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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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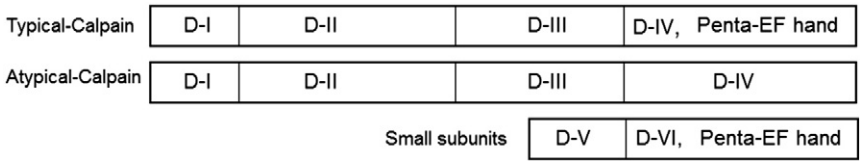

Fig. 1. Depiction of the domain structure of typical and atypical calpains. Compared to the typical calpains, the atypical calpains lack the Penta-EF hand in their domain IV. Calpain 4 only can bind with the typical calpains that contain the Penta-EF hand in their domain IV area.

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

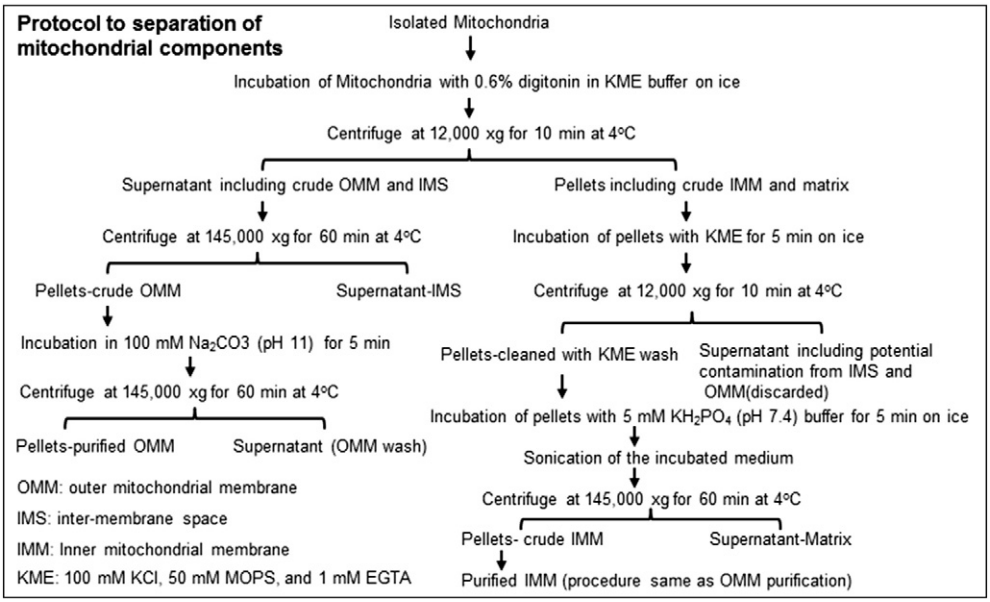

Fig. 2. Depiction of procedures to separate mitochondrial components. Briefly, mitochondria were isolated from pooled mouse hearts. Digitonin was used to permeabilize and rupture the outer mitochondrial membrane (OMM). After digitonin treatment, supernatant including OMM and IMS (intermembrane space) 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.