developed (Table II). Mitochondrial transplantation and mitochondrial replacement can fundamentally address the inadequate energy supply in pathological states and have been applied in clinical settings for the treatment of pediatric congenital heart disease (385).

Leber hereditary optic neuropathy (LHON), the most common primary mitochondrial disease, is a maternally-inherited bilateral-blinding optic neuropathy mainly caused by mtDNA mutations, including m.3460G>A (MT-ND1), m.11778G>A (MT-ND4) and m.14484T>C (MT-ND6), of which m.11778G>A is the most common mutation (387,388). These mutations can affect the mitochondrial respiratory chain complex I of retinal ganglion cells, impair mitochondrial function and increase the production of reactive oxygen species, leading to apoptosis and optic nerve degeneration and atrophy, which further leads to rapidly progressive loss of binocular vision (389-391). Treatment of LHON is mostly based on ectopic expression, that is, intravitreal injection of adenov-associated viral vectors with mitochondrial targeting sequences and then guiding the translated protein into mitochondria to restore mitochondrial function, which has been successfully and safely applied to cell models. Transplant into an inducible LHON animal model that preserves retinal ganglion cells and visual function (392,393).

The mitochondrial diseases associated with mtDNA deletion mainly include chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS) and Pearson syndrome. CPEO is mostly associated with m.3243A>G(MT-TL1) deletion, which manifests as progressive paralysis of the ocular muscles, resulting in ocular movement disorders and ptosis, which usually appear in late childhood or early adulthood (394,395). KSS is a more severe syndrome than CPEO and is mostly associated with m.8993T>G (APT6) deletion. Its main clinical manifestations are progressive ophthalmoplegia and retinitis pigmentosa, usually occurring before the age of 20 (396-399). Other symptoms may include mild skeletal muscle weakness, hearing loss, cognitive impaired cognitive function and diabetes. Pearson's syndrome is a syndrome caused by sideroblastic anemia and pancreatic exocrine insufficiency. There are very few cases (~100 cases worldwide) that may be related to the deletion of ATPase 6 and 8. Most patients die during infancy; however, a minority of patients who survive into adulthood tend to develop symptoms of KSS syndrome. Due to the double-membrane structure of mitochondria and the inability of foreign nucleic acids to recombine on endogenous mtDNA (168,400,401), there is currently no effective method to directly import nucleic acids into mitochondria and the localization of proteins to mitochondria is a routine practice in the treatment of mitochondrial diseases. In principle, expression of mitochondrial-targeted DNases that specifically recognize mutated sequences can remove mutated mtDNA, or at least reduce its abundance in a heterogeneous background. Restriction endonucleases, zinc finger nucleases and transcription activator-like effector nucleases have been tested and proven effective; these specific enzymes can be used to eliminate aberrant mtDNA and thereby reduce the rate of aberrant mtDNA in cells (402-406).

In addition, mitochondrial neurogastrointestinal encephalomyopathy, a rare mitochondrial disease, is often associated with TYMP gene mutations, manifesting as...
Table II. Treatment of mitochondrial diseases.

| Author, year | Mitochondrial diseases | Treatment method | Representative intervention | Mechanism | Effect on mitochondria | Application status | (Refs.) |
|--------------|------------------------|------------------|----------------------------|-----------|------------------------|--------------------|---------|
| Feng et al, 2019 | Primary mitochondrial disease | Edit mtDNA | AAV, CRISPR-Cas9 | Reduce mtDNA damage | Protection | Pre-clinical | (379-415) |

Dabravolski et al, 2022
Hamel et al, 2021
Karshovska et al, 2020
Gao et al, 2019
Grady et al, 2018
Bozi et al, 2020
Jing et al, 2019
Amore et al, 2021
Chen and Bhatti, 2021
Mejia-Vergara et al, 2020
Newman et al, 2021
Stenton et al, 2021
Wang et al, 2021
Yu-Wai-Man et al, 2020
Heighton et al, 2019
Wu et al, 2019
Del Monte et al, 2021
Di Mambro et al, 2021
Di Nora et al, 2019
Nguyen et al, 2019
Ashton et al, 2018
Bonora et al, 2019
Ni et al, 2018
Porporato et al, 2018
Qi et al, 2019
Ramachandra et al, 2020
Soukas et al, 2019
Bonora et al, 2021
D'Angelo et al, 2020
Hirano et al, 2021
Kripps et al, 2020
Parés et al, 2021
Jackson et al, 2020

MSC-EVs
Table II. Continued.

| Author, year                  | Mitochondrial diseases                      | Treatment method                      | Representative intervention | Mechanism                      | Effect on mitochondria | Application status | (Refs.) |
|-------------------------------|---------------------------------------------|---------------------------------------|------------------------------|--------------------------------|------------------------|--------------------|---------|
| Jiang and Shen, 2022          |                                             |                                       |                              |                                |                        |                    |         |
| Mok et al, 2020               |                                             |                                       |                              |                                |                        |                    |         |
| Ng et al, 2021                |                                             |                                       |                              |                                |                        |                    |         |
| Fang et al, 2019              |                                             |                                       |                              |                                |                        |                    |         |
| Gong et al, 2021              |                                             |                                       |                              |                                |                        |                    |         |
| González et al, 2021          |                                             |                                       |                              |                                |                        |                    |         |
| Gu et al, 2017                |                                             |                                       |                              |                                |                        |                    |         |
| Feng et al, 2019              | Pediatric congenital heart disease          | Mitochondrial transplantation         | Mitochondrial replacement    | Mitochondrial numbers          | Protection             | Clinical           | (379)   |
|                               |                                             |                                       |                              |                                |                        | evaluation         |         |
| Feng et al, 2019              |                                             |                                       |                              |                                |                        |                    |         |
| Li et al, 2017                | Metabolic disease, neurodegenerative disorder |                                       |                              |                                | Ubiquinone             |                    |         |
| Bhatti et al, 2017            |                                             |                                       |                              |                                |                        |                    |         |
| Li et al, 2017                |                                             |                                       |                              |                                | Ubiquinone             |                    |         |
| Bhatti et al, 2017            |                                             |                                       |                              |                                | Ubiquinone             |                    |         |
| Li et al, 2017                |                                             |                                       |                              |                                | Ubiquinone             |                    |         |
| Bhatti et al, 2017            |                                             |                                       |                              |                                | Ubiquinone             |                    |         |
| Li et al, 2017                |                                             |                                       |                              |                                | Ubiquinone             |                    |         |
| Bhatti et al, 2017            |                                             |                                       |                              |                                | Ubiquinone             |                    |         |
| Gong et al, 2021              | Drugs                                       |                                       |                              |                                | Protection             | Have been approved  |         |
| González et al, 2021          |                                             |                                       |                              |                                |                        |                    |         |
| Gu et al, 2017                |                                             |                                       |                              |                                |                        |                    |         |
| He et al, 2019                |                                             |                                       |                              |                                |                        |                    |         |
| Gong et al, 2021              |                                             |                                       |                              |                                |                        |                    |         |
| González et al, 2021          |                                             |                                       |                              |                                |                        |                    |         |
| Gu et al, 2017                |                                             |                                       |                              |                                |                        |                    |         |
| Russell et al, 2020           | Heart and kidney disease, sepsis, diabetes  |                                       |                              |                                | SS-31                  | Protection          | (437-451) |
| Saeb-Parsy et al, 2021        |                                             |                                       |                              |                                |                        |                    |         |
| Kelly and Pearce, 2020        |                                             |                                       |                              |                                |                        |                    |         |
| Rahman and Rahman, 2018       |                                             |                                       |                              |                                |                        |                    |         |
| Tabish and Narayan, 2021      |                                             |                                       |                              |                                |                        |                    |         |
| Yuan et al, 2021              |                                             |                                       |                              |                                |                        |                    |         |
| Ballarò et al, 2021           |                                             |                                       |                              |                                |                        |                    |         |
| Bhatti et al, 2021            |                                             |                                       |                              |                                |                        |                    |         |
| Saeb-Parsy et al, 2021        |                                             |                                       |                              |                                |                        |                    |         |
| Kelly and Pearce, 2020        |                                             |                                       |                              |                                |                        |                    |         |
| Rahman and Rahman, 2018       |                                             |                                       |                              |                                |                        |                    |         |
| Tabish and Narayan, 2021      |                                             |                                       |                              |                                |                        |                    |         |
| Yuan et al, 2021              |                                             |                                       |                              |                                |                        |                    |         |
| Ballarò et al, 2021           |                                             |                                       |                              |                                |                        |                    |         |
| Bhatti et al, 2021            |                                             |                                       |                              |                                |                        |                    |         |

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| Author, year       | Mitochondrial diseases | Treatment method | Representative intervention | Mechanism | Effect on mitochondria | Application status | (Refs.)         |
|-------------------|------------------------|------------------|----------------------------|-----------|------------------------|--------------------|----------------|
| Deng et al, 2021  |                        |                  |                            |           |                        |                    | Pre-clinical     |
| Le Gal et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| He et al, 2022    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| Labarta et al, 2019|                       |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| He et al, 2022    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| Labarta et al, 2019|                       |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| He et al, 2022    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| He et al, 2021    |                        |                  |                            |           |                        |                    |                 |
| Labarta et al, 2019|                       |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| He et al, 2022    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| Labarta et al, 2019|                       |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| He et al, 2022    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| Labarta et al, 2019|                       |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| He et al, 2022    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| Labarta et al, 2019|                       |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| He et al, 2022    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2021    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| Labarta et al, 2019|                       |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Bhatti et al, 2021|                        |                  |                            |           |                        |                    |                 |
| Grosser et al, 2021|                       |                  |                            |           |                        |                    |                 |
| Heighton et al, 2019|                      |                  |                            |           |                        |                    |                 |
| Wu et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| Del Monte et al, 2021|                      |                  |                            |           |                        |                    |                 |
| Di Mambro et al, 2021|                      |                  |                            |           |                        |                    |                 |
| Di Nora et al, 2019|                      |                  |                            |           |                        |                    |                 |
| Nguyen et al, 2019|                      |                  |                            |           |                        |                    |                 |
| Ashton et al, 2018|                      |                  |                            |           |                        |                    |                 |
| He et al, 2019    |                        |                  |                            |           |                        |                    |                 |
| He et al, 2020    |                        |                  |                            |           |                        | Pre-clinical       |                 |
| He et al, 2020    |                        |                  |                            |           |                        |                    |                 |
| Zhao et al, 2021  |                        |                  |                            |           |                        |                    |                 |
| Macdonald et al, 2018|                      |                  |                            |           |                        |                    |                 |
| Tan et al, 2013   |                        |                  |                            |           |                        | Pre-clinical       |                 |
|                   |                        |                  |                            |           |                        |                    |                 |

Table II. Continued.
splanchnic neuropathy and marked motor impairment, often combined with CPEO, sensorimotor polyneuropathy and white matter encephalopathy (407-409). With advances in gene editing technology, CRISPR/Cas9 has been proposed for the treatment of mitochondrial diseases, aiming to eliminate abnormal mtDNA sequences through the principles of bacterial immunology (410,411).

