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Review

Modern methods of detecting mitophagy

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Abstract

Inhibition of mitophagy is one of the signs of chronic disease pathogenesis. Detection and measurement of mitophagy levels under in vitro and in vivo models provide a better understanding of the role of mitophagy disorder in disease development and serve as prerequisites for creating a clinically applicable system test. The development of such a system is potentially feasible, but taking into account a number of factors that will be discussed in detail in this article. Here it is considered the main models of mitophagy-based test systems and an analysis is carried out showing their advantages and disadvantages. The future potential for the development of mitophagy-based diagnostic test systems is also discussed here.

Keywords: Mitophagy, Oxidative stress, Mitochondria, PINK1, Parkin.

1. Introduction

Mitophagy is the process of selective destruction of damaged and dysfunctional mitochondria (those that poorly perform their inherent functions) [1]. Mitophagy is a special case of autophagy - a long-standing evolutionary process aimed at the destruction of cellular proteins, as well as damaged cellular organelles by creating a two-membrane structure - an autophagosome in which final lysis occurs [2]. Autophagy can be selective and non-selective. In the first case, autophagy is activated in the presence of excess or damaged protein macromolecules or organelles at any energy balance of the cell. Non-selective autophagy occurs when the cell is in a state of nutrient deficiency and energy deficiency, in such a situation the cell uses its resources to restore [2]. As this is clear from the above mitophagy is a variant of selective autophagy, it is one of the most studied variants of autophagy. The importance of mitophagy is due to the important functions of the mitochondria themselves, which are necessary to ensure the normal functioning of the cell. Mitophagy refers to the processes of mitochondrial dynamics, which in addition to it also include division, fusion and mitochondrial transport. The main function of mitochondria is the energy supply of the cell, which is carried out by oxidative phosphorylation, during which "molecular accumulators" - ATP [3] are formed. In addition to the energy function, mitochondria take part in the synthesis of fatty acids and amino acids, the formation of heme and iron-sulfur clusters, as well as regulate calcium flow, are one of the links in the apoptosis initiation chain and produce active oxygen species (ROS) [3]. ROS at low concentrations are useful signaling molecules, but at high concentrations, they become dangerous highly reactive molecules that cause damage to organic macromolecules: proteins, lipids and nucleic acids, which leads to the destruction of cellular structures, including mitochondria themselves, and subsequently to cell death and the development of an inflammatory reaction [2]. ROS are formed as a by-product of oxidative phosphorylation, when disturbances occur in reactions in electron transfer along the electron transport chain, the efficiency of ATP formation decreases, while ROS generation increases [4]. That is, dysfunctional and damaged mitochondria are the main initiators of the development of oxidative stress, therefore they are undesirable and even dangerous structures

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for maintaining normal cellular homeostasis. During mitophagy, the destruction of unhealthy mitochondria occurs and is an obstacle to the development of oxidative stress in the cell. However, the mitophagy process itself is complex, involving the coordinated operation of various proteins, and if at least one of the components of the mitophagic reaction is disrupted, mitophagy can be interrupted.

Disorder of mitophagy at the macro level is one of the factors for the development of various chronic diseases. The role of mitophagy is described in the pathogenesis of cardiovascular, neurodegenerative, oncological, metabolic and inflammatory diseases [5]. The heart for its work needs a large influx of energy, which occurs mainly by oxidative phosphorylation [5]. Mitochondria are very important structures for the proper functioning of the heart muscle: the proportion of mitochondria by volume in cardiomyocytes reaches 30-40% [6]. In the study [7] it is noted that the level of mitophagy in cardiomyocytes decreases with age. Mitophagy disorder has been described for cardiovascular diseases such as atherosclerosis, coronary heart disease, heart failure and cardiomyopathy, as well as arrhythmias and hypertension [6]. The association of mitophagy disorder with the development of two main neurodegenerative diseases: Parkinson's disease and Alzheimer's disease has been shown [5]. Thus, PINK1 and Parkin proteins involved in the implementation of mitophagy are the main recessive risk factors for Parkinson's disease [5]. The association of mitophagy with neurodegeneration is due to the polar structure of neurons needed to transmit the nerve impulse. The polar structure of neurons requires localization of a large number of mitochondria at synaptic transmission sites, which makes neurons highly vulnerable to mitochondrial dysfunction [8]. When mitophagy is disrupted, mitochondria, which are not able to provide energy to the synaptic transmission process, accumulate, which leads to disruption of neurons. The role of mitophagy is also studied as a factor in the development of cancer. Accumulation of dysfunctional mitochondria in tumor cells leads to Warburg effect - shift of preferential metabolism of tumor cells from oxidative phosphorylation to glycolysis [9]. The increase in the number of dysfunctional mitochondria is also the cause of cell metabolism disorders and, as a result, the development of metabolic diseases such as type 2 diabetes mellitus and obesity [10]. However, perhaps, the main group of diseases, one of the pathogenesis factors of which is a disruption of mitophagy are inflammatory diseases, for example, Crohn's disease, sepsis, and type 1 diabetes [11, 12]. This is due to the fact that insufficient work of mitophagy proteins leads to an excess of unhealthy mitochondria producing a large amount of ROS, which are the main initiators of the inflammatory reaction. Secondly, damaged mitochondria, breaking down, secrete antigenic molecules associated with internal damage - DAMPs, which are similar to microbial PAMPs, initiate the triggering of innate immunity. Based on the above, mitophagy disturbance can be considered a potential predictor of the development of various diseases. Therefore, methods of detecting this process can bring assistance in the diagnosis and treatment of mitochondrial dysfunction-related diseases. In this article, we will discuss the existing methods for detecting mitophagy, as well as the possibility of creating full-fledged diagnostic test systems based on them.

