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Review

Heart mitochondria and calpain 1: Location, function, and targets

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ABSTRACT

Calpain 1 is an ubiquitous Ca^{2+}-dependent cysteine protease. Although calpain 1 has been found in cardiac mitochondria, the exact location within mitochondrial compartments and its function remain unclear. The aim of the current review is to discuss the localization of calpain 1 in different mitochondrial compartments in relationship to its function, especially in pathophysiological conditions. Briefly, mitochondrial calpain 1 (mit-CPN1) is located within the intermembrane space and mitochondrial matrix. Activation of the mit-CPN1 within intermembrane space cleaves apoptosis inducing factor (AIF), whereas the activated mit-CPN1 within matrix cleaves complex I subunits and metabolic enzymes. Inhibition of the mit-CPN1 could be a potential strategy to decrease cardiac injury during ischemia–reperfusion.

1. Introduction

Calpains are a family of calcium-dependent cysteine proteases [1]. The detailed structure of the calpain family can be found in recent excellent reviews [2,3]. Calpains are divided into ubiquitous and tissue specific isoenzymes [1]. The ubiquitous calpains include calpain 1 (u-calpain), calpain 2 (m-calpain), calpain 4 (a regulatory unit of calpains 1 and 2) [4,5], calpain 5, calpain 7, calpain 10, and calpain 14 [1–3]. The tissue specific isoforms include calpain 3 (skeletal muscle), calpain 6 (stomach), calpain 8 (smooth muscle), calpain 9 (stomach), calpain 11 (testes), calpain 12 (skin after birth), and calpain 13 (tests and lung) [1–3]. Based on domain IV structure, calpains can also be defined as typical and atypical calpains [6]. The typical calpains (1, 2, 3, 8, 9, 11, 12, and 14) have a penta-EF hand in domain IV at their COOH-terminus to bind with calcium, small regulatory units (calpain 4) [6,7], and calpastatin (an endogenous inhibitor) [8,9], whereas the atypical calpains (5, 6, 7, 10, and 13) lack the penta-EF hand [6] (Fig. 1). Calpains are found in all cells of vertebrates and are implicated in pathophysiological processes [1–3].

Calpains are traditionally considered as cytosolic proteins [1]. Activation of cytosolic calpains is involved in myocardial injury during ischemia and reperfusion [3,10]. Ischemia–reperfusion increases cytosolic calpain 1 activity in isolated rabbit hearts [11,12] and calpain 2 activity in isolated rat hearts [13]. Activation of cytosolic calpains increases cardiac injury during ischemia–reperfusion by cleaving full length bid to truncated bid [11], Na^+,K^+-ATPase [14,15], Ca^{2+}-ATPase [16], α-fodrin [17], and troponin T [18].

Mitochondrial dysfunction plays a critical role in cardiac injury following ischemia–reperfusion [19–21] or during heart failure [22,23]. Recently, calpains have been localized within mitochondria [24–26]. The activity of mitochondrial localized calpain 1 is increased in the mouse heart following ischemia–reperfusion [25]. This review will focus on mitochondrial localized calpains and their role in cardiac injury.

2. Localization of calpain within mitochondria

Calpains are known to be cytoplasmic enzymes [1], but we and other investigators have shown that calpains also exist within mitochondria [24–26]. Calpain 1 is identified within mitochondria and involved in cleavage of apoptosis-inducing factor (AIF) within mitochondria [24,25]. The large subunit of calpain 1 contains a mitochondrial leader sequence in its N-terminus [27]. The small subunit of calpain 1 (calpain 4) can be imported into mitochondria with the corresponding large subunit [27]. The biochemical characteristics of mitochondrial calpain 1 are similar to cytosolic calpain 1 with an 80 kDa large catalytic subunit as well as a 28 kDa regulatory small subunit-calpain 4 [4]. Mitochondrial calpain 1 was initially localized within the mitochondrial intermembrane space in liver mitochondria [24]. Our previous study showed that calpain 1 is also present in the intermembrane space. We also

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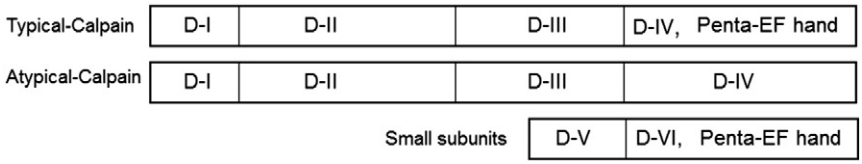

Fig. 1. Depiction of the domain structure of typical and atypical calpains. Compared to the typical calpains, the atypical calpains lack the Penta-EF hand in their domain IV. Calpain 4 only can bind with the typical calpains that contain the Penta-EF hand in their domain IV area.

found that calpain 1 immunoactivity was detected in a component including inner membrane and matrix in cardiac mitochondria [25].

