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Grupo # 8 Cardiovascular Physiology

The role of complex MICOS and its associated proteins in the heart

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Introduction

Mitochondria are cellular organelles located in the cell membrane dedicated to generating the necessary chemical energy to impulse the biochemical reactions of the cell. The chemical energy produced by mitochondria is stored in a small molecule called ATP (adenosine triphosphate). Mitochondria are very present in the heart cells due to the vast demand of energy this organ consumes; they are responsible for the daily production of 6kg of ATP, which is created through a process called oxidative phosphorylation. The heart muscle cells have approximately 5,000 mitochondria per cell. As a result of their high energy demand, the cardiac muscle cells contain more mitochondria than any other organ in the body (Vakifahmetoglu-Norberg, Ouchida, & Norberg, 2017).

The heart is a fist-sized muscle located behind and slightly to the left of the sternum. The heart pumps blood through the network of arteries and veins, this is called the cardiovascular system. The heart's job is to pump sufficient blood to supply a continuous amount of oxygen and other nutrients to the brain. Mitochondria plays a vital role in cellular energy, metabolism, and various alert pathways in the cell. Malfunctions in the architecture of mitochondria could result in severe damages to the human body, which could subsequently result in diseases involving both the nervous and cardiovascular system. Therefore, a stable mitochondrial structure is essential for its function. The membrane-level architecture of the mitochondria, specifically the inner

mitochondrial membrane (IMM), is dedicated to the preservation of a complex called mitochondrial contact site and cristae organizing system or MICOS. Studying the composition of this complex at a functional level, as well as the proteins associated with it, will help us understand how defects in the mitochondria influence the development of cardiovascular diseases.

Objective

The objective of this paper is to carry out scientific research related to the proteins responsible for the structure of the IMM and how these have an impact on cardiovascular function. Mitochondria are characterized by their structural compartment division, which allows them to adapt to changes in their morphology. Mitochondria contain two different membranes, the outer mitochondrial membrane (OMM) and the IMM. The latest, further divides into two sub compartments, the inner boundary membrane, and the cristae membrane, which contains independent functions. While the inner boundary membrane relies on protein transport, the cristae membrane has all the proteins necessary for energy production. To promote proper maintenance of these separate compartments to maintain stability, a complex named MICOS come into play. This complex is enriched in the cristae junctions, preserving their characteristic structure, therefore, when this complex is disrupted, defects in mitochondrial architecture are seen as well as functional limitations. MICOS is composed of multiple subunits, as well as different associated proteins that aid in maintaining IMM integrity. For this work, only the following proteins will be discussed: Mic-60, Mic-19, OPA1, ATP synthase, and Prohibitins.

Mic-60

Imagine anxiously wanting to arrive at an important meeting at a certain location; however, when arriving you encounter a building with no doors and no entry. This would surely not be a pleasant experience and when talking about millions of little buildings without doors inside the

human body, it would be chaos. Fortunately, these buildings, mitochondria, do have a door called the mitochondrial contact site and cristae organizing system, also known as the MICOS subcomplex. In this wide multi-subunit protein, the Mic60 protein plays an essential role for mitochondria prosperity. Mic-60 also identified as the heart protein, or mitofilin, is known to be the most important protein complex in the mitochondrial contact site and cristae organizing system (Feng, Madungwe, & Bopassa, 2019). It can be easily referred to as the "door-knob" of the door that represents the MICOS complex.

This protein complex is not only a core component for the assembly and maintenance of MICOS (Feng et al., 2019) but it was also found to be the most ancient subunit of the system located in the inner membrane of the mitochondria. Composed of an amino-terminal transmembrane segment and a mitofilin domain in the intermembrane space, the Mic-60 protein can interact with diverse outer membrane protein complexes, support the import of mitochondrial proteins, establish contact sites between the two mitochondrial membranes, and play a crucial role in mitochondrial architecture (Hessenberger et al., 2017).

As previously stated, the Mic-60 protein can be thought of as the door-knob of the MICOS protein, being a key interactor with multiple other complexes and proteins outside the inner membrane. Interaction with the translocase of the outer membrane complex (TOM) and the kinase PINK 1, which oversees protecting the cells that normalize cristae structure from stress induced mitochondrial dysfunction are just two in the list of proteins Mic-60 co-relates with to achieve mitochondrial stability. Furthermore, it is thought that this heart protein facilitates the passageway of mitochondrial proteins that serve as an essential function in cargo transport and signaling and are also significantly important for membrane biogenesis processes, b-barrel proteins, seals the necessity of having an intact Mic-60 protein.