To treat primary mitochondrial diseases, gene therapy based on ectopic expression is still the first choice; however, the application of viral vectors in live animals to correct any gene mutation still has the following significant problems: High cost (390,412-415), carcinogenicity and immunogenicity. Non-viral vector-mediated in situ mitochondrial gene therapy may be a promising approach to overcome the bottleneck of existing gene therapy LHON, such as liposome-based nanoparticles, which require further investigation (416-421).

Mesenchymal stem cell-derived EVs are a promising nanotherapeutic strategy to effectively attenuate mitochondrial damage and the inflammatory response by promoting mitochondrial transcription factor A expression and preventing mtdNA damage and leakage from target cells (422).

Oxidative stress caused by mitochondrial dysfunction is one of the etiologies of metabolic disease and is a potential target for the treatment of metabolic and neurodegenerative disorders (55,168,423-426). A number of antioxidants, such as vitamin E, ubiquinone, N-acetylcysteine, glutathione and melatonin, can effectively scavenge mitochondrial ROS and regulate redox processes, thus alleviating or curing disease. Antibiotics (e.g., tetracyclines and actinomycins), drugs (e.g., creatine and ursodeoxycholic acid) and exercise can significantly improve oxidative stress and balance mitochondrial fission and fusion, thus increasing the number of mitochondria, contributing to the treatment of cancer (400-406,426-442). SS31 and mitoTEMPO are novel mitochondrial-targeted antioxidants that have a scavenging effect on ROS (443-446). In addition, SS31 accumulates in the mitochondrial membrane to protect and restore the mitochondrial structure without affecting healthy mitochondria (162,447-453). Thus, SS31 and mitoTEMPO have protective effects on a variety of diseases, including heart and kidney-related diseases, as well as sepsis and diabetes, which have been demonstrated in a variety of animal models (454-457). The use of nanomaterials for mitochondrial targeting therapy has become a recent focus of research. Nanomaterials are materials with at least one of three spatial dimensions at the nanometer scale (1-100 nm). They are a new generation of materials composed of nanoparticles with sizes between atoms, molecules and macroscopic systems and are widely used in the medical field owing to their large specific surface area and excellent biocompatibility. Ideally, medical nanomaterials should remain quiescent in normal tissues but accumulate precisely and act in mitochondria under pathophysiological conditions (404,458,459). Delocalized lipophilic cations (DLCs), such as triphenylphosphine (TPP) and mitochondria-penetrating peptides (MPPs), serve a major role in mitochondria-targeted therapies. DLCs can accumulate specifically in the mitochondria of tumor cells and increase their MMP, leading to altered mitochondrial membrane permeability and inducing apoptosis (56,130,400,403,428,458-470). Studies have shown that
graphene has a large specific surface area, good targeting and high biocompatibility, making it a promising nanodelivery system (441,471-473). Mitochondrial biogenesis is driven by PcG-1α, which can increase the number of mitochondria in the cell and thus meet the evolving energy demands of the cell, alleviating ATP deficiency in patients with mitochondrial diseases. Promoting mitochondrial biogenesis is also an important component of mitochondrial therapeutics (474). Resveratrol, 5-aminoimidazole-4-carboxamide riboside, epicatechin and RTA-408 have significant pro-mitochondrial biogenesis effects; the treatment of mice with these drugs enhances the expression of mitochondrial electron transport chain proteins and mitochondrial transcription factors and increases the abundance of mitochondrial cristae (54,401,402,405,406,441, 471-478).

6. Summary and outlook

As the powerhouses of the cell, mitochondria are at the center of cellular oxidative phosphorylation and are critical for growth and development as well as the development of a number of diseases. Mitochondrial abnormalities can cause disturbances in the intracellular environment and can lead to a variety of diseases, such as mitochondrial heart disease, mitochondrial encephalopathy, mitochondrial myopathy and even various pathologies of the reproductive and respiratory systems. Therefore, the accurate detection of mitochondrial abnormalities is essential for clinical guidance.

Since the beginning of the last century, a number of methods for mitochondrial research have been developed (Fig. 3), from the discovery of mitochondria as intracellular granular structures to the observation of mitochondrial microstructures via EM and the use of fluorescent probes to detect physiological indicators within mitochondria. The application of these methods has provided theoretical foundations for the detection and treatment of mitochondrial diseases. Accordingly, the treatment of mitochondrial diseases has gradually evolved from drug-based therapy to multidisciplinary combination therapies, such as the use of nanomaterials to precisely transport therapeutic drugs into mitochondria for targeted drug delivery, substantially improving therapeutic efficiency. However, the methods by which therapeutic efficacy is achieved still warrant investigation. The combined application of biomedicine and material science may be a promising means of detection and treatment. Notably, the specific molecular mechanism underlying the pathogenesis of the mitochondrial disease remains unclear. Current monitoring and treatment strategies cannot completely cure mitochondrial disease but only alleviate symptoms or slow disease progression. Therefore, methods for detection and treatment that are specific to the molecular mechanisms are needed. Using multi-omics and artificial intelligence, artificial mitochondrial models can be established through molecular co-assembly technology and mitochondria-targeted drugs can be screened to conduct in-depth discussions on abnormal mitochondria, which may elucidate the pathogenesis of mitochondrial diseases at the molecular level and provide new treatments for mitochondrial diseases.
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Availability of data and materials

Data sharing is not applicable to this article, as no data sets were generated or analyzed during the current study.

Authors' contributions

YY wrote the first draft of this review. HS provided valuable comments on this first draft. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Akbari M, Kirkwood TBL and Bohr VA: Mitochondria in the signaling pathways that control longevity and health span. Ageing Res Rev 54: 100940, 2019.
2. Bock EP and Tait SWG: Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol 21: 85-100, 2020.
3. Chakrabarty RP and Chandel NS: Mitochondria as signaling organelles control mammalian stem cell fate. Cell Stem Cell 28: 394-408, 2021.
4. Hood DA, Memme JM, Oliveira AN and Triolo M: Maintenance of skeletal muscle mitochondria in health, exercise, and aging. Annu Rev Physiol 81: 19-41, 2019.
5. Li L, Conradson DM, Bharat V, Kim MJ, Hsieh CH, Minhas PS, Papakyrikos AM, Durairaj AS, Ludlam A, Andreasson KI, et al: A mitochondrial membrane-bridging machinery mediates signal transduction of intramitochondrial oxidation. Nat Metab 3: 1242-1258, 2021.
6. Martinez-Reyes I and Chandel NS: Mitochondrial TCA cycle metabolites control physiology and disease. Nat Commun 11: 102, 2020.
7. Kim Hong HT, Bich Phuong TT, Thu Thuy NT, Wheatley MD and Cushman JC: Simultaneous chloroplast, mitochondria isolation and mitochondrial protein preparation for two-dimensional electrophoresis analysis of ice plant leaves under well watered and water-deficit stressed treatments. Protein Expr Purif 155: 86-94, 2019.
8. Boussandon C and Keech O: Cell type-specific isolation of mitochondria in Arabidopsis. Methods Mol Biol 2363: 13-23, 2022.
9. Elekeofehinti OO, Kamdem JP, Saliu TP, Faminwu CD, Boligon A and Teixeira Rocha JB: Improvement of mitochondrial function by Tapinanthus rochelii (A.Rich.) Tiegh. Against hepatotoxic agent in isolated rat's liver mitochondria. J Ethnopharmacol 242: 110206, 2019.
10. Gäbelein CG, Feng Q, Sarasjic E, Zambelli T, Guillaume-Gentili O, Kornmann B and Vorholt JA: Mitochondria transplantation between living cells. PLoS Biol 20: e0010156, 2022.
11. Lee DA, Lee YH, Lee KH, Lee BS, Alishir A, Ko YJ, Kang KS and Kim KH: Aviculin isolated from lespedeza cuneata induce apoptosis in breast cancer cells through mitochondria-mediated caspase activation pathway. Molecules 25: 1708, 2020.
12. Léger JL, Jougleux JL, Savadogo F, Pichaud N and Boudreau LH: Rapid isolation and purification of functional platelet mitochondria using a discontinuous percoll gradient. Platelets 31: 258-264, 2020.
13. Léger JL, Pichaud N and Boudreau LH: Purification of functional platelet mitochondria using a discontinuous percoll gradient. Methods Mol Biol 2276: 57-66, 2021.
14. Lin PC, Bergamini C, Fato R, Pon LA and Pallotti F: Isolation of mitochondria from cells and tissues. Methods Cell Biol 155: 3-31, 2020.
15. Lin YT, Chen ST, Chang JC, Teoh RJ, Liu CS and Wang GJ: Green extraction of healthy and additive free mitochondria with a conventional centrifuge. Lab Chip 19: 3862-3869, 2019.
16. Long Q, Huang L, Huang K and Yang Q: Assessing mitochondrial bioenergetics in isolated mitochondria from mouse heart tissues using oroboros 2k-oxygraph. Methods Mol Biol 1966: 237-246, 2019.
17. Rahman MH, Xiao Q, Zhao S, Wei AC and Ho YP: Extraction of functional mitochondrial DNA based on membrane stiffness. Methods Mol Biol 2276: 343-355, 2021.
18. Ramezani M, Samiei F and Pourramad J: Anti-glioma effect of pseudosyneansea melanostigma venom on isolated mitochondria from glioblastoma cells. Asian Pac J Cancer Prev 22: 2295-2302, 2021.
19. Ruzzenente B and Metodiev MD: Linear density sucrose gradients to study mitoribosomal biogenesis in tissue-specific knockout mice. Methods Mol Biol 2224: 47-60, 2021.
20. Yang J, Cao L, Li Y, Liu H, Zhang M, Ma H, Wang B, Yuan X and Liu Q: Gracillin isolated from reineckia carnea induces apoptosis of A549 cells via the mitochondrial pathway. Drug Des Devel Ther 15: 233-243, 2021.
21. Chandra K, Kumar V, Werner SE and Odomi TW: Separation of stabilized MOPS gold nanostars by density gradient centrifugation. ACS Omega 2: 4878-4884, 2017.
22. Chen BY, Sung CW, Chen C, Cheng CM, Lin DP, Huang CT and Hsu MY: Advances in exosomes technology. Clin Chim Acta 493: 14-19, 2019.
23. Ecija-Arenas A, Román-Pizarro V and Fernández-Romero JM: Luminescence continuous flow system for monitoring the efficiency of hybrid liposomes separation using multiphase density gradient centrifugation. Talanta 222: 121532, 2021.
24. Hu P, Fabyanic E, Kwon DY, Tang S, Zhou Z and Wu H: Dissecting cell-type composition and activity-dependent transcriptional state in mammalian brains by massively parallel single-nucleus RNA Seq. Mol Cell 68: 1006-1015.e7, 2017.
25. Jerri HA, Sheehan WP, Snyder CE and Velegol D: Prolonging tissue atrophy in skeletal muscle by disrupting a metabolic feedback loop. Proc Natl Acad Sci USA 112026, 2019.
26. Kwon J, Zhan X, Shen T, Huang X, Kumar GR, Chen X, Zeng Z, Zhang R, Chen R, Li T, Zhang Y, et al: Serum hepatitis B virus RNA is encapsidated progenome RNA that may be associated with persistence of viral infection and rebound. J Hepatol 65: 700-710, 2016.
27. Pužar Dominkuš P, Stenovec M, Sitar S, Lasič E, Zorec R, Plemenitaš A, Žagar E, Kreft M and Lenassi M: MicroRNA regulation of mitophagy. Methods Mol Biol 1966: 319-333, 2019.
28. Wang J, Chen R, Li T, Zhang T, et al: Serum hepatitis B virus RNA is encapsidated progenome RNA that may be associated with persistence of viral infection and rebound. J Hepatol 65: 700-710, 2016.
29. Sugiyama A, Nagashima S, Tokuyama T, Amo T, Matsuki Y, Ishido S, Kudo Y, McBride HM, Fukuda T, Matsuhashi N, et al: MITOL regulates endoplasmic reticulum-mitochondria contacts via Mitofusin2. Mol cell 51: 20-34, 2013.
30. Wang J, Shi B, Chong L, Qiao Y, Zhou R, He Y and Yeung ES: Separation of novel noradrons by density gradient centrifugation. J Chromatogr A 1218: 3823-3829, 2011.
31. Zheng X, Xu K, Zhou B, Chen T, Huang Y, Li Q, Wen F, Ge W, Wang J, Yu S, et al: A circulating extracellular vesicles-based novel screening tool for colorectal cancer revealed by shotgun and data-independent acquisition mass spectrometry. J Extracell Vesicles 9: 1750202, 2020.
Exosomes from nicotine‑stimulated macrophages accelerate migration and proliferation. Theranostics 9: 6901‑6919, 2019.

