

Mitochondrial proteins that connected with calcium: do their pathways changes in PAH?

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Abstract. Calcium can be regulated by mitochondria and also plays a significant role in mitochondrial pathways. Recent study showed mitochondrial protein changes in the right ventricle in pulmonary arterial hypertension, which affects calcium network at the same time. The specific objective of this study is to assess the pathway of calcium transport by permeable pore in mitochondria and investigate the regulation of mitochondrial proteins in order to find the connection between mitochondrial proteins and right ventricular dysfunction in PAH (pulmonary arterial hypertension). This literature-based review came out by searching articles in Pubmed and Science Direct. And the related flow chart is expressed by the form of PRISMA. There is a network between mitochondria and calcium through the transport chain called mitochondria permeability transition pore (MPTP) as well as different kinds of proteins that are located in the mitochondria. MPTP is a kind of mitochondria pore and can have conformational changes after protein phosphorylation or reaction between mitochondrial proteins to activate the apoptosis caspase cascade process in cell death. In addition, MPTP can be activated by other mitochondrial protein like signal transducer activator of transcription3 (STAT3) to activate cytochrome c in pro-apoptosis to initiate cell death at the same time. The most obvious finding from this study is the role of calcium regulation in therapeutic treatment in PAH patients, which suggest an imaginable role for calcium transporter like mitochondria calcium uniporter (MCU) promoting bio-markers in cardiovascular disease resulting from mitochondrial dysfunction. In addition, right ventricle is a target of PAH in which mitochondria in RV would play an essential role in pathways such as ATP production via mitochondria metabolism.

Keywords: Pulmonary arterial hypertension; mitochondria permeability transition pore; signal transducer activator of transcription3; mitochondria calcium uniporter.

1. Introduction

PAH is a kind of pulmonary vasculopathy results from myocardial proliferation, apoptosis resistance, vasoconstriction and inflammation. PAH is characterized by a decreased cardiac index ($< 2.5 \text{ L/min/m}^2$) and increased right-sided, cardiac filling pressures, including right atrial pressure (RAP) $\geq 8 \text{ mmHg}$. What's more, progressive anatomical distortion and enlargement of the RV were shown among 12% of PAH patients [64]. There are factors lead to right ventricular failure (RVF) and PAH: myocardial ischemia after reperfusion, mitochondrial oxidative metabolism shift and insufficient capacity to excessive transpulmonary gradient. In PAH, mitochondrial metabolism is activated and reversibly suppressed. There are kinds of anoxia and reoxygenation-induced injuries in heart, especially in RV, such as phosphorylation, oxidative stress in mitochondria, and ADP exchange induce MPTP opening. RV dysfunction is shown to be associated with mitochondrial dysfunction. Mitochondria is a vital

organelle that is involved in several important pathways for cellular function. Mitochondria is a central mediator in several metabolisms such as energy consumption and apoptosis pathways[55]. There is a connection between RV failure and mitochondrial regulation which acts as the source of ATP. The consumption of oxygen would decrease, which shows the ability to produce energy is connected with RV failure in PAH[6,24]. The mitochondria in RV is a useful target for PAH, and the dysfunction of mitochondria in RV could induce myocardial damage significantly. Present therapies focus on improving RV function including mitochondrial function for a higher survival rate[4]. Calcium transport in mitochondria is a central pathway that is connected with MPTP. It is a mitochondrial transition pore that mediates cell death and cardioprotection targeting in PAH[2,56]. Mitochondrial permeability transition is a phenomenon that the inner membrane suddenly allows free passage of solutes up to 1.5 kDa in size. Evidence indicated that the transition pore mediated by inner membrane and calcium threshold takes

part in mediating cardiac dysfunction and cell death, and causes the occlusion of pulmonary arterioles[55]. There 3 components for MPTP structure: adenine nucleotide translocator (ANT), voltage-dependent anion channel (VDAC) and cyclophilin D[5]. Mitochondrial proteins mediates cell death and other processes by regulating those units. Additionally, MPTP has an excessive opening when oxidative stress and calcium overload happen, which leads to an uncoupling respiratory chain and a potential collapse to cause cell death, mitochondrial swelling, rupture and a morphology change to mitochondria[55,56].

