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Proteolysis of mitochondrial calpain-13 in cerebral ischemia-reperfusion injury

Yusaku Chukai [a], Toru Sudo [a], Tomokazu Fukuda [b], Hiroshi Tomita [c], Eriko Sugano [c], Taku Ozaki [a,*]

[a] Laboratory of Cell Biochemistry, Department of Biological Science, Graduate School of Science and Engineering, Iwate University, Morioka, Iwate, Japan
[b] Laboratory of Cell Engineering and Molecular Genetics, Department of Biological Science, Graduate School of Science and Engineering, Iwate University, Morioka, Iwate, Japan
[c] Laboratory of Visual Neuroscience, Department of Biological Science, Graduate School of Science and Engineering, Iwate University, Morioka, Iwate, Japan

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ABSTRACT

Calpains are calcium-dependent cysteine proteases activated by intracellular Ca^{2+}. Although calpains mainly exist in the cytosol, calpain-13 is present in the mitochondria in mouse brains; however, the enzymatic properties and physiological functions of calpain-13 remain unknown. Hence, in this study, we predicted and evaluated the enzymatic properties of calpain-13. Based on our bioinformatic approaches, calpain-13 possessed a catalytic triad and EF-hand domain, similar to calpain-1, a well-studied calpain. Therefore, we hypothesized that calpain-13 had calpain-1-like enzymatic properties; however, calpain-13 was not proteolyzed in C57BL/6J mouse brains. Subsequently, cerebral ischemia/reperfusion (I/R) injury caused proteolysis of mitochondrial calpain-13. Thus, our study showed that mitochondrial calpain-13 was proteolyzed in the mitochondria of the I/R injured mouse brain. This finding could be valuable in further research elucidating the involvement of calpain-13 in cell survival or death in brain diseases, such as cerebral infarction.

1. Introduction

Calpains, calcium-dependent cysteine proteases, are activated by increased intracellular Ca^{2+} concentrations and are involved in various intracellular pathways [1–4]. Although most calpains are present in the cytosol, five isoforms of calpain (calpains-1, -2, -5, -10, and -13) exist in the mitochondria [5–9]. Recently, we reported that mitochondrial calpain-13 was present in the cerebral cortex and hippocampus of the mouse brain [9].

For most calpains, the binding of Ca^{2+} to the penta EF domain is essential for activation. Calpains-1 and -2 are "classical" calpains that are activated at micromolar and millimolar concentrations of Ca^{2+}, respectively [2,4]. The activated mitochondrial calpain-1 and calpain-2 co-operatively induce apoptosis [6], which is involved in oxytosis in mouse hippocampal HT22 cells [10]. Mitochondrial calpain-5 is activated during endoplasmic reticulum (ER) stress [11] and ischemia/reperfusion (I/R) injury, resulting in the activation of inflammatory caspase-4/11 [12,13]. Calpain-10 induces mitochondrial dysfunction via the cleavage of ND6 and NDUFV2, two electron transport chain complex I subunits [7]. However, unlike other mitochondrial calpains, enzymatic properties and physiological functions of calpain-13 are yet to be elucidated.

Cerebral I/R is a model for assessing the marked increases in intracellular Ca^{2+} concentrations that follow cerebral ischemia. Failure to clear excitatory amino acids induces depolarization and Ca^{2+} overload in neuronal cells after cerebral ischemia. Glutamate receptors and ER stress are triggers of intracellular Ca^{2+} influx [14–16], which involves mitochondrial calpain activation [17].

Therefore, in this study, we aimed to clarify the enzymatic properties of cytosolic and mitochondrial calpain-13 *in vitro*. Furthermore, we examined the involvement of calpain-13 in I/R injury of the mouse brain. This study could provide novel insights into the enzymatic properties of calpain-13.