Optimizing extracellular vesicles' isolation from chronic kidney disease. Kidney Int 92: 11051‑11057, 2017.

Fluorescence‑detected mid‑infrared photothermal therapy. ACS Nano 14: 14190‑14200, 2020.

Mitochondria‑targeting polydopamine nanoparticles to deliver doxorubicin for overcoming drug resistance. ACS Appl Mater Interfaces 9: 16793‑16802, 2017.

Multiview confocal microscopy‑based force spectroscopy and multiparametric imaging of biomolecular and cellular systems. Chem Rev 121: 11701‑11725, 2021.

Mitochondria‑inspired nanoparticles with microenvironment‑adapting capacities for on‑demand drug delivery after ischemic injury. ACS Nano 14: 14190‑14200, 2020.

Mitochondria‑targeting polydopamine nanoparticles to deliver doxorubicin for overcoming drug resistance. ACS Appl Mater Interfaces 9: 16793‑16802, 2017.

Multiview confocal microscopy‑based force spectroscopy and multiparametric imaging of biomolecular and cellular systems. Chem Rev 121: 11701‑11725, 2021.

Mitochondria‑inspired nanoparticles with microenvironment‑adapting capacities for on‑demand drug delivery after ischemic injury. ACS Nano 14: 14190‑14200, 2020.

Mitochondria‑targeting polydopamine nanoparticles to deliver doxorubicin for overcoming drug resistance. ACS Appl Mater Interfaces 9: 16793‑16802, 2017.

Multiview confocal microscopy‑based force spectroscopy and multiparametric imaging of biomolecular and cellular systems. Chem Rev 121: 11701‑11725, 2021.

Mitochondria‑inspired nanoparticles with microenvironment‑adapting capacities for on‑demand drug delivery after ischemic injury. ACS Nano 14: 14190‑14200, 2020.

Mitochondria‑targeting polydopamine nanoparticles to deliver doxorubicin for overcoming drug resistance. ACS Appl Mater Interfaces 9: 16793‑16802, 2017.

Multiview confocal microscopy‑based force spectroscopy and multiparametric imaging of biomolecular and cellular systems. Chem Rev 121: 11701‑11725, 2021.
75. Yordanov S, Neuhaus K, Hartmann R, Díaz-Pascual F, Vidakovic L, Singh PK and Dresecher K: Single-objective high-resolution confocal light sheet fluorescence microscopy for standard biological sample geometries. Biomed Opt Express 12: 3372-3391, 2021.

76. Zhao Y, Raghuram A, Kim HK, Hielscher AH, Robinson JT and Miyashita L, Foley G, Gill I, Gillmore G, Grigg J and Wertheim d: Rodriguez-Gallardo S, Peters AM, Sette P, Jansen HD, Mac Donald D, et al: An amino-nitro-derivatized branched polyvalent model for SARS-CoV-2 infection of human alveolar type II-like cells. EMBO J 40: e105912, 2021.

77. Guo R, Barnea I and Shaked NT: Limited-angle tomographic phase microscopy utilizing confocal scanning fluorescence microscopy. Biomed Opt Express 12: 1869-1881, 2021.

78. Lamers MM, van der Vaart J, Knoops K, Riesebosch S, Breugem Tl, Mykytn AZ, Beumer J, Schipper D, Bezestorasti K, Koopman CD, et al: Mitochondrial membrane potential. J Chem Inf Model 59: 702-712, 2019.

79. Guo R, Alpert NM, Guehr J, Praszek L, Pelletier-Galarneau M, Ruskin J, Mansour MC, Wooten D, Ma C, Takahashi K, Zhou Y, et al: Mitochondrial functionality modifies human sperm acrosin activity, acrosome reaction capability and chromatin integrity. Hum Reprod 34: 3-11, 2019.

80. Sakhthivel R, Malar DS and Devi KP: Phytol shows anti-angiogenic activity and induces apoptosis in A549 cells by dephosphorylating the mitochondrial membrane potential. Biomed Pharmacother 105: 742-752, 2018.

81. Alyasin A, Momeni HR and Mahdieh M: Aquaporin3 expression and the potential role of aquaporins in mitotidy and mitochondrial membrane potential in human spermatozoa. Andrologia 52: e13580, 2020.

82. Alpert NM, Guehr J, Praszek L, Pelletier-Galarneau M, Ruskin J, Mansour MC, Wooten D, Ma C, Takahashi K, Zhou Y, et al: Quantitative in vivo mapping of myocardial mitochondrial membrane potential. PLoS One 13: e0190968, 2018.

83. Kuwahara Y, Roudknar MH, Suzuki M, Urushihara Y, Fujimoto M: Comparison of mitochondrial membrane potential in cross-resistance between radiation and docetaxel. Int J Radiat Oncol Biol Phys 96: 556-565, 2016.

84. Marcondes NA, Terra SR, Laskis CS, Hlivac NC, Dalmolin ML, Lacerda LA, Faulhaber GAM and Gonzalez FHD: Comparison of JC-1 and MitoTracker probes for mitochondrial viability assessment in stored canine platelet concentrates: A flow cytometry study. Cytometry A 95: 214-218, 2019.

85. Poznanski RR, Cacha LA, Ali J, Rizvi ZH, Yuapin P, Salleh SH and Bandyopadhyay A: Induced mitochondrial membrane potential for modeling soliton conduction of electrotonic signals. PLoS One 12: e0183677, 2017.

86. Georgakopoulos ND, Wells G and Campanella M: The pharmacological regulation of cellular mitophagy. Nat Chem Biol 13: 134-140, 2017.

87. Bikas A, Jensen K, Patel A, Costello J, Kalsatsas G, Hoperia V, Wartofsky L, Burman K and Vasko V: Mitotane induces mitochondrial membrane depolarization and apoptosis in thyroid cancer cells. Int J Oncol 55: 7-20, 2019.

88. Gloria A, Wegher L, Carluccio A, Valorz C, Robbe D and Contini A: Factors affecting staining to discriminate between bull sperm with greater and lesser mitochondrial membrane potential. Anim Reprod Sci 189: 51-59, 2018.

89. Saraf KK, Kumaresan A, Chhillar S, Nayak S, Lathika S, Datta TK, Gahlot SC, Karan P, Verma K and Mohanty TK: Spermatogenesis with high mitochondrial membrane potential: low tyrosine phosphorylation preferentially bind to oviduct explants in the water buffalo (Bubalus bubalis). Anim Reprod Sci 200: 108318, 2021.

90. El Manna W, Duplan E, Goiran T, Laurizzen J, Vaillant Beuchot L, Lucas-Gervais S, Morais VA, You H, Qi L, Salazar M, et al: Transcription- and phosphorylation-dependent control of a functional interplay between XBPI and PINK1 governs mitophagy and potentially impacts Parkinson disease pathophysiology. Autophagy 17: 4363-4385, 2021.

91. Frañco-Iborra S, Plaza-Gala A, Montypo M, Sebastian D, Vila M and Martinez-Vicente M: Mutant HTT (huntingtin) impairs mitophagy in a cellular model of Huntington disease. Autophagy 17: 3686-3694, 2018.

92. Lee JY, Lim W, Ham J, Kim J, You S and Song G: Ivermectin induces apoptosis of porcine trophoderm and uterine luminal epithelial cells through loss of mitochondrial membrane potential, mitochondrial calcium ion overload, and reactive oxygen species generation. PLoS One 15: 144-153, 2019.

93. Tao L, Liu X, Da W, Tao Z and Zhu Y: Pycnogenol achieves neuroprotective effects in rats with spinal cord injury by stabilizing the mitochondrial membrane potential. Neuro R 42: 1591-1604, 2020.

94. Haider S, Mohanraj N, Markandeya YS, Joshi PG and Mehta B: Picture perfect: Imaging mitochondrial membrane potential changes in retina slices with minimal stray fluorescence. Exp Eye Res 202: 108318, 2021.

95. Zhang G, Yang W, Zou P, Jiang F, Zeng Y, Chen Q, Sun L, Yang H, Zhou X, Yang X, et al: Mitochondrial functionality modifies human sperm acrosin activity, acrosome reaction capability and chromatin integrity. Aging Cell 20: e13444, 2021.

96. Hamilton K, Krause K, Badr A, Daily K, Estfanous S, Eltobgy M, Khadieke A, Anne B, and Zebruh D, et al: Effective immunomodulation pathways in cystic fibrosis macrophages. J Cyst Fibros 20: 664-672, 2021.
113. Rabinovich-Nikitin I, Rasouli M, Reitz CJ, Posen I, Margulets V, Koseki K, Warabi E, Ohue T, Jakobsen E, Waagepetersen HS and Aldana BI: Mitochondrial oxygen consumption rate of human embryos is regulated by the circadian clock gene in cardiac myocytes during ischemic stress. Acta Biomater 19: 3794-3812, 2021.