There are four mitochondria proteins vary in RVF and PAH, which affect the opening of mitochondria permeability pore: cyclosporine A (CsA), signal transducer activator of transcription (STAT), glycogen synthase kinase (GSK) and dynamin-related protein (Drp1). CsA is a kind of MPTP blocker that mediates morphological change of mitochondrial, especially in RVF and RV morphology[12]. It is an MPTP related protein in mitochondria that reduces MCT- induced RV mitochondrial damage by inhibiting cyclophilin D. The inhibition produces the cardioprotection against ischemia-reperfusion injury and post-myocardial infarction HF, which can be served as adjunctive therapy to PAH treatment. Second, Drp1 translocates from the cytosol to mitochondria when being activated by phosphorylation and has a binding partner in the outer mitochondrial membrane (OMM) fission protein 1(Fis1). Then a multimeric fission apparatus will be formed to take part in mitochondria division. RV injury is mediated by the translocation. And 2 kinds of drugs Mdivi1 and P110 are used to inhibit ischemia-induced mitochondrial fission by blocking Drp1 action[64,65]. Third, the phosphorylation ratio of mitochondrial proteins also changes in RV dysfunction. STAT3 (signal transducer and activator of transcription 3) can mediate cell programmed death by ADP-mediated respiratory chain as well as cyclophilin D, which is a composition of MPTP[37]. There are STAT3 and STAT5 which both have a cardioprotective effect but only STAT3 acts on the respiratory chain and the opening of MPTP. Different members in this family might be homologous but have different functions[58]. As an inhibitor of STAT3, Static decreases STAT3 phosphorylation in both Tyr705 and Ser727 sites to downregulate the respiratory chain, which can mediate cell survival in RV failure in PAH[37]. Lastly, GSK3 (glycogen synthase kinase 3) is a kind of enzyme in mitochondria to mediate cell survival by regulating the threshold of apoptosis[33]. Both GSK3 and its receptor have an opposite effect on different apoptotic pathways that GSK3 accelerates intrinsic apoptosis but regulate extrinsic pathway negatively[33]. Due to this, GSK3 plays a role in mitochondrial integrity induced by oxidative stress in PAH[56]. What's more, the rate of non-phosphorylated GSK and phosphorylated GSK in Ser9 influences MPTP in the aspect of its 2 subunits and opening threshold which is connected with reactive oxygen species (ROS)[29].

Providing the importance of calcium regulation by mitochondria in RV, this review aims to sum up the calcium signaling system containing MPTP and MCU in

mitochondria that are associated with RV hypertrophy and failure in PAH, and provide a framework for these 3 mitochondria proteins to identify potential few future clinical therapies, which can suppress the overload of calcium in mitochondria and the resulting RV dysfunction in PAH[22,42]. Such molecular pathways like calcium transportation and mitochondrial dynamics change in RV and pulmonary vascular, which provides the possible treatment for both RV failure and PAH.