2. Mitophagy mechanism

Mitophagy is a complex process that involves several consecutive stages: initiation of mitophagy, preparation of mitochondria selected for degradation for recognition by autophagy mechanism, absorption of labeled mitochondria by autophagosome and final lysosomal sequestration and hydrolytic degradation of mitochondria [13]. Depending on molecular mechanisms, PINK1/Parkin-dependent mitophagy and mitophagy mediated by receptors are emphasized, both types of mitophagy are initiated in response to different stimuli [14]. PINK1 and Parkin are the most well-known proteins involved in maintaining mitochondrial quality control. Disruption of both these proteins is associated with the development of Parkinson's disease [15]. PINK1 is a serine/threonine kinase and Parkin is a cytosolic ubiquitin-E3 ligase [1]. In healthy mitochondria, the N-terminus of the PINK1 is transported to the inner mitochondrial membrane, binding to the TOM/TIM complex, the transfer efficiency depends on the value of the mitochondrial membrane potential, while the C-terminus of the protein molecule containing the kinase domain is pulled into the cytoplasm. Once on the inner mitochondrial membrane, the PINK1 is partially degraded by mitochondrial processing peptidase (MPP) and presenilin-associated diamond protease (PARL). The remaining undisturbed central domain of PINK1 possessing kinase activity undergoes proteasomal degradation in cytosol [1]. Stress factors, such as depolarization of the mitochondrial membrane, proteotoxicity, dysfunctional state of proteins of the electron transport chain complex in the mitochondrial matrix, and increased mutagenicity of mitochondrial proteins lead to the accumulation of undegradated molecules PINK1 on the outer mitochondrial membrane due to disruption of the intermembrane transfer of the N-terminal domain PINK1 to the outer mitochondrial membrane. The PINK1 molecules accumulated on the outer membrane of mitochondria homodimerize, which leads to autophosphorylation and subsequently promotes kinase activation and improved binding to PINK1: Parkin and ubiquitin substrates [15]. Due to the properties of rapid accumulation of molecules on the outer membrane of mitochondria and the ability to activate in response to mitochondrial stresses, PINK1 can be considered an effective sensor of mitochondrial damage or mitochondrial dysfunction. Parkin, as noted above, is a ubiquitin-E3 ligase and contains a ubiquitin-like domain and four RING domains that, due to intramolecular interactions, block the active site and show competitive activity for binding to the E2 ligase [16]. In the case of mitochondrial injury or dysfunction, PINK1 activates Parkin in two ways: firstly, through binding to ubiquitin and its subsequent phosphorylation at the Ser65 position, which in turn binds to Parkin and activates it; secondly, directly phosphorylating Parkin at the Ser65 position in the ubiquitin-like domain of Parkin, which leads to conformational changes in the Parkin protein and allows it to interact with the E2 ligase, which triggers the ubiquitinylation reaction [15]. Parkin acts as a signal enhancer for mitochondrial damage from PINK1, promoting the generation of chains from ubiquitin protein molecules, which leads to the attraction of even more Parkin molecules to mitochondria. By recruiting to the mitochondria, Parkin marks with ubiquitin the various mitochondrial proteins contained on the outer mitochondrial membrane, in the matrix and on the inner mitochondrial membrane [17].