In order to further localize calpain 1 (CPN1) within cardiac mitochondria, mitochondrial components were separated using mitochondria isolated from pooled mouse hearts (see protocol in Fig. 2). The large subunit of CPN1 (mit-CPN1, arrow pointed in Fig. 3) was found in mitochondria, crude outer membrane (c-OMM), intermembrane space (IMS), and matrix (MTR). Human calpain 1 (HCPN1) was used as a positive control to show that the top band was the mit-calpain 1, whereas the low and thick band was a non-specific band (Supplemental Fig. 1). The mit-CPN1 was not found in the purified OMM (p-OMM), indicating that the mit-CPN1 was loosely attached on the OMM or contaminated by cytosolic CPN1. The mit-CPN1 was not detected in both crude inner membrane (c-IMM) nor purified inner membrane (p-IMM), indicating that the mit-CPN1 was not an IMM protein (Fig. 3). Calpain 4 was found in mitochondria, IMS, and MTR corresponding, as expected to mit-CPN1 and confirming localization. Calpastatin was detected in mitochondria, c-OMM, IMS, c-IMM, and MTR, again present at least in compartments where mit-CPN1 exists. Calpastatin was not detected in p-OMM and p-IMM, indicating that calpastatin was loosely attached and a potential contamination from cytosol (c-OMM) or matrix (c-IMM). VDAC (voltage dependent anion channel) was used as an OMM marker. VDAC was detected in both OMM and IMM, consistent with the notion of contact sites where OMM and IMM merge [28,29]. Subunit α of complex V was used as an inner membrane marker. Complex V was found in the c-OMM but not in the p-OMM, indicating that the purification procedure removed potential contamination of the IMM from the OMM. Complex V was detected in both the c-IMM and the p-IMM. Cytochrome *c* [30] and PDH [31] were used as markers of the IMS and MTR, respectively. Cytochrome *c* was mainly present in the IMS, whereas pyruvate dehydrogenase (PDH) was mainly found in the MTR. However, some cytochrome *c* was detected in the c-OMM, and PDH was also found in the c-IMM. Thus, these results indicate that proper purification is critical to locate the mitochondrial proteins in the corresponding compartment.

In liver mitochondria, calpain 2 is also found in the IMS [26]. Activation of mitochondrial calpain 2 increases the permeability of the OMM by interacting with VDAC in liver mitochondria [26]. Calpain 2 is also identified in brain mitochondria isolated from the hippocampus, cerebellum, and cortex [32]. However, calpain 2 is barely detected in the trypsin-purified heart mitochondria [25]. These results indicate that calpain 2 is less likely to be located in the IMS and the MTR in heart mitochondria. Recently, calpain 2 has been detected in non-protease purified rat heart mitochondria and localized to the mitochondrial matrix [33]. Therefore, Percoll purification, rather than trypsin treatment, may need to be used to test if calpain 2 truly exists in mouse heart mitochondria. Similarly, evaluation of calpain 2 content in protease-purified intact rat mitochondria would exclude non-specific adsorption of calpain 2 to mitochondria in that model. Currently, calpains 1 and 2 are identified in mitochondria based on immunoblotting results. Calpain antibodies for different domains may generate variable results [24]. Therefore, a genetic approach may be needed to clarify the issue. Calpain 1 specific knockout mice are