2. Methods

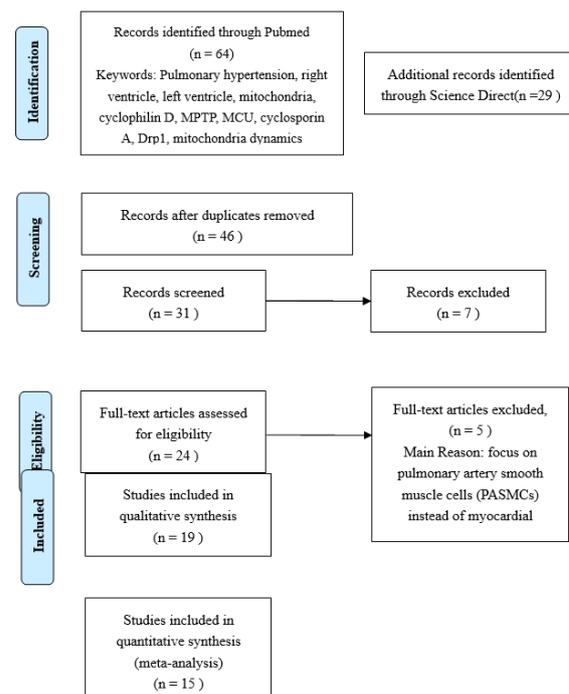


Fig.1 Methods

3. Results

There is an abnormal RV status in PAH, such as RV ischemia, which is a feature of pulmonary arterial hypertension (PAH) to contribute to RV failure. Evidence shows the RV failure in PAH increases the afterload, especially in PVR (pulmonary vascular resistance). In that reason, chronic RV pressure overload in PAH have the potential to trigger RV hypertrophy. With time, severe RV hypertrophy is able to cause RV dilatation and systolic dysfunction[64].

Mitochondria is the key organism in PAH, which acts as the ‘powerhouse’ to produce the energy required for cell metabolism mainly by oxidative phosphorylation (OXPHOS). It also takes part in other metabolic processes like cell programmed death, innate immunity and calcium homeostasis. Evidence showed that in such RV ischemia in PAH, mitochondria have a morphology change and can mediate RVF through cell death when mitochondria protein varies[65].

3.1 Calcium transportation related protein: MPTP and CsA

Transportation of calcium plays a central role in the mitochondrial pathway, including the regulation of energy production, and myocardial cell death in RV and causes the occlusion of pulmonary arterioles in severe situations.

MPTP is a non-selective channel[1] that can be activated by ROS dynamically and inhibited by CypD inhibitors like CsA. It is one of the factors of mitochondrial by regulating the opening of the channel after mitochondrial calcium metabolism[2]. The opening of MPTP is usually initiated by the mitochondrial permeability transition and could influence several pathways including the respiratory chain, the ATP synthesis, the potential collapse of IMM, mitochondrial swelling and sometimes apoptosis[3]. On the other hand, MPTP can be applied in disease prevention by targeting related mitochondrial proteins[4].

MPTP can be divided into 3 parts functionally: voltage-dependent anion channel (VDAC) in OMM, adenine nucleotide translocator (ANT) in IMM and cyclophilin D[5]. MPTP can be triggered by the increasing amount of calcium which is mediated by oxidative stress and decreasing ATP. Cyclophilin D is also a factor that activates MPTP opening. It binds with the F1-F0 ATP synthase. There is a kind of drug which can reduce the activity of MPTP by binding to cyclophilin D called cyclosporin A, which is also able to inhibit Casp3-dependent apoptosis[6,7].

The calcium transport can be assessed by NXAL and VDAC (voltage-dependent anion channel). In NXAL transport, the amount of sodium will increase, which makes calcium alteration happen to initiate the redox metabolism[8]. MPTP could also lead to mitochondrial dysfunction, which is a major cause of cell death and mainly happened in necrosis development. ROS can gain from 3 pathways: respiratory chain, the activation of monoamine oxidase located in the mitochondrial outer membrane and the phosphorylation of cytosolic protein p66. The generation of ROS can be achieved from ETC[9,10]. In addition, ROS burst can cause cell death morphologically[11]. Firstly, in RV in PAH, ROS will make an effort to RV gap junction after the mitochondrial protein connexin43 targeted to PKC in mitochondrial inner membrane and the plasma membrane in the process of ETC. Secondly, the dysfunction of MPTP could lead to cell apoptosis and regulate the amount of calcium in some ways[12].

3.2 Cell Programmed Death

Calcium transport can control the opening of MPTP. The apoptosis can be mediated by MPTP in mitochondrial, which leads to the morphology change to support the heart failure in RV in PAH[17]. The ER-mitochondrial network also shows relevance in cell death with the attendance of calcium[18].