114. Rovini A, Heslop K, Hunt EG, Morris ME, Fang d, Gooz M, Samuvel d J, Li L, Krishnasamy Y, Gooz M, Takemoto K, Woster PM, Lemasters JJ and Zhong Z: Mitochondrial depolarization after acute ethanol treatment drives mitophagy in living mice. Autophagy: 1-15, 2022 (Epub ahead of print).

115. Samavel d J, Krishnasamy Y, Gooz M, Takemoto K, Woster PM, Lemasters JJ and Zhong Z: Mitochondrial depolarization after acute ethanol treatment drives mitophagy in living mice. Autophagy: 1-15, 2022 (Epub ahead of print).

116. Wang Q and Hutt KJ: Evaluation of mitochondria in mouse oocytes following co-injection exposure. J Ovarian Res 14: 65, 2021.

117. Yazdankhah M, Ghosh S, Shang P, Stepicheva N, Hose S, Liu H, Chamling X, Tian S, Sullivan MLG, Calderon MJ, et al: BNIP3L-mediated mitophagy is required for mitochondrial remodeling during the differentiation of optic nerve oligodendrocytes. Autophagy 17: 3410-3419, 2021.

118. Young VC and Artigas P: Displacement of the Na+/K+ pump’s transmembrane domains demonstrates conserved conformational changes in P-type 2 ATPases. Proc Natl Acad Sci USA 118: e2029371118, 2021.

119. Cui Y, Duan W, Jin Y, Wu F, Xi F and Wu J: Graphene quantum dot-decorated porous silicon dressing for theranostics of diabetic wounds. Acta Biomater 131: 544-554, 2021.

120. Kambe Y and Yamaoka T: Initial immune response to a FRET-based MMP sensor-immobilized silic fibroin hydrogel in vivo. Acta Biomater 130: 199-210, 2021.

121. Fei Y, Guo L, Fang J, Jia Y, Wang X, Wei Q and Yu X: Construction of the FRET pairs for the visualization of mitochondria membrane potential in dual emission colors. Anal Chem 91: 3704-3709, 2019.

122. Lee H, Kim SJ, Shin H and Kim YP: Collagen-immobilized extracellular FRET reporter for visualizing protease activity secreted by living cells. ACS Sens 5: 655-665, 2020.

123. Liu L, Chu H, Yang J, Sun Y, Ma P and Song d: construction of mitochondrial membrane potential. Anal chem 89: 11514‑11519, 2017.

124. Zhan Y, Ling S, Huang H, Zhang Y, cheng G, Hardy M, Ouari O, Lopez M, Pawling J, Khatib A, Parrella R, Zhao H, El Hajj Y, et al: Graphene quantum dots for the assessment of mitochondrial toxicity in HepG2 cells using the Seahorse extracellular flux analyzer. Curr Protoc Chem Biol 45: 102055, 2021.

125. Nishida M, Yamashita N, Kikuchi M, Kunitoki Y, et al: Mitochondrial reactive oxygen species trigger mitoflin-dependent antitumor immunity via activation of Nrf2/mitTORS/p62 axis in tumor-infiltrating CD8 T lymphocytes. J Immunother Cancer 9: e002954, 2021.

126. Espinosa JA, Pohan G, Arkin MR and Markossian S: Real-time measurement of mitochondrial oxygen consumption in adherent cells by Seahorse XF96 cell mito stress test. STAR Protoc 2: 100245, 2021.

127. Eagleson KL, Villanueva M, Southern RM and Levitt P: Proteomic and mitochondrial adaptations to early-life stress are distinct in juveniles and adults. Neurobiol Stress 13: 100251, 2020.

128. Maredon K, Rueda J and Rahman F: Role of internal mitochondrial protein OPA1 in mitochondrial dysfunction by tobacco smoking and in the pathogenesis of COPD. Redox Biol 45: 102055, 2021.

129. Campos JC, Veliquici BB, Bozli LH, Bechara LRG, Dourado PMM, Andres AM, Jannig PR, Gomes KMS, Zambelli VO, Rocha-Resende C, et al: Exercise reestablishes autophagic flux and mitochondrial quality control in heart failure. Autophagy 13: 1304-1317, 2020.

130. Rossow HA, Acetoze G, Champagne J and Ramsey JJ: Measuring liver mitochondrial oxygen consumption and proton leak kinetics to estimate mitochondrial respiration in holstein dairy cattle. J Vis Exp, 2018.

131. Morimoto Y, Hara K, Yamanaka M, Nakano T, Sato M, Nakao Y, Iwata H, Fukui A, Morimoto Y and Shibahara H: Mitochondrial oxygen consumption rate of human embryos declines with maternal age. J Assist Reprod Genet 37: 1815-1821, 2020.

132. Anderson JV, Jakobsen E, Waagepetersen HS and Aldana BI: Distinct differences in oxygen consumption and ATP synthesis of regionally isolated non-synaptic mouse brain mitochondria. J Neurosci Res 97: 961-974, 2019.
151. Hubbard WB, Joseph B, Spry M, Vekaria HJ, Saatman KE and Sullivan PG: Acute mitochondrial impairment underlies prolonged cellular dysfunction after repeated mild traumatic brain injury. Acta Neuropathol 136: 1252-1263, 2019.

152. McAlpin BR, Mahalingam R, Singh AK, Dharmaraj S, Chrisikos TT, Boukemoloue N, Kavelaars A and Heijnen CJ: HDAC6 inhibition reverses long-term doxorubicin-induced cognitive dysfunction by restoring microglia homeostasis and synaptic integrity. Theranostics 12: 603-619, 2022.

153. Raut S, Patel R and Al-Ahmad AJ: Presence of a mutation in PSEN1 or PSEN2 gene is associated with an impaired brain endothelial cell phenotype in vitro. Fluids Barriers CNS 18: 3, 2021.

154. Algieri C, Trombetti F, Pagliarani A, Ventrella V and Nesci S: The mitochondrial F$_{1}$F$_{0}$-ATPase exploits the dithiol redox state to modulate mitochondrial Ca$^{2+}$ entry to promote mitotic progression. J Biol Chem 297: 1090-1102, 2022.

155. Sun C, Liu X, Wang B, Wang Z, Liu Y, Di C, Ji S, Li H, Wu Q, Xu D, et al: Endocytosis-mediated mitochondrial transplantation: Transferring normal human astrocytic mitochondria into glioma cells rescues aerobic respiration and enhances radiosensitivity. Theranostics 9: 3595-3607, 2019.

156. NYON S, Pan Y, Shung K and Wang Y: FRET-based Ca$^{2+}$ biosensor single cell imaging interrogated by high-frequency ultrasound. Sensors (Basel) 20: 4998, 2020.

157. Chen J, Qi M, Zhang C, Li B, Li D, Huang X, Qian Z, Zhao J, Wang Z and Tang D: A calcium phosphate drug carrier loading with 5-fluorouracil achieving a synergistic effect for pancreatic cancer therapy. J Colloid Interface Sci 605: 263-273, 2022.

158. Fan Y and Simmen T: Mechanistic connections between endothelial reticulum (ER) Redox Control And Mitochondrial Metabolism. Cells 8: 1071, 2019.

159. Shoshan-Barmatz V, Nahon-Crystal E, Shteinzer-Kuzmine A and Gupta R: VDAC1, mitochondrial dysfunction, and Alzheimer's disease. Pharmaco Res 131: 87-101, 2018.

160. Country MW and Jonz MG: Mitochondrial K+ATP channels stabilize intracellular Ca$^{2+}$ during hypoxia in retinal horizontal cells of goldfish (Carassius auratus). J Exp Biol 224: jeb423643, 2021.

161. Boyman L, Karbowsk W and Lederer WJ: Regulation of mitochondrial ATP production: Ca$^{2+}$ signaling and quality control. Trends Mol Med 26: 21-39, 2020.

162. Bravo-Saguà R, Parra V, López-Crisosto C, Díaz P, Quez AF and Lavandero S: Calcium transport and signaling in mitochondria-targeted small peptide SS-31 in diabetes mellitus. J Physiol 599: 1291-1305, 2021.

163. Marchi S, Patergnani S, Missiroli S, Morciano G, Rimessi A, Marchi S, Patergnani S, Missiroli S, Morciano G, Rimessi A: calcium transport and signaling in mitochondrial dynamics in type 2 diabetes: Pathophysiological implications. Int J Mol Sci 21: 2852, 2020.

164. Boyman L, Karbowsk W and Lederer WJ: Regulation of mitochondrial ATP production: Ca$^{2+}$ signaling and quality control. Trends Mol Med 26: 21-39, 2020.

165. Nakamura T, Ogawa M, Kojima K, Takayanagi S, Ishihara S, Takayanagi S, Ishihara S: MiCUI1, mitochondrial dysfunction, and oxidative stress in metabolic disorders—a step towards mitochondrial permeability transition pore: An overview. Biochimie 152: 85-93, 2018.

166. Liu M, Chen Y, Wu Y, Li Q, Ma T, Gao J, Xia Y, Fan M, et al: Protective effect of HINT2 on mitochondrial function via repressing MCU complex activation attenuates cardiac microvascular ischemia-reperfusion injury. Basic Res Cardiol 116: 65, 2021.

167. Mollazadeh H, Tavana E, Fanni G, Bo S, Banach M, Pirro M, van Haelst J, Jamilahmadi T and Sahlekar A: Effects of statins on mitochondrial pathways. J Cachexia Sarcompenia Muscle 12: 237-251, 2021.

168. Nakamura T, Ogawa M, Kojima K, Takayanagi S, Ishihara S, Takayanagi S, Ishihara S: MiCUI1, mitochondrial dysfunction, and oxidative stress in metabolic disorders—a step towards mitochondrial permeability transition pore: An overview. Biochimie 152: 85-93, 2018.

169. Chen M, Mu L, Wang S, Cao X, Liang S, Wang Y, She G, Yang J, Wang Y and Shi W: A single silicon nanowire-based ratiometric sensor analysis reveals caveolae are spatially distinct Ca$^{2+}$ stores in endothelial cells. Cell Calcium 54: 395–403, 2013.

170. Zhao H, Li T, Wang K, Zhao F, Chen J, Xu G, Zhao J, Li T, Chen L, Li L, et al: AMPK-mediated activation of MCU stimulates mitochondrial Ca$^{2+}$ entry to promote mitotic progression. Nat Cell Biol 21: 476-486, 2019.

171. Calvo-Rodriguez M, Hou SS, Snyder AC, Khartonova EK, Ramírez-Rojas J, Bogdanov A, and Akhmedova E: Neuroprotective effects of a new FRET-Based Ca$^{2+}$ sensor targeted to the nucleus. Int J Mol Sci 22: 9945, 2021.
Baines CP and Gutiérrez-Aguilar M: The still uncertain identity of the channel-forming units of the mitochondrial permeability transition pore. Cell Calcium 73: 121‑130, 2018.

Benecke R, Kreuzer J, Kumsta C, Wu L, Kamer KD, Czifra L, Zhang Y, Li S, Kaczergis MC, Webster CM, et al: Mitochondrial permeability uncouplings elevated autophagy and lifespan extension. Cell Metab 31: 1014‑1024.e16, 2016.