The appearance of numerous ubiquitin chains leads to the attraction and binding to the mitochondrial surface of autophagy mediators: RABGEF1, RAB5, RAB7A, OPTN, NDP52, TAX1BP1 and p62, called adapter proteins. Like the mechanism of general autophagy, light chain 3 (LC3) of protein 1 associated with microtubules recognizes and interacts with these adapter proteins, which subsequently initiates the formation of mitophagosomes in which unhealthy mitochondria undergo final degradation [18]. It is reported that for PINK1 and Parkin, there are a number of natural proteins of activators and inhibitors present in cells, which are considered possible targets for the therapy of diseases associated with mitochondrial dysfunction [19].

Some proteins that are contained in the inner and outer mitochondrial membrane are receptors of mitophagy and can directly induce mitophagic destruction of mitochondria. Examples of such proteins are cardiolipin (CL), deflibitin 2 (PHB2) - on the internal mitochondrial membrane, FUNDC1, BNIP3L/NIX, FKBP8 - on the external mitochondrial membrane [1]. Regulation of receptor-mediated mitophagy occurs at the transcriptional or post-transcriptional level in response to the occurrence of various mitochondrial stress states. The mitophagy receptor is FUNDC1 activated in response to hypoxia. In the conditions of a normoxia, FUNDC1 is phosphorylated on the Tyr18 by a kinase of SRC and on Ser13 CK2 kinase that leads to suppression of activity of FUNDC1 LIR motive responsible for activation of a mitofagiya. When hypoxia occurs, LIR is activated, which binds to LC3, promoting mitophagosome formation [20]. In addition, phosphatase PGAM5 in hypoxia dephosphorylates FUNDC1, which leads to separation between FUNDC1 and OPA1, which leads to inhibition of mitochondrial fusion, Drp1 recruitment additionally occurs, which promotes mitochondrial division, which is a prerequisite for further mitophagy [20]. Receptor BNIP3 is activated in hypoxia through interaction with hypoxic-inducible factor 1 (HIF-1), which leads to activation, LC3-dependent mitophagy and prevention of excessive production of mitochondrial ROS, thus blocking the possibility of oxidative stress [21]. Phosphorylation of BNIP3 by amino acid residues Ser17 and Ser24, and phosphorylation of NIX by amino acid residues Ser34 and Ser35 leads to increased binding of these proteins to LC3, which also leads to activation of NIX/BNIP3-mediated mitophagy [21]. In addition, for BNIP3, it has been shown that this protein enhances mitophagy through inhibition of cleavage of protein PINK1, which shows that different pathways leading to mitochondrial sequestration can intersect [22]. FKBP8 is the most recently discovered mitophagy receptor, it is able to interact with LC3 like other mitophagy receptors, activating mitochondrial degradation without the participation of the enzymes PINK1 and Parkin, however, further study is required to better understand its mechanism of action [23]. The role of the inner mitochondrial membrane protein PHB2 in mitophagy activation in mitochondrial membrane depolarization has been shown. PHB2 stabilizes PINK1 on the internal mitochondrial membrane and promotes the recruitment of Parkin in the mitochondria through inhibition of the action of the PARL protease, which takes part in the cleavage of PINK1, and the prevention of degradation of the enzyme PGAM5 - one of the mitophagy enhancers. These events lead to rupture of the outer mitochondrial membrane by proteasomes. Then PHB2 is transported from internal mitochondrial membrane to external one, where it interacts with LC3, which activates the final stages of mitochondrial degradation [24]. Cardiolipin (CL) is a phospholipid of the internal mitochondrial membrane and under normal conditions serves to maintain the structure of the internal folds of the mitochondrial membrane - crist. At the same time, there is evidence of his participation in the mitophagy process. The study [25] showed that the NDPK-D hexamer protein localized in mitochondrial intermembrane space enhanced CL transfer to the outer mitochondrial membrane in mitochondrial injury. For different cell types, it has been shown that CL localized to the outer mitochondrial membrane is the signal to degradation of damaged and dysfunctional mitochondria. CL can thereby directly interact with LC3 for subsequent mitophagy [25]. Depending on the physiological state of cells mitophagy is divided into basic, programmed or induced by stress [26]. Basic mitophagy occurs during normal cell homeostasis and is a continuous process that is in equilibrium with other processes of mitochondrial dynamics, during which old damaged and dysfunctional mitochondria are removed, and new ones are synthesized in place by biogenesis [27]. Baseline mitophagy levels correlate highly with cell type depending on their energy needs. So, the highest levels of mitophagy were shown in the kidneys and heart, organs that consume a large amount of ATP [28]. Programmed mitophagy is realized in cellular differentiation and maturation of tissues and organs. The main role of mitophagy in this case is to remove the excess number of mitochondria [1]. For example, mitochondrial receptor NIX participates in mitochondrial removal in erythrocyte differentiation [29]. In addition, programmed mitophagy has been shown to remove "paternal" mitochondria from fertilized oocytes [30]. Programmed mitophagy is also observed in maturing myoblasts, in which there is a shift of energy exchange from glycolysis to oxidative phosphorylation [31]. Stress-induced mitophagy occurs in pathological states to adapt the cell to changed conditions. This type of mitophagy protects the cell from impaired energy metabolism, development of oxidative stress and cell death [32]. Mitophagy disorder under stress conditions is associated with the development of various chronic diseases described in the previous section, as well as with the aging of the body.