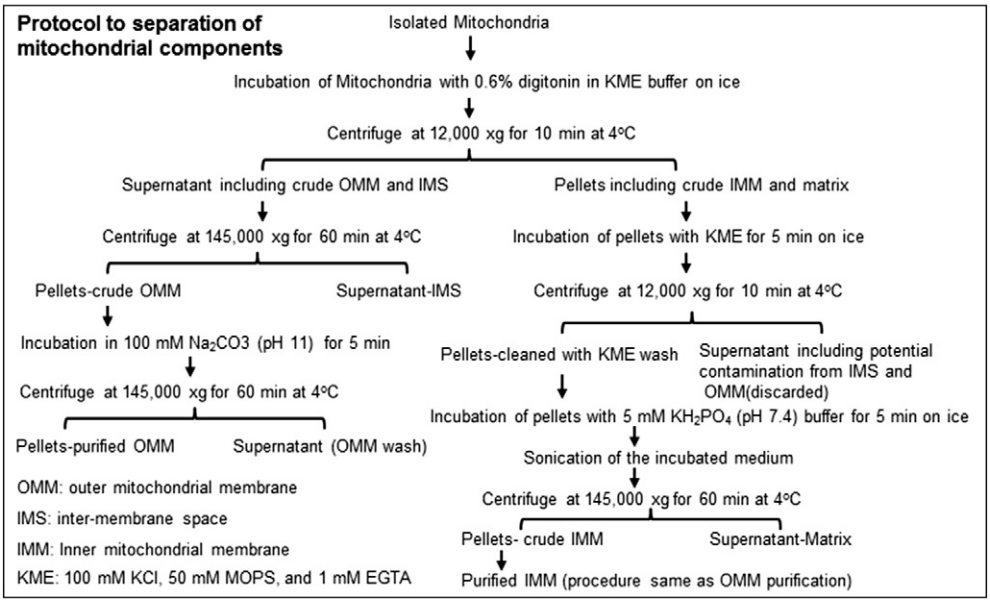

Fig. 2. Depiction of procedures to separate mitochondrial components. Briefly, mitochondria were isolated from pooled mouse hearts. Digitonin was used to permeabilize and rupture the outer mitochondrial membrane (OMM). After digitonin treatment, supernatant including OMM and IMS (intermembrane space) was separated from pellet including IMM (inner membrane) and MTR (matrix) by centrifugation. The ultra-centrifuge was used to separate OMM from IMS. Sonication was used to permeabilize the IMM. The IMM and MTR were separated by ultra-centrifugation. The OMM and IMM were purified by linearization. Ultra-centrifuge was used to pellet the purified OMM and IMM. These components were used for immunoblotting.