Borges P, Mone P, Plarre A, Behrens M1 and Quintanilla RA: Mitochondrial permeability transition pore contributes to mitochondrial dysfunction in fibroblasts of patients with sporadic Alzheimer’s disease. Redox Biol 19: 290‑300, 2018.

Kalani K, Yan SF and Yan SS: Mitochondrial permeability transition pore: A potential drug target for neurodegeneration. Drug Discov Today 23: 1983‑1989, 2018.

Naryzhnaya NV, Maslov LN and Oeltgen PR: Pharmacology of mitochondrial permeability transition pore inhibitors. Drug Dev Res 80: 1013‑1020, 2019.

Shah SS, Lannon H, Dias L, Zhang JY, Alper SL, Pollak MR and Naryzhnaya NV, Maslov LN and Oeltgen PR: Pharmacology of mitochondrial permeability transition pore opening modulates histone lysine methylation at the early phase of somatic cell reprogramming. Cell Metab 28: 935‑945.e5, 2018.

Burke PJ: Mitochondria, bioenergetics and apoptosis in cancer. Trends Cancer 3: 857‑870, 2017.

Pérez MJ, Gao G, Tricaud N, Aqawi M, Sionov RV, Gallily R, Friedman M and Steinberg d: daniyal M, Li c, Ge Y, Wang c, Zhang W, Lai S, Wang d and Wang L: Analysis of mitochondria for evaluating the toxicity of α‑synuclein in transgenic mice and isolated preparations by atomic force microscopy. Biomed Pharmacother 96: 1380‑1388, 2017.

Ghosh P, Bhoomik A, Saha S, Mukherjee S, Azmi S, Ghosh JK and Dungdung SR: Spermical efficacy of VRP, a synthetic cationic antimicrobial peptide, inducing apoptosis and membrane disruption. J Cell Physiol 233: 1041‑1050, 2018.

Jiang S, Zu Y, Wang Z, Zhang Y and Fu Y: Involvement of mitochondrial permeability transition pore opening in 7‑xylol‑10‑deacetylpaclitaxel‑induced apoptosis. Planta Med 77: 1005‑1012, 2011.

Tricaud N, Gao G, Lu L, Duan C, Wang X and Yang H: Morphological analysis of mitochondria for evaluating the toxicity of α‑synuclein in transgenic mice and isolated preparations by atomic force microscopy. Biomed Pharmacother 96: 1380‑1388, 2017.

Mukherjee R, Mareninova OA, Odinkovka IV, Huang W, Murphy J, Chvanov M, Javed MA, Wen L, Booth DM, Cane MC, et al: Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: Inhibition prevents acute pancreatitis by protecting production of ATP. Gut 65: 1333‑1346, 2016.

Urbani A, Giorgio V, Cerrara J, Franchin C, Arrigoni G, Jico K, Abe K, Maeda S, Shinzawa‑Itoh K, Bogers JFM, Ruiz I, Brillet R, Lafüth F, Teixeira‑Cercef F, Nguyen CT, Rizzi M and de Petro G: Small‑molecule inhibitors of cyclophilins block opening of the mitochondrial permeability transition pore and protect mice from hepatic ischemia/reperfusion injury. Gastroenterology 157: 1368‑1382, 2019.

Winquist RJ and Gripkoff VK: Targeting putative components of the mitochondrial permeability transition pore for novel therapeutics. Biochem Pharmacol 177: 113995, 2020.

Wu S and Zou MH: AMPK, mitochondrial function, and cardiovascular disease. Int J Mol Sci 21: 4967, 2020.

Lee P, Chandel NS and Simon MC: Cellular adaptation to hypoxia through hypoxia inducible factors and beyond. Nat Rev Mol Cell Biol 21: 268‑280, 2020.

Tan KY, Li CY, Li YF, Fei J, Yang B, Fu YJ and Li F: Real‑time monitoring ATP in mitochondrion of living cells: A specific fluorescent probe for ATP by dual recognition sites. Anal Chem 89: 1749‑1756, 2017.

Leu Y, Kircher V, Pachler W, Gehrke H, Urso P, et al: Purified F‑ATP synthase forms a Ca2‑dependent high‑conductance channel matching the mitochondrial permeability transition pore. Nat Commun 10: 4341, 2019.

Agawi M, Sionov RV, Gallily R, Friedman M and Steinberg D: Anti‑bacterial properties of cannabinoids toward streptococci mutants. Front Microbiol 12: 656471, 2021.

Aspert E, Bellini S, Grillo E, Gryptik M, Cantamessa L, Rebuli R, Meloni F, Salvi A, De Petro G, Arosio P, et al: H‑ferritin suppression and pronounced mitochondrial respiration through voltage dependent anion channel 1. Biomedicines 10: 1447, 2022.

Denu M, Liu Y, Yang Y, Xiao F, Fan J, Yu H, Qiu Y, Liu B, Liu B, Xiao F and Yuhui Q: Anti‑gastric cancer activity and mechanism of natural compound ‘Heilaohulignan c’ isolated from Kadsura coccinea. Phytother Res 35: 3977‑3987, 2021.

Rambold AS and Pearce EL: Mitochondrial dynamics at the membrane‑embedded FO2 of ATP in single cells. Angew Chem Int Ed Engl 57: 10873‑10878, 2018.

Fattori M, Pardi S, Bocchi N, Forni R, et al: Mechanism of mitochondrial permeability transition pore opening and damage in the pancreas: Inhibition prevents acute pancreatitis by protecting production of ATP. Gut 65: 1333‑1346, 2016.

Potter M, Newport E and Morton KJ: The Warburg effect: 80 Years On. Biochem Soc Trans 44: 1499‑1505, 2016.

Nesici S, Pagliarani A, Algieri c and Trombetti F: Mitochondrial F‑type ATP synthase: multiple enzyme functions revealed by the membrane‑embedded F0 structure. Crit Rev Biochem Mol Biol 55: 309‑321, 2020.

Schönfeld P and Wojtczak L: Short‑ and medium‑chain fatty acids in energy metabolism: The cellular perspective. J Lipid Res 57: 943‑954, 2016.

Chistikova DA, Shukat TP, Melnichenko AA, Grechko AV and Orekhov AN: The role of mitochondrial dysfunction in cardiovascular disease: A brief review. Ann Med 50: 121‑127, 2018.

Costa R, Peruzzo R, Bachmann M, Montà GD, Vicario M, Santinon G, Mattarei A, Moro E, Quintana‑Cabrera R, Scorrano L, et al: Impaired mitochondrial ATP production downregulates Wnt signaling via ER stress induction. Cell Rep 28: 1949‑1960.e6, 2019.

Rambold AS and Pearce EL: Mitochondrial dynamics at the interface of immune cell metabolism and function. Trends Immunol 39: 6‑18, 2018.

Roque AJ, Muñoz‑González SA and Kamikawa R: The origin and diversification of mitochondria. Curr Biol 27: R1177‑R1192, 2017.
238. Fernström J, Mellon SH, McGill MA, Picard M, Reus VI, Murata O, Shindo Y, Ikeda Y, Iwasawa N, Citterio D, Oka K and Mita M, Sugawara I, Harada K, Ito M, Takizawa M, Ishida K, Midelfort CF and Rose IA: A stereochemical method for detecting enzymatic radical reactions. Curr Opin Chem Biol 8: 462-467, 2004.

237. Chen H and Zhang YP: Enzymatic regeneration and conservation of ATP: Challenges and opportunities. Crit Rev Biochem Mol Biol 51: 285-302, 2016.

239. Dorr BM and Fuerst EA: Enzymatic amidation for industrial applications. Curr Opin Chem Biol 43: 127-133, 2018.

240. Finley D and Prado MA: The proteasome and its network: Engineering for adaptability. Cold Spring Harb Perspect Biol 12: a039985, 2020.

241. Hammel D, Marx A and Zumbusch A: Fluorescence-lifetime-sensitive probes for monitoring ATP cleavage. Chemistry 24: 15329-15335, 2018.

242. Ishida A, Yamada Y and Kamidate T: Colorimetric method for enzymatic screening assay of ATP using Fe(III)-xylanol orange complex formation. Anal Bioanal Chem 409: 5881-5887, 2017.

243. Midelfort CF and Rose IA: A stereochemical method for detection of ATP terminal phosphate transfer in enzymatic reactions. Glutamate synthetase. J Biol Chem 251: S881-S887, 1976.

244. Utaj M, Morello R, Vemula V, Salhotra A and Månsson A: Single molecule turnover of fluorescent ATP by myosin and actomyosin unveil elusive enzymatic mechanisms. Cell Commun Signal 15: 102, 2017.

245. Klier PEZ, Martin JG and Miller EW: Imaging reversible mitochondrial membrane potential dynamics with a masked rhodamine voltage reporter. J Am Chem Soc 143: 4095-4099, 2021.

246. Vasta JD, Corona CR, Wilkinson J, Zimpich CR, Hartnett JR, Ingold MR, Zimmerman K, Machleidt T, Kirkland TA, Hawliker KG, et al.: Quantitative, wide-spectrum kinase profiling in live cells for assessing the effect of cellular ATP on target engagement. Cell Chem Biol 25: 206-214.e1, 2018.

247. Billelton LH, Stoolman JS, Vasan K, McElroy GS, Martinez-Reyes I, Gao P, Helmin KA, Abadala-Velazquez HC, Sela NA, et al.: Mitochondrial complex III is essential for suppressive function of regulatory T cells. Nature 565: 495-499, 2019.

248. Wu M, Gu J, Song S, Guo R, Liu T and Yang M: Research journey of respirasome. Protein Cell 11: 318-328, 2020.

249. Yamada S, Ozaki H and Noguchi K: The mitochondrial respiratory chain maintains the photosynthetic electron flow in Arabidopsis thaliana leaves under high-light stress. Plant Cell Physiol 61: 283-295, 2020.

250. Yashihata K, Miyazaki T, Fukuda Y, Mitsuyma S, Saito J, Shimamura S, Yamamoto Y, Izumikawa K, yanagihara K, et al.: The novel arylamide T-2307 selectively disrupts yeast mitochondrial function by inhibiting respiratory chain complexes. Antimicrob Agents Chemother 63: e0374-19, 2019.

251. Fernandez-Vizarra E and Zeviani M: Mitochondrial disorders of the OXPHOS system. FEBS Lett 595: 1062-1106, 2020.

252. Hernandez-Agustín P, Choya-Foces C, Carregal-Romero S, Ramos E, Oliva T, Villa-Piñá T, Moreno L, Izquierdo-Alvarez A, Cabrera-Garcia JD, Cortés A, et al.: Na+ controls hypoxic signalling by the mitochondrial respiratory chain. Nature 586: 287-291, 2020.

253. Kobayashi A, Azuma K, Ikeda K and Inoue S: Mechanisms underlying the regulation of mitochondrial respiratory chain complexes by nuclear steroid receptors. Int J Mol Sci 21: 6683, 2020.