3. *In vitro* mitophagy detection models

The main methods used to detect mitophagy in mammalian cells are electron microscopy, with the purpose of searching for autophagosomes, fluorescence microscopy based on the use of specific mitophagy labels, and western blotting, measuring the degree of degradation of mitochondrial proteins [33]. Here we look at the main mitophagy study models used on cell culture using predominantly fluorescence microscopy as the most complex and more commonly used method in the field.

3.1. Measurement of mitochondrial membrane potential

Since the depolarization of the mitochondrial membrane is one of the main stress factors that initiate the launch of mitophagy, the use of potentiometric dyes can reveal the development of mitophagy in the early stages. The main dyes used for this purpose are TMRM, TMRE and JC-1 [34]. TMRM and TMRE dyes are not able to

persist for a long time in the depolarized mitochondrial membrane, therefore their use in long-term studies is limited [35]. Dye JC-1 looks like a more promising option. In healthy mitochondria with a normal level of membrane potential, JC-1 at high concentrations penetrates and accumulates in mitochondria, where it begins to form molecular complexes - J aggregates, the maximum fluorescence of which is in the red zone. In unhealthy mitochondria, the dye JC-1 accumulates in lower concentrations due to disruption of transport through the depolarized membrane, JC-1 molecules do not collect into complexes and preserve the maximum fluorescence in the green spectrum [36]. A successful choice of dye can allow a fairly accurate measurement of the membrane potential of mitochondria, however, depolarization of the mitochondrial membrane does not always directly correlate with the level of mitophagy.

3.2. Detection of mitochondrial colocalization with autophagosomes and lysosomes

Since unhealthy mitochondria in the process of mitophagy connect to structures called autophagosomes, where they are sequestered from healthy mitochondria for further degradation, a quantitative assessment of mitochondrial colocalization and autophagosomes can serve as an effective marker of mitophagy. For labeling mitochondria in living cells, the dye MitoTrackerRed is most often used for this purpose, and for labeling autophagosomes, a hybrid protein GFP-LC3 is used, giving a green color. Colocalized mitochondria and autophagosomes are observed in photographs in the form of yellow dots [2]. When analyzing mitophagy for fixed cells in addition to MitoTracker, antibodies with a fluorescent label to mitochondrial proteins: Tom20, VDAC or a protein subunit of the IV complex of the electron transport chain [2] can be used to visualize mitochondria. Since at the final stage of mitophagy autophagosomes with mitochondria merge with lysosomes, where mitochondria are degraded, the colocalization of mitochondria and lysosomes is also the marker of mitophagy [2]. To visualize this process, dyes MitoTracker - for staining mitochondria and LysoTracker - for staining lysosomes are used, but it is need to know that LysoTracker is not a strictly specific dye for lysosomes, but stains all compartments of a cell with a low level of pH, so as an alternative, antibodies with a fluorescent label to lysosomal proteins LAMP-1 and LAMP-2 [2] can be used. The advantage of this mitophagy evaluation model is the ability to trace the development of mitophagy in dynamics on living cells, however, the accuracy of the analysis may not be high enough due to possible non-target binding of dyes [2].