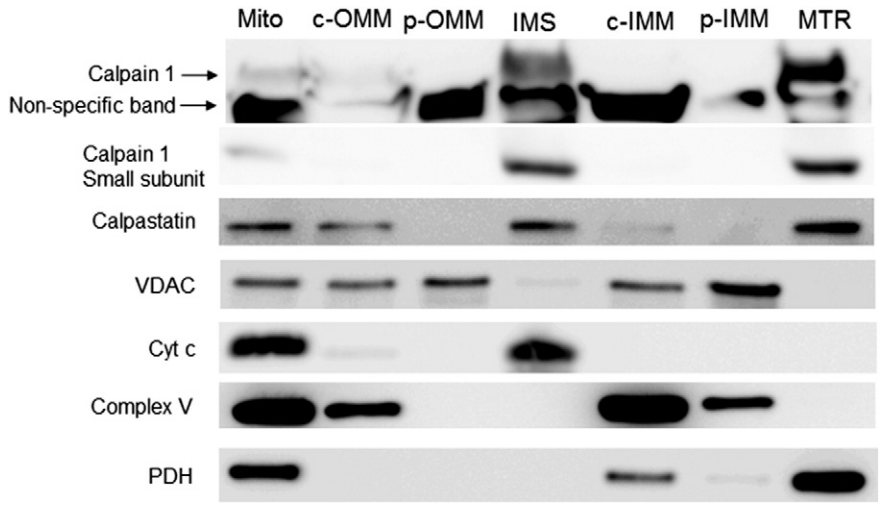

Fig. 3. Localization of mitochondrial calpain 1 (mit-CPN1) in different mitochondrial components isolated from pooled mouse hearts. The mit-CPN1 band (top band, over 75 kDa) was very close to a non-specific band (under 75 kDa) [24]. The mit-CPN1 was detected in mitochondria, crude OMM (c-OMM), the IMS, and the MTR. The mit-CPN1 was removed in the purified OMM (p-OMM), suggesting that the mit-CPN1 was loosely attached on the crude OMM. The mit-CPN1 on the c-OMM may come from the IMS because some cytochrome *c* was also found on the c-OMM. Calpain 4 (small subunit of calpain 1) was localized to mitochondria, the IMS, and the MTR. Calpastatin was found in mitochondria, the c-OMM, the IMS, c-IMM (crude IMM), and the MTR. Calpastatin was not detected in the p-OMM and p-IMM (purified IMM), suggesting that calpastatin detected in the c-OMM and c-IMM was contaminating from the IMS and the MTR. VDAC (voltage dependent anion channel), cytochrome *c* (Cyt *c*), complex V, and PDH (pyruvate dehydrogenase) were used as the marker of the OMM, the IMS, the IMM, and the MTR, respectively. Some Cyt *c* and PDH were detected in the c-OMM and the c-IMM, indicating that proper purification is critical to separate mitochondrial components. VDAC was detected in the p-OMM and the p-IMM, indicating that contact size is present in these components.

available [34]. Since knockout of calpain 2 is embryonic lethal, development of conditional calpain 2 knockout mice is needed to clarify if calpain 2 is located in mouse heart mitochondria.

Calpain 10 is another mitochondrial localized calpain involved in calcium-induced mitochondrial dysfunction [35]. In renal cortex mitochondria, calpain 10 is found in outer membrane, intermembrane space, inner membrane and matrix [35]. Calpain 10 is present in cardiac mitochondria [36]. Activation of calpain 10 increases cell injury by impairing the mitochondrial respiratory chain [35] and is also involved in the disruption of ryanodine receptor-mediated apoptosis [36]. Therefore, activation of the mit-CPN1 and calpain 10 each appears to contribute to cell injury during ischemia–reperfusion.

2.1. Regulation of mitochondrial calpain activity

Calpains are calcium-dependent proteases and their activities are regulated by intracellular calcium concentration [37]. Calcium is imported into mitochondria mainly through the calcium uniporter (H^+/Ca^{2+}) in the IMM [38,39]. Calcium is extruded from mitochondria into cytosol through Na^+/Ca^{2+} exchange [40,41] and MPTP opening [42] (Fig. 4). Calpain 1 is activated in the presence of 3–50 μmol/L Ca^{2+}, whereas activation of calpain 2 requires high calcium concentrations (400–800 μmol/L Ca^{2+}) [24,43,44]. In the resting cardiac myocyte, the calcium concentration within the mitochondrial matrix is estimated to be approximately 100 nM, similar to the calcium concentration in