254. Martinez-Reyes I, Cardona LR, Kong H, Vasan K, McElroy GS, Werner M, Kishchen H, Rezcek CR, Weinberg SE, Gao P, et al.: Mitochondrial ubiquinol oxidation is necessary for tumour growth. Nature 585: 288-292, 2020.

255. Castellana S, Biagini T, Petrizziello F, Parca L, Panzironi N, Castelli V, Vescovi A, Centonze D and Marzà T: MiR-33: Modelling the residue interaction network of the respiratory chain subunits. Nucleic Acids Res 49 (D1): D1282-D1288, 2021.

256. Wang M, Ren X, Wang L, Lu X, Han L, Zhang X and Feng J: A functional analysis of mitochondrial respiratory chain cytochrome bc1 complex in gynecaeomycetes tritici by RNA silencing as a possible target of carbamone. Mol Plant Pathol 21: 1529-1544, 2020.

257. Mira R, Botham A, Voisin V, Xu C, St-Germain J, Sharon D, Hoff FW, Qi Y, Hurren R, Gronda M, et al.: The mitochondrial peptidase, neurolysin, regulates respiratory chain supercomplex formation and is necessary for AML viability. Sci Transl Med 12: eaaz8264, 2020.

258. Heyman E, Daussin F, Wieczorek V, Caiazzo R, Matran R, Berthom P, Aubertier J, Berthoin S, Descatoire A, Leclerc E, et al.: Muscle transcript and supply and use in type 1 diabetes, from ambient air to the mitochondrial respiratory chain: Is there a limiting step? Diabetes Care 43: 209-218, 2020.
278. Lobo-Jarne T, Pérez-Pérez R, Fontanesi F, Timón-Gómez A, Ndi M, Formosa LE, Gao M, Yi J, Zhu J, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB, Cysewski D, Wasilewski M, Benincà C, Cysewski D, Dou Y, Chu Y, Yu J, Zou H: Mitochondrial ROS production that dictates cell fate of ovarian cancer cells. Cell Death Dis 10: 851, 2019.

279. Vankayala R and Hwang KC: Near-infrared-light-activatable nanomaterial-mediated phototheranostic nanomedicines: An emerging paradigm for cancer treatment. Adv Mater 30: e1706320, 2018.

300. Wang S, Zhang Z, Wei S, He F, Li Z, Wang HH, Huang Y and Nie Z: Near-infrared light-controllable MXene hydrogel for tunable on-demand release of therapeutic proteins. Acta Biomater 130: 114-126, 2021.

301. Zhan Y, Shi Y, Cao Y, Liu Q, Chen X, Zhang L, Wang Y, Yang X and Wang H: Biochemical basis and metabolic interplay of redox regulation. Redox Biol 32: 101284, 2020.

302. Zhai M, Liu G, Gu Y, Yang L, Wang W, Shi X, Wang Z and Zou H: In situ monitoring of mitochondria regulating cell viability by the RNA-specific fluorescent photosensitizer. Anal Chem 92: 10815-10821, 2020.

303. Blanco FJ, Valdes AM and Rego-Perez I: Mitochondrial DNA damage and the pathways of osteoarthritides. Nat Rev Rheumatol 14: 327-340, 2018.

304. Fuhrmann DC and Brüne B: Mitochondrial composition and function under the control of hypoxia. Redox Biol 12: 208-215, 2017.

305. van der Reest J, Meierhold J, van der Meijden M, de Jongh J, van der Lee JH and Paull TT: Mitochondria at the crossroads of oxidative stress and metabolism. EMBO Mol Med 12: 101-112, 2020.

306. Zhang L, Kaplan P, Tatarkova Z, Sivonova MK, Racay P and Lehotsky J: Mitochondrial DNA oxidation and the electron transport chain to polarize macrophages for tissue repair. Cell Metab 29: 443-456.e5, 2019.

307. Jiang H, Zhang XW, Liao OL, Wu WT, Lin YL and Huang WH: Electrochemical monitoring of paclitaxel-induced ROS release from mitochondria inside single cells. Small 15: e1901787, 2019.

308. Kaplan P, Tatarkova Z, Sivonova MK, Racay P and Lehotsky J: Homocysteine and mitochondria in cardiovascular and cerebrovascular systems. Int J Mol Sci 21: 7698, 2020.

309. Koch RE, Josefson CC and Hill GE: Mitochondrial function, ornamentation, and immunocompetence. Biol Rev Camb Philos Soc 92: 1459-1477, 2017.

310. Zhang L, Wang X, Cueto R, Effi C, Zhang Y, Tan H, Qin X, Ji Y, Yang X and Wang H: Biochemical basis and metabolic interplay of redox regulation. Redox Biol 26: 101284, 2020.

311. Madreiter-Sokolowski T, Thomas C and Ristow M: Mitochondrial DNA damage and the pathways of osteoarthritides. Nat Rev Rheumatol 14: 327-340, 2018.

312. Angelova PR, Esteras N and Abramov AY: Mitochondria and lipid peroxidation in the mechanism of neurodegeneration: Finding ways for prevention. Med Res Rev 41: 770-784, 2021.

313. van der Reest J, Nardini Cecchi G, Hoogendoorn A, Hoogenboom J and Kordovitzki P: Mitochondria: Their relevance during oocyte ageing. Aging Cell 20: 101-117, 2021.

314. Martins WK, Santos NF, Rocha CS, Barcellos FB, Tsurome T, Viotto AC, Matsukuma AY, Abrantes ABP, Siani P, Dias LG and Tsubone TM: Biosensors for synaptic inactivity: Implications for synapse pruning. Redox Biol 36: 101678, 2020.

315. Kleih M, Boppel K, Dong M, Gaißler A, Heine S, Oloyajo MA, Aulitzky WE and Essmann F: Direct impact of cisplatin on mitochondria induces ROS production that dictates cell fate of ovarian cancer cells. Cell Death Dis 10: 851, 2019.

316. Sidlauskaitė E, Gibson JW, Megson IL, Whitfield PD, Tovmasyan A, Batinic-Haberle I, Murphy MP, Moult PR and Cobley JN: Mitochondrial ROS cause motor deficits induced by synaptic inactivity: Implications for synaptic pruning. Redox Biol 16: 344-351, 2018.

317. Weinberg S, DeBerge M, Gainullina A, Schipma M, Kinchen JM, Ben-Sahra I, Gius DR, Yvan-Charvet L, Markusse M, Beart PM and Ottersbach K: Mitochondrial DNA oxidation and the electron transport chain to polarize macrophages for tissue repair. Cell Metab 29: 443-456.e5, 2019.

318. Jiang H, Zhang XW, Liao OL, Wu WT, Lin YL and Huang WH: Electrochemical monitoring of paclitaxel-induced ROS release from mitochondria inside single cells. Small 15: e1901787, 2019.

319. Kaplan P, Tatarkova Z, Sivonova MK, Racay P and Lehotsky J: Homocysteine and mitochondria in cardiovascular and cerebrovascular systems. Int J Mol Sci 21: 7698, 2020.

320. Koch RE, Josefson CC and Hill GE: Mitochondrial function, ornamentation, and immunocompetence. Biol Rev Camb Philos Soc 92: 1459-1477, 2017.

321. Lee JH and Paull TT: Mitochondria at the crossroads of oxidative stress and metabolism. EMBO Mol Med 12: 101-112, 2020.
323. Emoto Mc, Sato-Akaba H, Hamaue N, Kawanishi K, Koshino H, He L, Hinoshita M, Abe T, Sato A, Maeda Y and Takeyoshi M: Development of novel photosafety test method based on singlet oxygen generation detected using electron spin resonance. J Appl Toxicol 41: 247-255, 2021.

324. Mendoza c, désert A, Khrouz L, Páez c A, Parola S and Okazaki Y, Ishidzu Y, Ito F, Tanaka H, Hori M and Toyokuni S: Mitochondrial DNA in innate immune responses and inflammatory pathology. Nat Rev Immunol 17: 363-375, 2017.

325. Li MX, Chen F, Yang SS, Ding J, Ding L and Ren NQ: trap? Free Radic Biol Med 154: 84-94, 2020.

326. Mendoza c, désert A, Khrouz L, Páez c A, Parola S and Okazaki Y, Ishidzu Y, Ito F, Tanaka H, Hori M and Toyokuni S: Mitochondrial DNA in innate immune responses and inflammatory pathology. Nat Rev Immunol 17: 363-375, 2017.

327. Prasad A, Wei W and chinnery PF: Inheritance of mitochondrial DNA in the APPswe/PS1dE9 mouse model of Alzheimer's disease by electron paramagnetic resonance spectroscopy: To probe or to trap? Free Radic Biol Med 154: 84-94, 2020.

328. Kasahara T and Kato T: What can mitochondrial DNA analysis in the APPswe/PS1dE9 mouse model of Alzheimer's disease by electron paramagnetic resonance spectroscopy: To probe or to trap? Free Radic Biol Med 154: 84-94, 2020.

329. Heinrichs B: Heterogeneous singlet oxygen generation: Site-specific selection reveals selective effects of datural sunscreens on the formation of singlet oxygen. Free Radic Res 55: 450-460, 2021.

330. Zhang K, Lazo S, Noren Hooten N, Green J, Eitan E, Mode NA, Liu QR, Kasahara T and Kato T: Unbiased P cR‑free spatio‑temporal mapping of plasma‑induced hydrogen peroxide. Arch Biochem Biophys 700: 108762, 2021.

331. Zonderman AB, Ezike N, Mattson MP, Ghosh P and Evans MK: Mitochondrial DNA in extracellular vesicles declines with age. Adv Exp Med Biol 20: 663, 2019.

332. Du X, Guo X, Zhan L, Li X, Yin C, Chen C, Li M, Li B, Wang Y and Xing J: Site-specific selection reveals selective constraints and functionality of tumor somatic mtDNA mutations. J Exp Clin Cancer Res 36: 168, 2017.

333. Medeiros TC and Graef M: Autophagy determines mtDNA copy number dynamics during starvation. Autophagy 15: 178-179, 2019.

334. Yang H and Xing J: Mitochondrial DNA in innate immune responses. Nat Commun 10: 4624, 2019.

335. Acevedo-Arozena A et al: Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy 17: 1-382, 2021.

336. Motaño H, Ito J, Matsuki N, Uchi T, Onodera O and Kakita A: Cytosolic dsDNA of mitochondrial origin induces cytotoxicity and neurodegeneration in cellular and zebrafish models of Parkinson's disease. Nat Commun 12: 3011, 2021.

337. Wiessner M, Semerikova SA, Khrunyk YY and Putintseva YA: Influenza A virus M2 protein triggers mitochondrial DNA-mediated antiviral immune responses. Nat Commun 10: 4624, 2019.