3.3. Mitochondrial mass assessment

Mitochondrial mass change analysis can be used to assess mitochondrial degradation by mitophagy. FACS (Fluorescence-Activated Cell Sorting Analysis) can be used with introduction of the already mentioned dye MitoTracker, which was successfully demonstrated in analysis of programmed mitophagy at maturation of red blood cells [37]. The second method of evaluating mitochondrial mass is immunostaining of mitochondrial proteins using specific antibodies [2]. The third method of mitochondrial mass assessment is determination of mitochondrial and nuclear DNA ratio measured using quantitative PCR [38]. The advantage of this model for assessing mitophagy is that it is based on accurate quantitative methods so that the results are highly objective. At the same time, the selection of mitochondrial proteins as targets for the antibody should be carried out more carefully, since some mitochondrial proteins (primarily membrane proteins) can be cleaved by direct proteasome degradation without mitophagy [39].

3.4. Analysis of mitochondrial enzyme activity

Since mitochondria are the center of cell energy exchange, they contain a large number of important enzymes involved in the tricarboxylic acid cycle and oxidative phosphorylation. Mitochondrial reduction due to mitophagy action can be measured by measuring the activity of some mitochondrial enzymes. A suitable variant is the enzyme citrate synthase, whose activity is most often independent of mitochondrial dysfunction, but correlates with a change in mitochondrial mass, as demonstrated in various studies [40], [41]. The change in enzyme activity can be easily quantified, but its mitophagy specificity is not fully known.

4. *In vivo* mitophagy detection models

The use of animal mitophagy detection models allows us to expand the understanding of the role of mitophagy in the functioning of tissues and organs, as well as to better understand how mitophagy disorder affects the pathogenesis of the studied diseases. There are several characterized models of transgenic reporter mice that can be used to study mitophagy.

4.1. Autophagosome imaging

GFP-LC3 is the first transgenic reporter line of mice designed to study autophagy. The fusion protein GFP-LC3 binds to the membranes of autophagosomes and gives color in the green region of the spectrum [42]. Mitophagy detection requires additional immunostaining on mitochondrial proteins. However, the disadvantage of this model is also associated with this: even using the immunostaining of mitochondrial proteins, the presented model well characterizes general autophagy [42].

4.2. pH-dependent localization in lysosomes

The mt-Keima protein targets the mitochondrial matrix and is able to change the color of fluorescence depending on the pH medium: in neutral (for example, in autophagosomes), the color is green, and in acidic (for example, in lysosomes) the color changes to red. Thus, it is possible to estimate the degree of mitochondrial degradation in lysosomes that emit a red signal [33]. The advantage of this protein is its high stability in lysosomes [33]. Transgenic mt-Keima mice proved to be a useful model for studying the pathogenesis of neurodegenerative diseases in several studies [43], [44]. However, several disadvantages of using this model have been identified. Firstly, the obtained samples cannot be fixed due to a disruption of the pH gradient, secondly, the fluorescence spectra of the mt-Keima protein overlap partially, which can lead to an orange color in lysosomes, thirdly, it is difficult to determine the location of the fluorescence signal in mouse tissues [33].

The mito-QC model of transgenic mice is a more advanced variant of pH-dependent mitophagy detection. Mito-QC contains a fusion fluorescent protein mCherry-GFP with a targeting sequence of mitochondrial membrane protein FIS1. In cytoplasm at neutral pH mCherry

and GFP show stable fluorescence of red and green color, however at hit of mitochondrions in lysosomes the signal of GFP is extinguished therefore during lysosomal degradation of mitochondrion is visible only a red luminescence of a signal from mCherry [45]. This model has a number of advantages. So, it is convenient to compare the level of mitophagy in various tissues and organs with the help of this model. So, in the study [45] it was shown that mitophagy passed at the same speed in skeletal muscle and heart, the highest degree of mitophagy was found in liver and kidneys, and the smallest in spleen. In addition, tissue samples can be fixed at neutral pH values and there is no overlap between fluorescence spectra mCherry and GFP, which allows a clear interpretation of the results [43]. A disadvantage of this system is that these fluorescent proteins can be destroyed by proteasomes. In addition, it is difficult to analyze individual cell populations in the same tissue, which requires the use of an additional reporter [33].