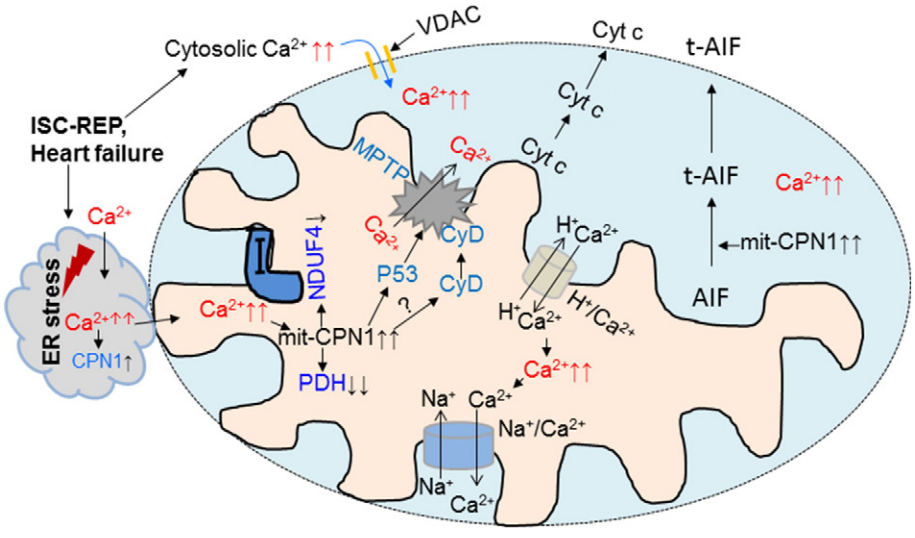

Fig. 4. Depiction of the potential role of mit-CPN1 within mitochondria. Calcium enters into the IMS through VDAC. Calcium within the IMS is imported into the MTR through H^+/Ca^{2+} exchange located at the IMM. Calcium within the MTR is exported through Na^+/Ca^{2+} exchanger. Calcium overload within the MTR can trigger MPTP opening that leads to an outflow of calcium from the MTR. The increased calcium concentration in cytosol during ischemia–reperfusion (ISC–REP) or heart failure leads to calcium overload within mitochondria. ISC–REP or heart failure can increase mitochondrial calcium concentration through ER stress. Mitochondrial calcium overload activates the mit-CPN1 that in turn cleaves AIF within the IMS. Activation of the mit-CPN1 within the MTR may damage the complex I through cleavage of subunits and degradation of PDH. Activation of the mit-CPN1 can facilitate the MPTP opening through interaction with the cyclophilin D (CyD) and p53.

cytosol [45–47]. Calcium concentration is increased to 1–2 μM during myocyte contraction [48]. Taken together, the calcium concentration within the matrix is barely sufficient to activate the mit-CPN1 under physiologic conditions. In buffer-perfused heart, the calcium concentration in the matrix is around 250 nM during equilibration perfusion [49]. Calcium concentration is increased to approximately 1 μM at the end of 25 min ischemia and to 2.6 μM at the end of 30 min reperfusion [49,50]. The calcium concentration in the matrix is still lower than the threshold to activate the mit-CPN1 even at the peak calcium that occurs early in reperfusion. These measured calcium concentrations would appear even less likely to activate mitochondria-localized calpain 2. However, the calcium threshold to activate calpain is dramatically decreased during oxidative stress [51,52]. Ischemia–reperfusion damages the mitochondrial respiratory chain and markedly increases the ROS generation [53–56]. Therefore, mit-CPN1 and possibly calpain 2 [33] can be activated in the presence of mitochondrial calcium overload combined with oxidative stress during ischemia–reperfusion.

Calcium overload also activates calpain 10, although some isoforms of calpain 10 are not solely calcium-dependent [35]. As discussed above, calpain 1 and calpain 2 belong to typical calpains, and their activity is regulated by calpain 4 (a small subunit of calpains 1 and 2) [4]. Genetic ablation of calpain 4 completely eliminates the activities of calpain 1 and calpain 2, supporting that calpain 4 is necessary for the activation of calpain 1 and calpain 2 [4,57,58]. Calpastatin is an endogenous inhibitor of typical calpains [1]. Calpain 4 and calpastatin have already been identified within mitochondria [24] (Fig. 3). Thus, the CPN1 and CPN2 activities, including in mitochondria, can be regulated by genetic knockout of calpain 4 [58] or overexpression of calpastatin [59]. Calpain 10 is an atypical calpain [6], and its activity is not subject to regulation by calpain 4 and calpastatin. The mechanism to regulate calpain 10 activity remains unclear, although post-translational modification including phosphorylation may affect calpain 10 activity [60]. In addition to endogenous regulatory mechanisms, calpain activity is also affected in different physiological conditions. The activities of calpains are decreased under acidic conditions [10,13,24,61], although sensitivity to acidification is different among calpains. In liver mitochondria, calpain 1 activity is regulated through a chaperone protein ERp57 [10,13,24,26,61]. A number of calpain inhibitors are available to manipulate calpain activity under pathophysiologic conditions [11,24,62]. MDL-28170 is a typical calpain inhibitor that is used to inhibit calpain 1 and calpain 2 [11,24,62]. Although PD150606 is a more selective calpain 1 inhibitor, it is still not an exclusive CPN1 inhibitor [63]. A selective inhibitor of calpain 10 is also available [64].