338. Bavmann K: mtDNA in nuclear DNTPs. Nat Rev Mol Cell Biol 20: 663, 2019.

339. Lazo S, Noren Hooten N, Green J, Ito T, Mode NA, Liu QR, Kasahara T and Kato T: Unbiased P cR‑free spatio‑temporal mapping of plasma‑induced hydrogen peroxide. Arch Biochem Biophys 700: 108762, 2021.

340. Kreisel K, Navarrete c, Feldberg AL, Watt dL, Nilsson AK, Papanastasiou M, Wang Y, Elia I, Perlin JR, Hoi K, Chan V, Abraham BJ, Yang S, Mullahoo J, capin G, Paetau A, Terzioglu M, Euro L and Suomalainen A: Loss of c 2orf69 ‑mediated autophagy drives erythropoiesis. Nature 592: 737-746, 2021.

341. Yang H and Xing J: Site-specific selection reveals selective constraints and functionality of tumor somatic mtDNA mutations. J Exp Clin Cancer Res 36: 168, 2017.

342. Moscatelli and Kato T: Unbiased P cR‑free spatio‑temporal mapping of plasma‑induced hydrogen peroxide. Arch Biochem Biophys 700: 108762, 2021.

343. Yang H and Xing J: Site-specific selection reveals selective constraints and functionality of tumor somatic mtDNA mutations. J Exp Clin Cancer Res 36: 168, 2017.

344. Rossmann MP, Hoi K, Chan V, Abraham BJ, Yang S, Mullahoo J, Aveedo-Arozena A et al: Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy 17: 1-382, 2021.

345. Wiessner M, Maroofian R, Ni MY, Pedroni A, Müller JS, Stucka R, Beetz C, Efthymiou S, Santorelli FM, Alfaires AA et al: Biallelic variants in HPDL cause pure and complicated hereditary spastic paraplegia. Brain 144: 1422-1434, 2021.
365. Goncalves VF, Mendes-Silva AP, Koyama E, Vieira E, Kennedy JL and Diniz B: Increased levels of circulating cell-free mtDNA in plasma of late life depression patients. J Psychiatr Res 139: 29-39, 2021.

366. Liu Y, Zhou K, Guo S, Wang Y, Ji X, Yuan Q, Su L, Guo X, Gu X and Xing J: NGS-based accurate and efficient detection of circulating cell-free mitochondrial DNA in cancer patients. Mol Ther Nucleic Acids 23: 657-666, 2021.

367. Maresca A, Del Dotto V, Romagnoi M, La Morgia C, Di Vito L, Cavaletti M and Capelli V: ER-MITO Study Group: Expanding and validating the biomarkers for mitochondrial diseases. J Mol Med (Berl) 98: 1467‑1478, 2020.

368. Nie S, Lu J, Wang L and Gao M: Pro-inflammatory role of cell-free mitochondrial DNA in cardiovascular diseases. JMBB Life 72: 19890‑19890, 2020.

369. Valenti D, Vacca RA, Moro L and Atlan A: Mitochondria can cross cell boundaries: An overview of the biological relevance, pathophysiological implications and therapeutic perspectives of intercellular mitochondrial transfer. Int J Mol Sci 22: 3312, 2021.

370. Zong X, Guo Y and Fan Z: Increased level of free-circulating mtDNA in maintenance hemodialysis patients: Possible role in systemic inflammation. J Clin Lab Anal 36: e24558, 2022.

371. Zhong XY, Guo Y and Fan Z: Increased level of free-circulating mtDNA in maintenance hemodialysis patients: Possible role in systemic inflammation. J Clin Lab Anal 36: e24558, 2022.

372. Angelova PR, Andruska KM, Midei MG, Barilani M, Atwal P, Tucher O, Milner P, HeERICX F and ShchePINov MS: RT001 in progressive supranuclear palsy: Phase 2 trial for leber hereditary optic neuropathy treated within 6 months of disease onset. Front Aging Neurosci 13: 2019, 2021.

373. Tucher O, Milner P, Heerinckx F and Shchepinov MS: RT001 in patients with clinically mild Leber hereditary optic neuropathy. Eur J Ophthalmol 31: 13962‑13969, 2019.

374. Shahinian A, Gallos A, Karshovska E, Langton AK, Ayer J, Griffiths CEM, 398. di Nora C, Paldino A, Miani D, Finato N, Pizzolitto S, de Maglio V, Vendramin I, Caporali L, et al: Impaired complex I repair causes recessive leber's hereditary optic neuropathy. J Clin Invest 131: e138267, 2021.

375. Wang L, Ding H, Chen T, Olong M, Long M, Wang Z, Shi D, Yu C and Qin W: Occult primary white matter impairment in leber hereditary optic neuropathy. Eur J Neurolog 28: 2871‑2881, 2021.

376. Newman NJ, Yu-Wai-Man P, Carelli V, Moster ML, BiUSSe V, Vignal-Clermont C, Sergott RC, Klopstock T, Sadan AA, Barboni P, et al: Efficacy and safety of intravitreal gene therapy for leber hereditary optic neuropathy treated within 6 months of disease onset. Front Aging Neurosci 13: 2019, 2021.

377. Zambelli VO, Camillo GA, Agrimi F, Carelli V, di Nora C, de Maglio V, Vendramin I, Caporali L, et al: Bilateral visual improvement with unilateral gene therapy injection for leber hereditary optic neuropathy. Sci Transl Med 12: eaa47243, 2020.

378. Heighton JN, Brady LI, Sadikovic B, Bulman DE and Tarnopolsky MA: Genotypes of chronic progressive external ophthalmoplegia in a large adult-onset cohort. Mol Genet Metab 48: 237, 2019.

379. Yu W, Yang L, Wu HL, Hou Y and Wang Zx: Optical coherence tomography findings in chronic progressive external ophthalmoplegia. Chin Med J (Engl) 132: 1202‑1207, 2019.

380. Newman NJ, Yu-Wai-Man P, Carelli V, Moster ML, BiUSSe V, Sadan AA, Klopstock T, Vignal-Clermont C, Sergott RC, DURPHOL D and GOLDFELD D and GOLDFELD D: The pathogenesis of leber hereditary optic neuropathy: Is vision truly rescued? Science Transl Med 12: eaa47243, 2020.

381. AshtoN TM, McKenna WG, Kunz-Schughart LA and Higgins GS: Oxidative phosphorylation as an emerging target in cancer therapy. Clin Cancer Res 24: 2482‑2490, 2018.

382. Bonara M, Wierczkowski MR, Sinclair DA, Kroemer G, Pinton P and Galluzzi L: Targeting mitochondrial dynamics: Therapeutic potential and obstacles. Nat Rev Cardiol 16: 33‑55, 2019.

383. Chen B, Wang Z, Du H, Liu D, Yin Q, Liu B, Zhang Q and ZHANG Z: A pH-activatable nanoparticle for dual-stage precisely targeted mitochondrial delivery to restore PDriven senescence inAML therapy. Cancer Discov 11: 3198‑3213, 2021.

384. Feng B, Wang K, Liu J, Mao G, Cui J, Xuan X, Jiang X, Zhang K and Zhang H: Ultrafast photoluminescence of Au@mesoporous SiO2 nanocomposites for photodynamic therapy. Anal Chem 91: 5658‑5667, 2019.

385. Rekelo AP, Eiflot H, Cintres PV, Guillo‑Noel L, Pereira CV, Timmann D, Schätzch S, Schütz L, Coarelli G, Durr A, et al: Biallelic loss-of-function variations in PRX3 lead to cerebellar ataxia. Brain 144: 1467‑1481, 2021.

386. Wu HC, Rérolle D, Berthier C, Hiehlé R, Sakamoto T, Quentin S, Benhenda S, Morganti C, Wu C, Conte L, et al: Actinomyacin D targets NPM1c-primitive mitochondria to restore PML-driven senescence in AML therapy. Cancer Discov 11: 3198‑3213, 2021.

387. Hamel Y, Mojtaba A, Windows M, Renard P, LEopP R, NEMezany I, Pellé O, Goudin N, Tang X, Rodero MP, et al: Compromised mitochondrial quality control triggers lipol-induced rhabdomyolysis. Cell Rep Med 2: 100370, 2021.

388. Karshovska E, Wei Y, Subramanian P, Mohibullah R, Geißler C, Camillo GA, Agrimi F, Carelli V, di Nora C, Paldino A, Miani D, Finato N, Pizzolitto S, de Maglio V, Vendramin I, Caporali L, et al: Bilateral visual improvement with unilateral gene therapy injection for leber hereditary optic neuropathy. Sci Transl Med 12: eaa47243, 2020.

389. Qi T, Chen B, Wang Z, Du H, Liu D, Yin Q, Liu B, Zhang Q and Wang Y: A pH-activatable nanoparticles for dual-stage precisely targeted mitochondria-targeted photodynamic anti-cancer therapy. Biomaterials 213: 119219, 2020.

390. Ramachandra CJA, Hernandez-Resendiz S, Crespo-Avalin GE, Lin YH and Hausenloy DJ: Mitochondria in acute myocardial infarction and cardioprotection. ElBioMedicine 57: 102884, 2020.

391. Soukas AA, Hao H and Wu L: Metformin as anti-aging therapy: Is it for everyone? Trends Endocrinol Metab 30: 745‑755, 2019.
407. Bonora E, Chakraborty S, Kellaris G, Tsutsumi M, Bianco F, Bergamini C, Ullah F, Isidori F, Liparulo I, Diighiugiovanni C, et al.: Biallelic variants in LIG3 cause a novel mitochondrial neuro‑gastrointestinal syndrome. Brain 144: 14646, 2021.

408. D’Angelo R, Boscetti E, Amore S, Costa R, Pugliese A, Caporali L, Gramena LL, Papa V, Vizioli L, Caprizzo M, et al.: Liver transplantation in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): Clinical long‑term follow‑up and pathogenic implications. J Neurol 267: 3702‑3710, 2020.

409. Hirose M, Yamada I, De Giorgio R, Pironi L, Accornero A, Canacci G, D’Alessandro R, Filosto M, Martì R, Nonino F, et al.: Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): Position paper on diagnosis, prognosis, and treatment by the MNGIE international network. J Inherit Metab Dis 44: 376‑387, 2021.

410. Kripps N, Nakayuemongysuk W, Shayota BJ, Bergquist W, Gomez‑Ospina N, Esquivel CO, Concepcion W, Sampson JB, Cristin DJ, Jackson WE, et al.: Successful liver transplantation in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Mol Genet Metab 150: 58‑64, 2020.

411. Parés M, Fornaguera C, VilalJulia F, Oh S, Fan SHY, Tam YK, Comes N, Vidal F, Martí R, Borrós S and Barquínero J: Preclinical assessment of a gene‑editing approach in a mouse model of mitochondrial neurogastrointestinal encephalomyopathy. Hum Gene Ther 32: 1210‑1223, 2021.

412. Jackson WE, Mullall DM, Minot CM and Gammage PA: Therapeutic manipulation of mtDNA heteroplasmacy: A shifting perspective. Trends Mol Med 26: 698‑709, 2020.