Now that the importance of the heart protein and its influential role in mitochondrial prosperity has been stated, the idea of said protein not being present or functioning properly can be clearly seen as unfavorable to biological processes, thus having a direct effect on human health. Deletion of the heart protein may result in irreversible damage to the mitochondrial inner membrane architecture including loss of cristae junctions and detachment of cristae membranes (Feng et al., 2019). Moreover, lack of Mic-60 results in an augmented rate of ATP synthase oligomerization and poor ultrastructure (Wollweber, von der Malsburg, & van der Laan, 2017). Additionally, an overexposure of the Mic-60 protein causes unregulated cristae branching and an increased number of cristae junctions (Wollweber et al., 2017). The effects of this overexposure were researched through a yeast model that evaluated mitochondrial structure with the presence of an altered mitofilin protein. The results showed that an increase in the size of the mitofilin protein altered the whole internal mitochondrial structure, including an eye-opening loss of cristae junctions, again evidencing the importance of a stable mitochondrial contact site for energy and other proteins to be distributed efficiently throughout the whole cell. These mitochondrial abnormalities, although seem small, may spawn life-threatening diseases, such as cardiovascular dysfunction. For instance, Diabetes type 1 patients were found to have disorganized mitochondrial cristae on skeletal muscle and cardiac tissue, while Ischemia/Reperfusion IR (stroke) patients were found to have a reduction or loss of Mic-60 that was associated with increased ROS production. However, ROS production is found to have both positive and negative outcomes in cell death events surrounding I/R, leaving an unclear role for Mic-60 during IR (Feng et al., 2019). This and previous evidence prove how alterations in microscopic protein complexes, such as the mitofilin

protein, are highly likely to be responsible for respiratory deficiencies, unwanted cell deaths surrounding the IR among other health complications (Rabl et al., 2009).

Mic-19

As mentioned before, the MICOS is organized by structures called cristas that are essential for mitochondria to work. The deletion or knockdown of MICOS components cause defects in mitochondrial morphology and mitochondrial disfunction. Deletion of subunits of the MICOS complex cause partial or almost the entire loss of crista junction. MICOS complex builds a protein interaction network with different mitochondrial localizations and functions. One of these proteins is called Mic-19. Mic-19 is a peripheral protein of the inner mitochondrial membrane and component of the MICOS complex. This protein maintains mitochondrial morphology and structure of the inner membrane to make mitochondrial work. Mic-19 protein is related to the mitochondria in many ways. It undergoes oxidation in the mitochondria and provides stability in the contact sites and cristas (Sakowska et al., 2015). Mic-19 is one of the mitochondrial intermembrane space bridging (MIB) components. Mic-19 works together with SAMM50 protein by undergoing a process named N-myristoylation. This has a very important role in protein-protein functional bonding in the mitochondria. Mic-19 is critical and essential for the structure and the correct function of the mitochondria, working as a mediator between the IMM and the OMM membranes (Utsumi et al., 2018). Mic-19 is also related to cardiolipin, a component of the inner membrane of the mitochondria that is widely located in the heart mitochondrial membranes. Cardiolipin joins with Mic-19 and helps to assemble and stabilize MICOS complex and provides membrane stability (Dudek, Hartmann, & Rehling, 2019). A study entitled The Oxidation Status of Mic19 Regulates MICOS Assembly shows that human and Saccharomyces cerevisiae Mic-19

undergo oxidation in mitochondria and requires the mitochondrial intermembrane space assembly pathway, which couples the oxidation and import of mitochondrial intermembrane space protein for mitochondrial localization (Sakowska et al., 2015). In this investigation it was identified Mic19 in yeast and Mic-19 in humans in the MICOS complex of both of them. Human Mic-19 possesses CHCH domain that is essential for the import of Mic-19 into mitochondria. In yeast Mic19 the arrangement is different from the human Mic-19. However, both are essential for the maintenance of mitochondrial morphology. For this investigation it was used yeast strains, *S. cerevisiae*, plasmids and siRNA constructs and transfection. Yeast cells were grown at 19-37℃ on YPG medium. To isolate mitochondria, differential centrifugation was used according to standard procedure. Mitochondrial samples were prepared for electrophoresis. For isolation of human mitochondria, human 293 embryonic kidney cells were cultured in Dulbecco's modified eagle medium. Cells were harvested and homogenized in a Dounce homogenizer in isolation buffer. The results of this study were that human Mic-19 forms two disulfide bonds in the CHCH domain, and its mitochondrial localization depends on the MIA pathway. Import of yeast Mic19 into mitochondria is dependent on the MIA pathway. Although the redox state of Mic19 does not influence its mitochondrial localization, the MICOS complex contains oxidized Mic19 and the oxidation of Mic19 is important for MICOS stability. The lack of this protein or its deletion caused destabilization of the MICOS complex and altered the mitochondrial internal membrane morphology affecting the mitochondrial functions (Sakowska et al., 2015).