3. Mitochondrial calpain 1 in intermembrane space

Calpains are involved in many pathophysiological processes including embryonic development, cell function, intracellular signal transduction, cell cycle, and ischemia–reperfusion injury [2,3,65]. Many proteins are substrates of cytosolic calpain 1 and calpain 2 [66]. Calpain cleaves the cytoskeleton and membrane associated proteins including α_2-spectrin, which is used as a biological marker of calpain activation [10,13,15,67]. Intracellular calcium overload during ischemia–reperfusion activates cytosolic calpains 1 and 2 [10,11,13,15,67]. The activated cytosolic calpains 1 and 2 in turn increase calcium overload by cleaving calcium regulator proteins including Na^+–K^+-ATPases, Ca^{2+}-ATPases, H^+-ATPases, Na^+/H^+-exchanger, and Na^+/Ca^{2+}-exchanger [10,15,68,69]. Thus, calpain activation plays a critical role in the failure of intracellular calcium control mechanisms that result in an excessive intracellular calcium accumulation [2,3]. Intracellular calcium overload eventually leads to mitochondrial calcium overload that may activate mitochondrial localized calpain 1 and possibly calpain 2 with the higher calcium requirement.

Activation of mit-CPN1 likely contributes to cardiac injury during ischemia–reperfusion [25,70]. A translocation of intermembrane space proteins including cytochrome *c*, AIF, Smac/DIABLO, and Omi/HtrA2 from mitochondria into cytosol and nucleus triggers apoptotic cell death [20,21,65,71,72]. Loss of cytochrome *c* from mitochondria also enhances necrotic cell death by reducing bioenergy production through inhibition of oxidative phosphorylation [54,73–75]. Among these intermembrane proteins, the relationship between activation of mit-CPN1 and loss of AIF from mitochondria is well established [24,25]. Translocation of AIF from mitochondria to the nucleus triggers caspase-independent cell death by inducing DNA damage [76–78]. The mature form of AIF (62 kDa) is anchored at the inner mitochondrial membrane within the intermembrane space [79]. In order to release AIF from the mitochondria into cytosol, the mature AIF first must be detached from the inner membrane. In the isolated liver or heart mitochondria, incubation of mitochondria with exogenous calcium cleaves the mature AIF (62 kDa) to a truncated AIF peptide (about 57 kDa). Administration of a calpain inhibitor prevents the calcium-mediated AIF cleavage [24]. These results support that activation of mitochondrial calpain leads to AIF cleavage. As discussed above, only the mit-CPN1 is identified in the intermembrane space of cardiac mitochondria [25]. Therefore, activation of mit-CPN1 leads to AIF cleavage in heart mitochondria. However, it has been reported that mitochondrial calpain 1 is not involved in AIF cleavage in brain mitochondria [80]. Thus, the link between mitochondrial activation of calpain 1 and AIF cleavage may be tissue dependent.

Following detachment from the inner membrane, permeation of the outer mitochondrial membrane is required for the release of cleaved AIF from the intermembrane space [24]. In isolated liver mitochondria, activation of mitochondrial calpain 2 increases the permeability of the outer mitochondrial membrane by cleaving VDAC and inducing accumulation of bax on the outer membrane [24]. Although calpain 2 has not been identified in murine cardiac mitochondria [25], it has been identified in rat heart mitochondria [33]. Ischemia–reperfusion is known to trigger mitochondrial permeability transition pore (MPTP) opening that in turn increases the outer membrane permeability [71]. In addition, ischemia can increase the permeability of the outer membrane through induction of imbalance between anti-apoptotic and pro-apoptotic bcl-2 family proteins [81,82]. Taken together, a key step for AIF release from mitochondria into cytosol is to detach the AIF from the inner membrane through activation of mit-CPN1.

4. Mitochondrial calpain 1 within the matrix

Administration of calpain inhibitors decreases cardiac injury during ischemia–reperfusion [2,3]. In isolated rabbit hearts, inhibition of calpain improves oxidative phosphorylation in cardiac mitochondria following ischemia–reperfusion, supporting that the activation of calpain contributes to mitochondrial energy generation [83,84]. In addition, calpain inhibition attenuates the increased state 4 respiration in mitochondria following ischemia–reperfusion, indicating that activation of calpain increases the permeability of the inner mitochondrial membrane [83]. In liver mitochondria, inhibition of calpain using Cbz-Leu-Leu-Tyr-CHN2 delays the exogenous calcium-induced depolarization of mitochondrial inner membrane potential [85], suggesting that activation of calpain triggers the MPTP opening.