Apoptosis and necrosis are 2 processes in cell death. They can be triggered by death receptors on the cell surface or other mitochondrial proteins, which is to induce the activation of caspase to cut the aspartic acid

residues[19,20,21]. In detail, the Apaf-1 signal will bind with cytochrome c to activate caspase-9 in the process, which is a member of the intracellular cysteine protease family[22,23]. Both intrinsic and extrinsic pathways could lead to caspase, which has initiator caspase including caspase-8 and -9 and -10 as well as effector caspase[22]. The receptor pathway is mainly regulated by RIP1 (receptor interacting protein) and RIP3 which are serine kinases and are functional to targeting to acting area[11].

Apart from RIP, mitochondrial proteins are trigger factors in cell death as well. Both OMM (outer mitochondrial membrane) and IMM (inner mitochondrial membrane) take steps. OMM can mediate apoptosis through its permeabilisation while IMM performs a starting point of oxidative phosphorylation in electron transport with the participation of cytochrome c[25]. Besides, BAX can act as OMM components to influence necrosis and MPTP during mitochondrial fusion dynamically.

The transmembrane gradient that triggers the opening of MPTP is generated by protons pumping in the process of substrate oxidation in the Krebs cycle[11]. MPTP will impact in 2 general ways: the function of ATP and matrix swelling. Firstly, apoptosis might have a negative influence to stop DNA respiration and proteasome function of ATP while ATP still has a large amount in necrosis. Secondly, more water will be delivered into the matrix osmotically, which has a high amount of solute. Then matrix swelling happens. Being affected by this, the extra IMM will leave and OMM would be ruptured, which makes it possible for apoptogens to enter into the cytoplasm to activate the caspase[11].

BCL-2 family has a pro-apoptosis effect which is made up of BAX and BAK and BH3 families. When BIM and BID in BH3 family deliver the signal to BAX and BAK, the conformational change of them shows a transmembrane domain in α -helix to initiate the permeabilisation of OMM and let the caspase happen[26].

3.3 Possible treatment: CsA

CsA (cyclosporine A) is a MPTP blocker which down-regulates MPTP opening and change the morphology of mitochondrial to play a role in PAH that is induced by MCT (monocrotaline)[42]. It is a factor that mediates the level of right heart failure through the combination of MCT [42]. Acting as an MPTP blocker, the process of inhibition is effective to prevent reperfusion injury and myocardial infarction in RVF[43].

To prove the positive effect of CsA and the effect in the MCT-induced PAH model, 3 groups of mice were injected with 3 MCT levels according to a certain gradient: the NC group (normal saline), the MCT group (60mg/kg) and MCTCsA (10mg/kg/day) group. After a 4-week measurement, those mice were killed for the observation of their lung and heart tissues. Firstly, the degree of RVH (right ventricular hypertrophy) can be measured by recording the related weight of the ventricle septum ratio. Then, myocardial fibrilla hypertrophy can be compared by their BW (body weight) and the weight of the whole lung (TLW)[42]. Although the CsA treatment induced myocardial fibrilla hypertrophy even more by increasing

RV mass, it significantly reduced TLW (total lung weight) in the PAH model. What's more, due to the influence of MCT in parts of integrity loss and the disruption of the membrane, substrates have different levels in those groups. Csp3 and AIF both have a higher level in MCT group than CsA group[42]. And such proteins like VDAC, ANT and cyclophilin D which is the consumption of MPTP would not change under the treatment of different CsA environments but have a conformational change in groups[44]. Therefore, CsA prevents MCT-induced mitochondrial damage significantly by reducing Casp3 expressions without MPTP component (ANT, VDAC and cyclophilin D) change.

3.3.1 Mitochondria dynamics proteins: Drp1

The impaired coronary perfusion pressure caused the RV ischemia response by inducing Drp1 activation and translocation, which include increasing myocardial depolarisation and mitochondria swelling, mitochondrial fission and oxidative damage. Then Drp1-induced fission triggered the RV diastolic dysfunction as well as PAH impaired function[64]. Drp1 is activated by dephosphorylation in serine 637 or phosphorylation in serine 616 which reflects the pathologic rate of mitochondrial fission[63]. Then these proteins will accumulate together and bind with the binding partner on OMM called Fis1 due to the myocyte injury mechanism[61,62]. Finally, a multimeric fission apparatus is created in mitochondrial dynamics (Figure 2).