The role of mitochondria in cardiovascular diseases is prone to be a potential therapeutic target. If the Mic-19 and MICOS complex are affected, then the mitochondria may not function properly which can result in cardiovascular diseases. Mitochondrial dysfunction is a factor in the pathogenesis of several chronic human diseases, including cardiovascular diseases. Dysfunctional

mitochondria compromise cellular respiration and energy production and lead to oxidative stress causing cell damage and death. Examples of cardiovascular diseases if the mitochondria are affected are arteriosclerosis of vital arteries, altered blood lipid profile, atherosclerotic plaque development in the vascular wall, aneurysm, heart failure, hypertension, hyperlipidemia (Poznyak, Ivanova, Sobenin, Yet, & Orekhov, 2020). The correct function of the mitochondria has a direct impact in the execution of the heart. The way that the heart functions depend on the energy that the mitochondria forms, its structure, and its correct function. If one of these gets affected the repercussions can be cardiovascular diseases or sudden death. Therefore, Micc-19 is very important and essential for the mitochondria to function correctly. If this protein is altered, it affects the morphology of the mitochondria and can cause a mitochondrial dysfunction.

ATP Synthase

One of the important proteins related to MICOS is ATP synthase, also known as Complex V. This protein is dedicated to the production of adenosine triphosphate, or ATP for short, the main energy molecule used in all cells, and is in the IMM (Jonckheere, Smeitink, & Rodenburg, 2012). Complex V also serves as the opposing force for MICOS, making it a complementary enzyme for the structure of mitochondria. Crosstalk between MICOS and ATP synthase is crucial to preserve mitochondrial formation. This protein creates the positive curvatures or proton traps at the cristae rims by assembling into rows or other oligomeric configurations. The absence of Complex V may precipitate cell death. Therefore, it is critical to sculpt cristae and maintain mitochondrial function (Cadena, Gahura, Panicucci, Zikova, & Hashimi, 2021).

ATP synthase is organized into dimers and higher oligomers that provide stabilization for the complex; specifically, oligomerization facilitates ATP synthesis. The complex structure is

divided into two functional domains: F1 and Fo. F1 is a soluble portion located in the mitochondrial matrix and it is composed of 3 copies of subunits α and β: and one copy of γ, δ and ε. These last three make up the central stalk of the complex. Fo consists of a subunit c-ring with 8 copies, and one copy of subunits a, b, d, F6 and the oligomycin sensitivity domain. Each of the subunits in these domains have a specific function to produce adenosine triphosphate (Jonckheere et al., 2012).

As mentioned before, the main function of ATP synthase is to produce ATP adenosine diphosphate (ADP) and inorganic phosphate (Pi). This is achieved through a process called ATP synthesis. Although ATP synthase assembly and sequence remains partially hypothetical due to its biogenesis not being studied easily, the synthesis of ATP has been hypothesized to have a sequence like this one: first, energy derived from a gradient of protons crosses the inner mitochondrial membrane through the Fo portion of the enzyme. Then, the proton gradient establishes a proton motive force with two components: a pH differential and an electrical membrane potential. The released energy causes rotation of two rotary motions: the rings of subunit c; and subunits γ, δ, and ε. Protons then pass Fo through subunit α to the c-ring. Rotation of subunit γ provides energy for ATP synthesis, or rotary catalysis. Each side switches through conformations in which ADP and Pi bind. Rotating is performed by the γ subunit while α and β remain in a fixed position through peripheral stalk. This creates ATP and finally, it is released. ATP synthase, through all its subunits, uses the energy created by the proton electrochemical gradient to phosphorylate ADP to ATP. This takes place in the mitochondrial matrix (Jonckheere et al., 2012).

The relationship between MICOS and ATP synthase can be closely observed through the paper *MICOS and F1FO-ATP synthase crosstalk is a fundamental property of mitochondrial*

cristae. The investigators in this paper looked to discover if the crosstalk between MICOS and ATP synthase dimers is a fundamental property of cristae. To prove this theory, they used the model T. brucei, an organism that is part of the supergroup Discoba. Within the organism, they proceeded to deplete various subunits of MICOS and ATP synthase to observe each other reaction to the absence of the other. These scientists were able to conclude that the crosstalk between MICOS and ATP synthase was indeed a fundamental property of mitochondrial cristae.