413. Jiang Z and Shen H: Mitochondria: Emerging therapeutic strategies for oocyte rescue. Reprod Sci 29: 711‑722, 2022.

414. Morin BY, de Moraes MH, Zeng J, Bosch DE, Kotrys AV, Kripps K, Naganos A, Hsu F, Radey MC, Peterson SB, Mootha VK, et al.: A bacterial cytidine deaminase toxin enables CRISPR‑free mitochondrial base editing. Nature 583: 631‑637, 2020.

415. Ng YS, Bindoff LA, Gorman GS, Klopopstock T, Korblum C, Mancuso M, McFarland R, Sue CM, Suomalainen A, Taylor RW, et al.: Mitochondrial disease in adults: Recent advances and future promise. Lancet Neurol 20: 573‑584, 2021.

416. Fang H, Yao S, Chen Q, Liu C, Cai Y, Geng S, Bai Y, Tian Z, Zacharias AL, Takebe T, et al.: De novo‑designed near‑infrared nanomaterials for super‑resolution monitoring of lysosomes in cells, in whole organoids, and in vivo. ACS Nano 13: 14426‑14436, 2019.

417. Gong X, Pu X, Wang J, Yang L, Cui Y, Li L, Sun X, Liu J, Bai J and Wang Y: Enhancing of nanocatalyst‑driven chemodynamic therapy for endometrial cancer cells through inhibition of PDK1. Jparkin‑mediated mitophagy. Int J Nanomedicine 14426‑14436, 2019.

418. He C, Jiang S, Yao H, Zhang L, Yang C, Jiang S, Ruan F, Zhan D, Liu G, Lin Z, et al.: High‑content analysis for mitophagy response to nanoparticles: A potential sensitive biomarker for nanoassembly assessment. Nanomedicine 15: 59‑69, 2019.

419. González LF, Bevilacqua LE and Naves R: Nanotechnology‑based mitochondria‑targeted antioxidants MitoQ nanoaggregates for super‑resolution monitoring of lysosomes in cells, in whole organoids, and in vivo. ACS Nano 13: 14426‑14436, 2019.

420. He G, Pan X, Liu X, Zhu Y, Ma Y, Du C, Liu X and Mao C: HIF‑1α‑mediated mitophagy determines Zn(II) nanoparticle‑induced human osteosarcoma cell death both in vitro and in vivo. ACS Omega 6: 8874‑8883, 2020.

421. Zhao M, Liu S, Wang C, Wang Y, Wan M, Liu F, Gong M, Yuan Y, Chen Y, Cheng J, et al.: Mesenchymal stem cell‑derived extracellular vesicles attenuate mitochondrial damage and inflammation by stabilizing mitochondrial DNA. ACS Nano 15: 1519‑1538, 2021.

422. Macdonald R, Barnes K, Hastings C and Mortlibuy M: Mitochondrial abnormalities in Parkinson’s disease and Alzheimer’s disease: Can mitochondria be targeted therapeutically? Biochem Soc Trans 46: 891‑909, 2018.

423. Tan DX, Manchester LC, Liu X, Rosales‑Corral SA, Acuna‑Castroviejo D and Reiter RJ: Mitochondria and chloroplasts as the original sites of melatonin synthesis: A hypothesis related to melatonin’s primary function and evolution in eukaryotes. J Pineal Res 54: 127‑138, 2013.

424. Lee JH, Park A, Oh KJ, Lee SC, Kim WK and Bae KH: The role of adipocyte‑derived mitochondria: Regulation of mitochondrial function for the treatment of metabolic diseases. Int J Mol Sci 20: 4924, 2019.

425. Wallace DC: Mitochondrial genetic medicine. Nat Genet 50: 1642‑1649, 2018.

426. Strohbe D and Campaella M: Anxiety‑type therapy: A paradigm of successful mitochondrial pharmacology. Trends Pharmacol Sci 39: 437‑439, 2018.

427. Wang XQ, Peng M, Li CX, Zhang Y, Zhang M, Tang Y, Liu MD, Xie BR and Zhang XZ: Real‑time imaging of free radicals for mitochondria‑targeting hypoxic tumor therapy. Nano Lett 18: 3681‑3688, 2018.

428. Kim HK, Noh YH, Nilius B, Ko KS, Rhee BD, Kim N and Han J: Current and upcoming mitochondrial targets for cancer therapy. Semin Cancer Biol 47: 154‑167, 2017.

429. Lleonart ME, Grodzicki R, Graffer DM and Lyakhovich A: Mitochondrial dysfunction and potential anticancer therapy. J Med Res Rev 37: 1279‑1299, 2017.

430. Tian J, Huang B, Cui Z, Wang P, Chen S, Yang G and Zhang W: Mitochondria‑targeting and ROS‑sensitive smart nanoscale supramolecular organic framework for combinational amplified photodynamic therapy and chemotherapy. Acta Biomater 130: 447‑459, 2021.

431. Chen WW, Freinkman E and Sabatini DM: Rapid immunopurification of mitochondria for metabolite profiling and absolute quantification of matrix metabolites. Nat Protoc 12: 2215‑2231, 2017.

432. Jung HS, Lee JH, Kim K, Koo S, Verwilst P, Sessler JL, Kang C and Kim JS: A mitochondria‑targeted cytochrome‑based photothermogenic photosensitizer. J Am Chem Soc 139: 972‑9978, 2017.

433. Roth KG, Mambetsariy I, Kulkarni P and Salgia R: The mitochondrion as an emerging therapeutic target in cancer. Trends Mol Med 26: 119‑134, 2020.

434. Tabish TA and Narayan RJ: Mitochondria‑targeted graphene oxide for advanced cancer therapeutics. Acta Biomater 129: 43‑56, 2021.

435. Yuan P, Deng FA, Liu YB, Zheng RR, Rao XN, Qiu XZ, Zhang DW, Xu XY, Cheng H and Li SY: Mitochondria‑targeted O2‑oomerizer to alleviate tumor hypoxia for enhanced photo‑dynamic therapy. Adv Healthc Mater 10: e2100198, 2021.

436. Bhatti JS, Thamarai K, Kandimalla R, Manczak M, Yin X, Ramasubramanian B, Sawant N, Preepdeepkan JA, Vijayan M, Kumar S and Reddy PH: Protective effects of a mitochondria‑targeted small peptide S5ST against hyperglycemia‑induced mitochondrial abnormalities in the liver tissues of diabetic mice, Tallyho/JngJ mice. Mitochondrion 58: 49‑58, 2021.

437. Deng HF, Yue LX, Wang NN, Zhou YQ, Zhou W, Liu X, Ni YH, Huang CS, Qiu LZ, Liu H, et al.: Mitochondrial iron overload‑mediated inhibition of Nrf2‑HO‑1/GPX4 assisted AL‑induced nephrotoxicity. Front Pharmacol 11: 624529, 2020.

438. De Gal K, Wiel C, Ibrahim MX, Henricsson M, Sayin VI and Bergo MO: Mitochondria‑targeted antioxidants MitoQ and MitoTEMPO do not influence BRF‑driven malignant melanoma and KRAS‑driven lung cancer progression in mice. Antioxidants (Basel) 10: 163, 2021.

439. Bhatti JS, Thamarai K, Kandimalla R, Manczak M, Yin X, Kumar S, Vijayan M and Reddy PH: Mitochondria‑targeted small peptide, SS31 ameliorates diabetes induced mitochondrial dynamics in male Tallyho/JngJ mice. Mol Neurobiol 58: 795‑808, 2021.
Tuncer S, Akkoca A, Celec MC and Dalkilic N: Can MitoTEMPO provide protective effect against ischemia-reperfusion injury in rat kidneys? J Biomed Nanotechnol 17: 1679-1689, 2021.

He Y, Zhang R, Quan Z, He B, Yu X, Chen Z, Ren Y and Li K: Preparation of targeted mitochondrion nanoscale-release peptides and their efficiency on eukaryotic cells. J Biomed Nanotechnol 17: 1679-1689, 2021.

Sun M, Ma J, Ye J, Fan H, Le J and Zhui J: Protective effect of mitochondria-targeted antioxidant peptide SS-31 in sepsis-induced acute kidney injury. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 33: 1418-1422, 2021 (In Chinese).

Zhu Y, Luo M, Bai X, Li J, Nie P, Li B and Luo P: SS-31, a mitochondria-targeting peptide, ameliorates kidney disease. Oxid Med Cell Longev 2022: 1295509, 2022.

Olga Y, Bilir D, Tuncay E and Turan B: MitoTEMPO provides an antiarrhythmic effect in aged-rats through attenuation of mitochondrial reactive oxygen species. Exp Gerontol 160: 110961, 2020.

Tuncer S, Akkoa A, Celen MC and Dalkilic N: Can MitoTEMPO protect rat sciatic nerve against ischemia-reperfusion injury? Naunyn Schmiedebergs Arch Pharmacol 394: 545-553, 2021.

Vrijen S, Besora-Casals L, van Veen S, Zielich J, Van den Haute C, Hamouda NN, Fischer C, Ghesquière B, Tournev I, Besora-Casals L, van Veen S, Zielich J, Van den Haute C, Hamouda NN, Fischer C, Ghesquière B, Tournev I, Agostinis P, et al: ATP13A2-mediated endo-lysosomal polyamine export counters mitochondrial oxidative stress. Proc Natl Acad USA 117: 31998-32027, 2020.

Wang Y, Zhao Y, Wang Z, Sun R, Zou B, Li R, Liu D, Lin M, Zhou J, Ning S, et al: Peroxiredoxin 3 inhibits acetaminophen-induced liver pyroptosis through the regulation of mitochondrial ROS. Front Immunol 12: 652782, 2021.

Liu C, Liu B, Zhao J, Di Z, Chen D, Gu Z, Li L and Zhao Y: NAD3*-sensitized upconversion metal-organic frameworks for mitochondria-targeted amplified photodynamic therapy. Angew Chem Int Ed Engl 59: 2634-2638, 2020.

Lu M, Qu A, Li S, Sun M, Xu L, Kuang H and Xu C: Mitochondria-targeting plasmidic spisky nanorods increase the elimination of aging cells in vivo. Angew Chem Int Ed Engl 59: 8698-8705, 2020.

Li C, Zhang W, Liu S, Hu X and Xie Z: Mitochondria-targeting organic nanoparticles for enhanced photodynamic/photothermal therapy. ACS Appl Mater Interfaces 12: 30077-30084, 2020.

Zhang CX, Cheng Y, Liu DZ, Liu M, Cui H, Zhang BL, Mei QB and Zhou SY: Mitochondria-targeted cyclosporin A delivery system to treat myocardial ischemia reperfusion injury of rats. J Nanobiotechnology 17: 18, 2019.

Sun J, Zhang J, Tian J, Virzi GM, Digvijay K, Cueto L, Yin Y, Rosner MH and Ronco C: Mitochondria in sepsis-induced AKI. J Am Soc Nephrol 30: 1151-1161, 2019.