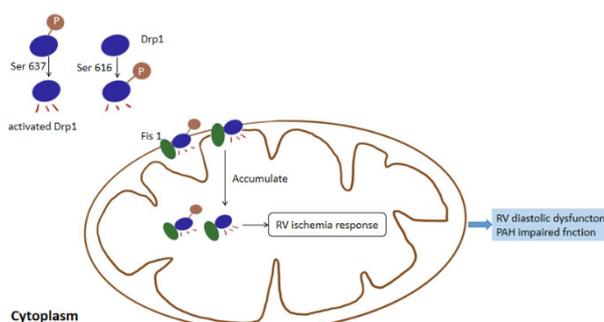


Figure 2. RV ischemia response is achieved by the binding between Drp1 and its partner. Firstly, the dynamin protein triggers the phosphorylation in Ser616 or dephosphorylation in Ser637. Then the activated Drp1 translocates from the cytoplasm to the outer mitochondrial membrane. Finally the aggregation of the binding lead to excessive mitochondria fission in RV ischemia.

3.3.2 Mitochondria dynamics proteins: GSK-3β in phosphorylation pathways

GSK is a mitochondrial protein that is connected to calcium transport in PAH of the pulmonary vascular smooth muscle cells and RV myocytes through cell death and the regulation of MPTP opening[29,30,31]. It mediates apoptosis resulting from mitochondrial toxins, ceramide and oxidative stress. A recent report found that

pharmacologic inhibition of GSK-3 reduced infarct size and improved postischemic function which provides the possibility for pulmonary hypertension treatment[60]. What's more, GSK3 reduction by ER stress leads to mitochondrial dysfunction, which inhibits the opening of MPTP[31]. GSK3 is a kind of serine or threonine kinase that has α and β form ubiquitously. What's more, it mainly acts in regulating glycogen synthesis by phosphorylation[30]. The phosphorylation of almost 50 substrates can mediate several processes like metabolism apoptosis and gene expression[32]. Apart from the receptor extrinsic pathway, mitochondrial integrity is disrupted by cell stress resulting from the apoptotic signaling pathway in cell death. Here, the cell death is mediated when mitochondrial pathway changes[31,33]. For example, the process of re-oxygenation reduces the threshold of ROS to MPTP especially in depolarized mitochondrial[29]. What's more, such mitochondrial swelling inhibits the activity of kinases, such as PKC and GSK-3 β . GSK-3 β has opposite actions in the apoptosis pathway, which can both have a positive and negative effect on signaling pathway [33]. GSK-3 β has several mediators. Apart from PKB and mTOR signaling pathway, the activity of GSK-3 β is regulated by its inhibitor extracellular signal-related kinase (Akt) through phosphorylating related residues[34]. Therefore, GSK is a kind of kinase that is activated by phosphorylation in serine9 to regulate MPTP for cell survival as well as gene expression while STAT is a transcription factor. GSK-3 β can cause a protein-protein reaction to down-regulation to VDAC and ANT, which can regulate the activity of MPTP indirectly. However, STAT has the possibility to change MPTP directly when the potential of the mitochondrial membrane varies. It was also recognized as a factor in cell apoptosis after the depolarisation happens, which we will explain in the next part.

GSK-3 β takes part in Ca-induced MPTP opening in mitochondrial. MPTP opens when Ca influx with a large amount (510.0+26.5 μ mol/mg), which is induced by cyclosporine. There are 3 groups of mice: LETO, OLEFT and TUD. Through the treatment of EPO (erythropoietin), GSK-3 β can be phosphorylated. And the amount of GSK-3 β in nonphosphorylated situations in OLEFT is higher than LETO group. Experiment showed that calcium needed before in the normal group is less than the counterpart which is treated by cyclosporin A[33], and the connection between GSK-3 β and the needed calcium. Therefore, it is improved that GSK-3 β would mediate MPTP by controlling calcium concentration. Then, the immunoblotting showed that the ratio of GSK and phosphorylated GSK is connected with the concentration of calcium in the process of MPTP opening. Additionally, this ratio can also influence 2 subunits of MPTP. The activated GSK-3 β can form both ANT and VDAC to cut down the threshold of the permeable channel while the activity of the kinase will be decreased to accelerate the opening of MPTP after being phosphorylated at Ser9[33]. Therefore, the regulation of GSK-3 β also plays a role in MPTP opening (Figure 3).