The heart has a high production and consumption rate of ATP which is required to maintain its continuous mechanical work. Problems in all the ATP generating processes may severely affect contractile function directly. Changes in substrate utilization, which often occur in Ischemia/Reperfusion IR alterations, eventually leads to mitochondrial dysfunction, resulting finally in ATP deficiency which further impaired contractility. Therefore, alterations in cardiac metabolism can affect the progression to heart failure by mechanisms beyond ATP supply (Doenst, Nguyen, & Abel, 2013).

OPA1

Another protein that is associated with the MICOS complex is OPA. OPA1 is the only protein responsible for the fusion of the IMM. This protein can be mutated and produce hereditary diseases. OPA1 undergoes subsequent dynamic changes and interacts in a pathway called mitochondrial dynamics that involves processes of fission and fusion. OPA1 is found in the IMM and plays a really important role in the mitochondria. OPA1 preserves cellular death and organizes mitochondrial cristae. By undergoing fusion this protein can control the mitochondrial shape. Moreover, it also plays a role in the mitochondrial regulation of apoptosis and the maintenance of (mtDNA) (MacVicar & Langer, 2016). OPA1 has a dual role in maintaining mitochondrial

morphology and energetics through mediating inner membrane fusion and maintaining the cristae structure. OPA1 is composed of two different isoforms, a long OPA1 (L-OPA1), which promotes mitochondrial fusion and a short OPA1 (S-OPA1) which is created by the proteolysis of L-OPA1, that is involved in mitochondrial fission (Hu et al., 2020).

Excessive OPA1 processing creates fragmentation, and this may cause cell death and tissue degeneration. If the balance of fusion (combining mitochondria) and fission (mitochondria fragmentation into smaller pieces) is interrupted it can cause stress, pathologic conditions and can result in cell death. The mitochondrial fusion secures the distribution of mitochondrial DNA and is associated with respiratory efficiency. Loss of the OPA1 fusion property may make the mitochondria more susceptible to develop apoptosis and can cause the collapse of the mitochondrial network (Hu et al., 2020). Diseases associated with OPA1 include, but are not limited to Optic Atrophy, Optic Atrophy with or without Deafness, Ophthalmoplegia, Myopathy, Myopathy, and Ataxia. Optic Atrophy is a condition in which the vision slowly starts to decrease. Optic Atrophy with or without deafness is a disorder of visual loss and sensorineural loss. Ophthalmoplegia is a condition in which the eye muscle becomes paralyzed. Myopathy is a general term for any condition that affects the muscles that are responsible for voluntary movement in the body. Ataxia is a term used for a group of disorders that damage coordination, balance, and speech.

In an experiment performed by Hu, C. et alin 2020, they found that cristae dynamics is regulated by OPA1. The goal of this project was to investigate the role of OPA1 in mitochondrial crista dynamics. These researchers used mouse embryonic fibroblasts (MEF/s) as models and did a genetic modification to delete OPA1 gene (OPA1 KO). They arrived at the conclusion that lack

of OPA1 increases fragmentation and that the cristae showed a morphology crooked and disordered (Hu et al., 2020).

Prohibitins

Prohibitin 1 & 2 are proteins which are “ubiquitously” expressed in different tissues, and are present in the various organanelles, besides the mitochondria like the nucleus and cytosol. PHB proteins are highly expressed in cells depending on the role they play during the mitochondrial function. Prohibitin 1 & 2 form a Ring-Like structure of 1 MDa, consisting of 12-20 PHB heterodimers. Prohibitin 1 was identified as an Anti-Proliferative in mammalian cells, whereas Prohibitin 2 was identified by its binding to the IgM antigen receptor. Depending on the cellular localization, Prohibitin 1 & 2 have distinctive functions, but are more hard working on the mitochondrial function. Prohibitins have a huge impact when it comes to cellular proliferation and mitochondrial housekeeping. The different functions of Prohibitins are mediated by cell compartment-specific attributes (Signorile, Sgaramella, Bellomo, & De Rasmo, 2019).