The activity of mitochondrial calpain 1 is increased in cardiac mitochondria following ischemia–reperfusion [25]. The administration of a calpain inhibitor during ischemia–reperfusion to the buffer perfused heart improved oxidative phosphorylation using complex I substrates [84]. Inhibition of calpain also decreased the MPTP opening in mitochondria isolated from mouse heart following ischemia–reperfusion [84]. These results suggest that activation of mit-CPN1 contributes to electron transport damage including complex I and MPTP opening during ischemia–reperfusion. We propose that activation of calpain 1 within the mitochondrial matrix damages complex I by cleavage of subunits. In the rat heart, calpain 2 was localized to mitochondrial matrix, with activity favoring MPTP and complex I damage via cleavage of subunits [33]. Thus, during ischemia and reperfusion, multiple studies are

converging to support the role of mitochondrial calpain activation in processes that damage mitochondrial metabolism and favor cell death.

Mitochondrial calpain 10 is localized within mitochondrial matrix [35]. In renal mitochondria, activation of mitochondrial calpain 10 impairs the electron transport chain by proteolytic digestion of complex I subunits [35,86]. Genetic ablation of complex I subunits sensitizes to the MPTP opening in mouse heart mitochondria [87]. Thus, the damaged complex I not only can decrease the rate of respiration [20], but it also may sensitize mitochondria to undergo MPTP opening which is a key mechanism to induce cell death during ischemia–reperfusion [87].

In addition to damage to the ETC, ischemia–reperfusion impairs metabolic enzymes in the tricarboxylic acid (TCA) cycle in cardiac mitochondria [88–90]. Proteomic studies show that ischemia–reperfusion leads to degradation of metabolic enzymes including pyruvate dehydrogenase (PDH), malate dehydrogenase (MDH), and succinate dehydrogenase (SDH) in rat heart mitochondria [88–90]. Our ongoing study showed that ischemia–reperfusion led to decreased PDH content in buffer perfused mouse heart [91]. Incubation of isolated mouse heart mitochondria with exogenous calcium also decreased PDH, whereas inhibition of mit-CPN1 using MDL protected PDH content. These results suggest that activation of the mit-CPN1 contributes to degradation of PDH during ischemia–reperfusion, perhaps providing a mechanism for the previously observed decrease in the matrix protein content during ischemia–reperfusion.

Exogenous calcium treatment leads to depolarization of the inner mitochondrial membrane potential in a dose-dependent manner in isolated heart mitochondria. Inhibition of calpain using MDL-28170 attenuates the calcium-induced depolarization of the inner membrane potential [84]. These results clearly show that activation of mit-CPN1 contributes to inner membrane permeability increases in heart mitochondria. However, the mechanism by which activation of mitochondrial calpain 1 permeabilizes the inner membrane, and the potential role of MPTP, remains unclear.

Administration of a calpain inhibitor to the intact heart can decrease MPTP opening through regulation of intracellular calcium status via inhibition of cytosolic calpain [10,13,15]. As discussed above, activation of cytosolic calpain during ischemia–reperfusion can increase intracellular calcium concentration by cleaving Na^+, K^+-ATPase, Ca^{2+}-ATPase, H^+/Na^+ exchanger, and Na^+/Ca^{2+} exchanger [2,3]. Activation of cytosolic calpain can increase the permeability of the outer mitochondrial membrane through cleavage bid to t-bid [11]. Calpain 1 is also found on the endoplasmic reticulum (ER) [92]. Therefore, calpain activation can cause calcium overload through ER stress [93]. Taken together, activation of cytosolic calpains can sensitize MPTP opening through induction of intracellular calcium overload and subsequent mitochondrial calcium overload.