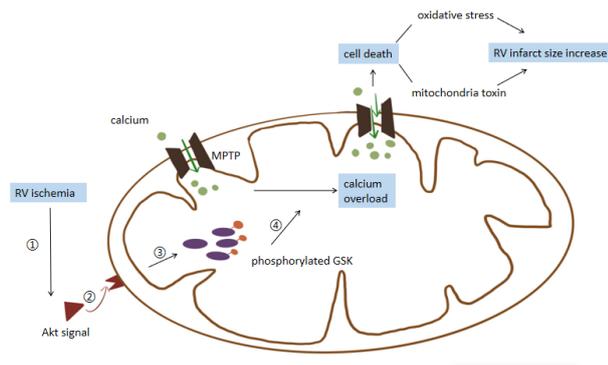


Figure 3. The induced apoptosis by increased phospho-GSK3-to-total GSK3 ratio. Firstly, the Akt signal in glycolysis bind with receptor to increase the phosphorylation ratio of GSK, which would decrease the threshold when calcium is overload in RV reperfusion. Then the excessive opening of MPTP triggers cell death. The signalling apoptosis pathway increase mitochondria toxicity as well as cause oxidative stress to increase infarct size.

3.3.3 Mitochondria dynamics proteins: STAT3 in phosphorylation pathways

STAT is a mitochondrial protein that takes part in cardioprotective signaling pathways and regulates stress signals when acting as transcriptional molecules[35]. It locates in SSM (subsarcolemmal) as well as IFM (interfibrillar) mitochondrial[36]. Different proteins in STAT family shares homologous properties but act with different functions in cardioprotection. For instance, STAT3 locates in interfibrillar cardiomyocyte mitochondrial while STAT5 is not. Additionally, STAT1 and STAT3 can be detected in the mitochondrial of rat ventricular, which have similar proportions[37]. Mitochondrial STAT3 can be activated through the phosphorylation in tyrosine 705 as well as serine 727, which can be observed through immunoprecipitation, which usually mediate not only oxygen consumption through the respiratory chain that is stimulated by ADP and has a substrate of complex1 but also MPTP opening in mitochondrial[38].

Due to the location of STAT3, the cardioprotection of STAT3 in mitochondrial can be achieved by the regulation of MPTP opening and the respiratory chain, especially to complex1. An experiment of STAT3-KO mice improved that 2 groups of mice tolerated calcium at a different level before MPTP opening[37].

The combination of cyclophilin D delays MPTP opening, as the cyclophilin D binds to cyclosporine A, which is a kind of MPTP inhibitor. Cyclophilin D attends the swelling of mitochondrial, but the level of STAT remains. In addition, Tom20 (translocase of the outer membrane 20) is another protein comes from mitochondrial and can bind with STAT3 including phosphorylated STAT3 to mediate the opening of MPTP [37]. Post-translational modification by phosphorylation and dephosphorylation plays a central role in the regulation of STAT3 activity while transcription. These kinds of modifications would influence complex1 and complex2 in the mitochondrial respiratory chain. The respiration which is activated by

ATP with complex1 is reduced in STAT3-KO group while complex 2 remains in this process [38,40]. Experiments showed that the activity of complex1 and the consumption of oxygen can be mediated by mitochondrial STAT3. STAT3 may have a role in the prevention of MPTP opening as well, which is the main regulator of cell death and may be connected with cell survival in ischemia-reperfusion[37].