In the mitochondria, Prohibitin 1 & 2 assemble at the inner membrane to form a supra-macromolecular structure, also including biogenes, bioenergetics and dynamics, so they can determine the cell fate, death, or life. Prohibitin 2 is a highly conserved protein located in the mitochondrial inner membrane, which plays a key role in the cellular energy metabolic homeostasis. Mitochondria are highly dynamic structures that fuse and divide continuously, adjusting their shape and cellular distribution depending on cell type and the energy demands of the cell. Loss of Prohibitins has been shown to affect the mitochondrial morphology as it resulted in fragmented and disorganized mitochondria. Also, the loss of Prohibitins in mouse embryonic

fibroblast, resulted in an increased mitochondrial fission and the loss of mitochondrial cristae (Signorile et al., 2019; Wu et al., 2020; Yang, Li, & He, 2018). (1, 2, 3)

PHB alterations have been found in aging and cancer, as well as neurodegenerative, kidney and cardiac diseases. Overexpression of PHB1 has been found to protect cardiomyocytes from hypoxia-induced cell death by inhibiting cytochrome c release and decreasing levels of Bc12 protein. Also, overexpression of PHB1 in cardiomyocytes has been shown to protect cells from oxidative stress-induced mitochondrial apoptosis and preserve the cytochrome c release and mitochondrial membrane permeability. In another study, the goal was to see how PHB1 and PHB2 protect cardiomyocytes from hypoxia-induced cell death. The model that was used was spontaneous hypertensive rats, in left ventricles with left ventricular hypertrophy. They showed that anoxia treatment induces apoptosis associated with mitochondrial fission and down-regulation of the PHB2 in the mitochondria, and the enforced expression of PHB2 ameliorates mitochondrial fission, in which prevents apoptosis (Signorile et al., 2019; Yang et al., 2018).

Discussion

Mitochondria are essential organelles that play a central role in cellular energetics, metabolism and signaling. They are characterized by their structural differentiation and compartmentalization, which allows them to adapt their morphology dynamically in response to physiological requirements. We have seen that the MICOS complex is essential for mitochondrial inner membrane integrity and function, however, how this complex specifically plays a role and interacts, remains to be find. We do know that when it comes to problems of Ischemia/Reperfusion injury, all these proteins involved in the MICOS complex, as well as the associated proteins, play a big role. After an ischemic episode, different cell death pathways take place, within them, two important pathways are crucial and known to contribute greatly to a big part of the damage

observed. Apoptosis and Necrosis are mechanisms of cellular death, and they are central pathways that are connected to different biochemical and functional connections within the cell. Both Apoptosis and Necrosis can cause heart failure, thus, representing a therapeutic challenge.

Cardiac disease is the disease with major morbidity and mortality statistics worldwide. Inside the MICOS protein complex, all sub proteins must also be properly functioning to achieve stable mitochondrial architecture and overall human health. The Mic-60, for example, being the interactor and connector of the inner and outer mitochondrial membranes, plays a key role in mitochondrial stability. Deletion of this protein or it is presenting any abnormality may result in poor mitochondrial ultrastructure and little to any energy distribution throughout the cell. This may later lead to multiple health complications such as Diabetes type 1 and Ischemia/Reperfusion injury, restating the importance of healthy and stable sub-proteins in the MICOS complex. On the other hand, decreased expression of different proteins like PHBs in cardiac diseases has been studied. As well, has been associated overall with changes in the expression of non-coding RNAs. It was also observed that ATP synthase is needed for a fully functional and healthy mitochondria, and that MICOS and ATP synthase have opposing, but mutually important tasks to maintain mitochondrial function and stability.

In conclusion, all the proteins studied above are crucial for the stability of cristae structure, and therefore play a critical role in mitochondrial formation. For example, without ATP synthase, the whole mitochondria would fail to keep its form and would not be able to perform its function accordingly. It is also responsible for producing ATP and consequently, in charge of the production of the fuel for cellular processes (Belogrudov, 2009; Boyer, 1997). The PHB1 has pleiotropic

functions, such as cell proliferation, cell growth, cell signaling and cell death. Most of these cellular events require a proper signaling mechanism to function properly or effectively. Also, OPA1 is one of the most important proteins in the mitochondria because of all its roles that provides the cell to stay alive, ranging from mitochondrial IMM fusion to cristae morphological changes (Amati-Bonneau et al., 2009). Finally, every single subunit that composes the MICOS complex is essential for maintaining the cristae junctions that promote the dual role within the IMM. The two proteins mentioned above, Mic-60 and Mic-19, are key components of this core complex, establishing once more the importance of maintaining a proper and functional mitochondria crista, more specifically during problems of IR in which the mitochondria are susceptible.

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