The exact mechanism by which activation of the mitochondrial calpains sensitizes the MPTP opening in cardiac mitochondria remains unclear. p53 is a tumor suppressor protein and its content is normally maintained at a low level by interaction with Mdm2 (mouse double minute 2 homolog) that leads to degradation [94]. The p53 content rapidly increases in cytosol and nucleus during oxidative stress through phosphorylation and subsequent dissociation from Mdm2 [95]. The phosphorylated p53 is transferred to and accumulates within mitochondria to increase MPTP opening through formation of a complex with cyclophilin D [94,96]. Calpain plays a critical role in maintaining p53 level within cells [97]. Activation of cytosolic calpain facilitates the translocation of p53 from cytosol to brain mitochondria [98], indicating that cytosolic calpain activation may induce MPTP opening through facilitation of p53 translocation from cytosol to mitochondria (Fig. 4). Activation of mitochondria-localized calpain 1 [84] or calpain 2 [33] may sensitize the MPTP opening in cardiac mitochondria by impairing complex I [33,84]. Cyclophilin D is a critical regulator of the MPTP opening [99]. Cyclophilin D is located within the matrix and can be accessed by the mit-CPN1. Genetic inhibition of complex I sensitizes MPTP opening in heart mitochondria through increased protein acetylation [87]. Administration of calpain inhibitor attenuates complex I damage and decreases MPTP opening in heart mitochondria following ischemia–reperfusion [33,84], suggesting that activation of mit-CPN1 sensitizes MPTP opening by impairing complex I. Taken together, activation of mit-CPN1 may increase MPTP opening by facilitating translocation of cyclophilin D from the matrix to the inner mitochondrial membrane through interaction with p53 [97] or by impairing complex I in the respiratory chain [87].

Since there is no exclusive mit-CPN1 inhibitor available, pharmacological approaches are not sufficient to clarify the role of mitochondrial calpains in cardiac injury during ischemia–reperfusion. Genetic approaches are already used to manipulate calpain 1 and 2 activities. Although calpain 4 [4] or calpain 1 [34] knockout mice are available, and would distinguish the roles of calpains 1 and 2 in the mitochondria, these mice also have limitations in that global knockout of calpain 4 or calpain 1 affects both cytosolic and mitochondrial calpain activities. Therefore, selective overexpression of calpain 1 within mitochondria or development of a calpain inhibitor targeted into mitochondria could be a proper approach to investigate the role of mit-CPN1 activation in cardiac injury.

5. Other proteases within mitochondria

In addition to calpains, other proteases exist within mitochondria. Lon and Clp (caseinolytic protease) are proteases located within the mitochondrial matrix. The detailed role of Lon and Clp in maintaining mitochondrial protein integrity can be found in a recent excellent review [100]. Briefly, the critical role of the Lon protease is to remove the oxidized protein within mitochondrial matrix, whereas the Clp protease plays a key role in mitochondrial unfolded protein response [100]. In renal proximal tubular cells, oxidative stress leads to the degradation of calpain 10. Administration of Lon inhibitor decreases the calpain 10 degradation [101]. These results indicate that there is an interaction between Lon and mitochondrial calpain 10. These two proteases likely contribute a critical role in degradation of the damaged mitochondrial proteins, especially in stressed conditions.

6. Summary and conclusions

Although more than one calpain family is located within mitochondria, the focus of our current review is to identify the localization of mit-CPN1 and explore its potential function in cardiac mitochondria, especially during ischemia–reperfusion. Activation of mit-CPN1 within the intermembrane space induces a release of AIF from mitochondria into cytosol through cleavage of AIF to truncated AIF. In contrast, activation of the mit-CPN1 and mit-CPN2 within mitochondrial matrix inhibits mitochondrial metabolism by damaging complex I subunits or degrading metabolic enzymes. In addition, activation of the mit-CPN1 or mit-CPN2 may sensitize to MPTP opening in cardiac mitochondria following ischemia–reperfusion. Inhibition of cytosolic calpains decreases cardiac injury during ischemia–reperfusion. Modulation of mitochondrial localized calpain, especially mit-CPN1, may provide a complimentary, promising strategy to decrease cardiac injury during ischemia–reperfusion.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2015.08.004.

Conflict of interest

There is no conflict interest.

Abbreviations

CPN1	calpain 1
HCPN1	human calpain 1
Mit-CPN1	mitochondrial calpain 1
ETC	mitochondrial electron transport chain

AIF	apoptosis inducing factor
OMM	outer mitochondrial membrane
IMM	mitochondrial inner membrane
IMS	mitochondrial intermembrane space
MTR	matrix
MDL	MDL-28170
ROS	reactive oxygen species
$\Delta\Psi$	inner mitochondrial membrane potential
PDH	pyruvate dehydrogenase
MDH	malate dehydrogenase
Mdm2	mouse double minute 2 homolog
SDH	succinate dehydrogenase
VDAC	voltage dependent anion channel
TMRM	tetramethylrhodamine
CyD	cyclophilin D

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