Stattic is a STAT3 inhibitor that dramatically down-regulates the STAT3 phosphorylation in both Tyr705 and Ser727. The membrane potential of mitochondrial is affected by this inhibitor as well, which indicates that the toxic effect will not happen in mitochondrial[40]. Regarding the aspect of respiration, respiration activated by ADP we mentioned before will be down-regulated instead of the counterpart of basal respiration[38].

4. Discussion

Experiments improved the connection between phosphorylation ratio and MPTP opening level. Here is the detailed mechanism that how GSK-3 phosphorylation inhibits the opening of MPTP [31]. Firstly, the glycogen pathway and other triggers GSK-3 β phosphorylation which increases the ratio of phospho-GSK-3 β to nonphospho-GSK-3 β . Then there will have an infarct size limitation which increases the MPTP threshold to inhibit cell death in some ways[51]. On the other hand, experiments showed that a lower ratio could accelerate MPTP opening and necrosis [33]. Additionally, a GSK-3 β inhibitor can also regulate the process since it has a competitive effect with GSK-3 β [31]. Apart from influencing the threshold, the phosphorylation ratio could also affect the sub units of MPTP, which means the Ser9 phosphorylation can activate the combination between phospho-GSK-3 β and ANT. Then the combination of cyclophilin D and ANT will be blocked due to this interaction[2,29,30]. Finally, MPTP opening is inhibited since cyclophilin D is blocked, which plays a role in increasing the calcium sensitivity of ANT.

To sum up, the raising of Ca, ATP depletion and ROS burst will influence MPTP[2,29], which is induced by GSK-3 β phosphorylation. When the effect comes to the threshold and ANT activity, cell death would happen and lead to connected cardiovascular diseases like RVH and RVF in PAH [33].

The most basic category of STAT is STAT3 and STAT5 in this family, which both play roles in cardioprotection while only STAT3 exists in mitochondrial and losing STAT5 has a low possibility to affect PAH at a mitochondrial level[37]. What's more, STAT1 and STAT3 might have opposing functions in cell death that STAT3 would down-regulate cell apoptosis and STAT1 might accelerate that[49]. In our part, we mainly focus on STAT3 in this family as well as its inhibitor and another mitochondrial protein that combines with STAT3, which can both mediate the respiration chain and MPTP opening. Firstly, after the encoding of protein in the nucleus, STAT3 is then transported into the mitochondrial by Tom20 to play a role through a pathway that is dependent on Tom20. And during the process of transcription, the

post-translational modification can mediate STAT3 by phosphorylation. Among those 2 kinds of phosphorylation, which happens in Tyr705 is induced by a Janus kinase which is a kind of protein kinase and is responsible to accelerate molecular dimerization as well as transportation[37,39]. And this protein kinase can play roles indirectly to influence the respiration by targeting STAT3 in docking sites. Apart from that, the phosphorylation at Ser727 is able to regulate the rate of transcription of STAT3. But there is still a question about the precise degree of STAT3 phosphorylation[38]. Acting as a kind of motivator transcriptionally, STAT3 can also takes part in gene encoding which take place in the chondriome[37]. Secondly, the inhibitor of STAT3 has a negative effect on MPTP opening by STAT3 phosphorylation. Different from the phosphorylation in Ser727, Static has 2 kinds of regulation in the Tyr 705 site which can down-regulate phosphorylation in Tyr or decrease the amount of tyrosin directly[41]. Static acts in the mediation of respiration, especially in the process which is activated by ADP instead of basal respiration[38]. It is also improved that the inhibitor can accelerate the opening of voltage-dependent MPTP after reducing the threshold[50]. This can be achieved by the respiratory chain when only complex1 acts as the substrate. In the STAT3-KO group which had a STAT3 deletion, Static bound to STAT3 at the beginning and then change the potential of the channel, and finally down-regulated the opening[37]. This experiment showed that STAT3 has a great significance in cell survival since it can prevent the MPTP potential change through its binding to Static.

CsA is potential in PAH which can not only prevent right ventricular failure due to RV hypertrophy[42] but also dramatically regulate MPTP opening and the conformational change of MPTP proteins[45]. CsA also acts as a blocker in MPTP opening which has an effect on reperfusion injury. This can be achieved by blocking the combination of CypD and ANT[48].