5. Potential to create diagnostic mitophagy detection test systems

So far, the application of the mitophagy detection system in clinical practice has been extremely difficult. First of all, the transgene administration of fluorescent proteins, similar to that performed in mouse models, is impossible for humans on either the ethical or practical side, so a potential test system can only work in vitro without interference with the human body. In addition, for many diseases, especially neurodegenerative ones, for which the association of pathogenesis with mitophagy disorder is shown, the target population of cells in which pathology is present is in a difficult place for analysis, for example, in the brain. This means that the biomaterial for analysis in this case would be taken by biopsy, which seems to be an unnecessarily time-consuming procedure for this diagnosis. The creation of a test system for determining the level of mitophagy will be the optimal option for diagnosing the development of systemic inflammatory diseases, for example, sepsis, the intake of biomaterial in this case will be carried out from the bloodstream. An important feature of the test system for detecting mitophagy should be its complexity, that is, the test system should respond to molecular mitophagic targets differently in time intervals: premitophagic formation of ubiquitin chains in mitochondria, mitochondrial sequestration in autophagosomes, fusion of an autophagosome containing mitochondria with lysosome. This is necessary to understand which of the stages of mitophagy is disturbed and, accordingly, which proteins may be responsible for this disruption. Therefore, one of the main preliminary tasks is to find targets optimal for detecting mitophagy. The fluorescent dyes selected for detection should have non-overlapping spectra to better understand the results obtained, as well as be resistant to cellular proteases and not have an off-side effect on mitochondrial proteins. It is also important that the results should be both qualitative and quantitative with a clear definition of the boundaries of the norm. This step will require further in-depth study at what levels the reduction in mitophagy is associated with pathology. For high-quality fluorescent imaging of the results, the use of ultra-high resolution microscopy, which can reach 10 nm, can be promising, which will allow to obtain new data on changes to which mitochondria are subjected during pathogenesis [46]. A diagram illustrating the requirements for a potential test system for mitophagy diagnosis with clinical application is presented in Figure 1.

5. Discussion

A lot of research work is required to better understand the role of mitophagy in the development of a number of chronic diseases, but the fact that a mitophagy disorder is found in cells in pathological conditions is already a proven observation. Based on this, the disruption of mitophagy can be considered as one of the predictors or as one of the evidence of an already developed disease. It is worth understanding that a separate fact in itself associated with a change in the level of mitophagy in cells cannot be considered a sufficient argument for the correct diagnosis, however, it can help in the diagnosis of the disease if we consider it in conjunction with other factors. The degradation of mitochondrial proteins may not always be associated with the mitophagy process, the identification of new mitochondrial proteins that undergo mitophagic degradation is an important step in the search for mitophagy markers. Another important step is also the search for new mitophagy-detecting compounds that will allow more accurate interpretation of the results. Mito-QC mice are the most successful animal model of mitophagy study at the moment, which, however, should not stop researchers from developing new more advanced in vivo models, the application of which will allow a more thorough study of the role of mitophagy in the pathogenesis of diseases. An additional issue requiring close attention is the comparative analysis of the level of mitophagy in specific organs in the baseline state and in pathogenesis, which is necessary to better understand the normal values of mitophagy.

6. Conclusion

Mitophagy is a process of selective destruction of damaged and old mitochondria. Mitophagy suppression is one of the signs of the different chronic diseases development, which suggests the potential use of mitophagy disorder signs as predictors of these diseases. At the moment, the most promising methods for detecting and measuring mitophagy in vivo and in vitro include: measuring the potential of the mitochondrial membrane, detecting jointly localized mitochondria and autophagosomes, as well as mitochondria and lysosomes, measuring the activity of mitochondrial enzymes and assessing the change in mitochondrial mass in the cell. Creating a diagnostic system test to detect mitophagy in clinical practice is a challenging but potentially feasible task. Important factors here are: which disease is better diagnosed in this way, how the biomaterial is taken, which mitophagic targets and dyes

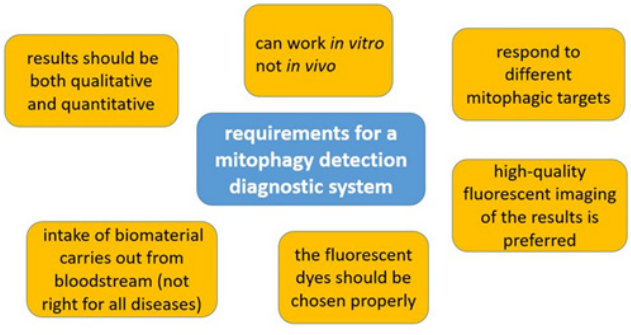

Fig. 1. Requirements for a mitophagy detection diagnostic system.

for detection will be selected, and on which equipment the analysis will be carried out.

Conflict of interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed consent
The authors declare that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
All authors had equal roles in study design, work, statistical analysis and manuscript writing.

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