There is serious mitochondrial damage in RV in PAH which is induced by MCT. CsA reduce the expression of Casp3 to prevent damage. When PAH happens, it will lead to an increase in RV afterload as well as RVF which is mentioned before[44]. There may have a therapy for PAH by controlling RVF prevention or by regulating the artery pressure in the pulmonary in the future[42]. Apoptosis is proved to be a central factor in not only PAH but also RVF since PAH usually results from the apoptosis of smooth muscle cells in lung tissue and the apoptosis in RV myocardial leads to its failure[46,47]. What's more, Casp3 which is induced by caspase-9 mediates the apoptosis when MPTP opening in mitochondrial happens. Besides, Csp3 can be inhibited by CsA, which proves that RVF induced by MCT has a connection with caspase pathway which can be regulated by CsA[44] (Figure 4). As mentioned, mitochondrial could have dysfunction such as swelling and rupture due to the imbalanced MPTP and MCU complex in calcium transport, which could lead to RV hypertrophy in PAH. In this part, there will have kinds of target therapy of mitochondrial protein like CsA. And it is expected to a conventional PAH treatment to be a new strategy[44].

Additionally, the intervention of 2 drugs Mdivi-1 and P110 can prevent mitochondrial morphology change and protect membrane potential in ischemia-reperfusion in PAH by decreasing the expression of Drp1. And studies showed the treatment of Mdivi-1 as well as P110 to myocardial cells can be subjected to IR injury and cardiac arrest. In RV ischemia-reperfusion, the excessive calcium and calcineurin will translocate from cytoplasm to mitochondria due to the activated MPTP opening. Then Drp1 is activated and bind with Fis1 to cause the morphology change to RV diastolic dysfunction. And in this process, studies have proved that both Mdivi-1 and P110 can effectively restore mitochondrial membrane potential and mitochondrial cristae integration and prevent mitochondrial swelling by preventing the translocation of Drp1 to mitochondria.

In conclusion, right ventricular (RV) function determines prognosis in pulmonary arterial hypertension and accelerates PAH[64]. The level of several mitochondrial proteins changes in this process which influences the threshold of calcium-permeable pore to cause RV ischemia response including myocardial depolarisation, glycolysis, cell death and mitochondrial fission[12]. Proposed mechanistic pathways have been improved in future treatment such as CsA which is the inhibitor of the MPTP component[4], Mdivi1 which is a Drp1 inhibitor as well as P110 to block the binding between the dynamic protein and the fission protein[61,62].

However, there is still a limitation to those studies. Some drugs were not studied in vivo in MCT PAH like Mdivi1 as well as some only affect PAH partly. In CsA rat groups, CsA prevented the myocardial damage by mediating the Casp3 process while there wasn't a change to cyclophilin D, which is blocked by CsA in mitochondria[4]. Additionally, the thickness of the arterioles wall hasn't changed by CsA which means it cannot reduce artery pressure. So a new treatment strategy is still expected in RV targeting therapy in PAH.

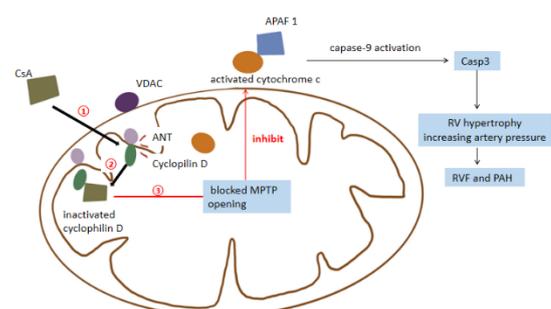


Figure 4. CsA as a possible treatment in RV hypertrophy PAH by blocking caspase-dependent pathway. 1-2, CsA blocks the combination of CypD and ANT to inactivate CypD. 3, the opening of MPTP is blocked after CypD inactivation, which inhibits the combination of APAF1 and cytochrome c. The caspase pathway is therefore down-regulated to decrease the artery pressure in RV and PAH.

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