2. Materials and methods

2.1. Animals

C57BL/6J mice (age: 8–10 weeks, body weight: 22–28 g) were used for preparing samples for an *in vitro* calcium assay and establishing an *in vivo* cerebral I/R mouse model. The animal experiments were approved

* Corresponding author.
E-mail address: tozaki@iwate-u.ac.jp (T. Ozaki).

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Abbreviations:

AIF	apoptosis-inducing factor
I/R	ischemia/reperfusion
ER	endoplasmic reticulum
COX IV	cytochrome *c* oxidase subunit 4
AK2	adenylate kinase 2
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

by the Committee for the Ethics of Animal Experimentation of Iwate University (approval number: A202326).

2.2. Antibodies

Anti-mouse calpain-13 and anti-mouse cytochrome *c* oxidase subunit 4 (COX IV) antibodies were prepared by Scrum Inc. (Tokyo, Japan). The antigen sequences were as follows; calpain-13, Cys-FKNQDFRTLRD-OH; COXIV, Cys-FPTYADRRDYPLPD-OH. Commercially available antibodies used in this study were anti-adenylate kinase 2 (anti-AK2, sc-28766, Santa Cruz Biotechnology), anti-β-actin (sc-47778, Santa Cruz Biotechnology), horseradish peroxidase-conjugated polyclonal goat anti-rabbit immunoglobulins (P0448, Dako, Carpinteria, CA, USA), and horseradish peroxidase-conjugated polyclonal rabbit anti-mouse immunoglobulins (P0260, Dako).

2.3. Subcellular fractionation

Subcellular fractionation was performed as previously described [9]. Mice were euthanized via cervical dislocation, and the mouse brain was soaked in a homogenizing buffer (20 mM Tris-HCl, 250 mM sucrose, pH 7.4). Each tissue sample was homogenized with 20 strokes and centrifuged at 600×*g* for 10 min, and the supernatant was centrifuged at 12,000×*g* for 15 min. Subsequently, the supernatant was centrifuged at 105,000×*g* for 30 min to obtain the cytosolic fractions, excluding the microsome fractions, and the pellet was resuspended in homogenizing buffer and centrifuged at 12,000×*g* for 15 min to obtain a pellet containing mitochondria. The pellet was solubilized in TEM buffer (20 mM Tris-HCl, pH 7.4) containing 1 % Triton X-100, and incubated for 1 h. Mitochondrial fractions were obtained from the supernatant after centrifuging the suspension at 105,000×*g* for 30 min. The homogenizing buffer and TEM buffers used in the *in vivo* assay included 1 mM ethylenediaminetetraacetic acid (EDTA) and 1 mM ethylene glycol tetraacetic acid (EGTA). All procedures of subcellular fractionation were performed at 4 °C.

2.4. *In vitro* Ca^{2+}-dependency of calpain-13

We performed an *in vitro* proteolysis assay to determine the minimum calcium concentrations required for the activation of cytosolic and mitochondrial calpain-13. Following the aforementioned subcellular fractionation procedure, we isolated the cytosolic and mitochondrial fractions. To identify the essential Ca^{2+} concentrations for the activation of cytosolic and mitochondrial calpain-13, different Ca^{2+} concentrations (final concentration range 0–1000 μM) were added to each sample of cytosolic and mitochondrial fractions, followed by incubation at 37 °C for 1 h. Moreover, we investigated whether the activation of calpain-13 was inhibited by calpeptin, which was added to the two fraction samples at a final concentration of 10 μM, followed by preincubation at 4 °C overnight. After the incubation, Ca^{2+} was added to each sample at a final concentration of 1 mM, followed by incubation at 37 °C for 1 h. Thereafter, EDTA and EGTA were added to the samples at final concentrations of 1 mM each to stop the reaction, and the resulting solutions were then boiled at 90 °C for 3 min. Proteolysis of calpain-13 was examined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis.

2.5. *In vitro* time-dependency of calpain-13

We performed a proteolysis assay by changing the reaction time to chronologically evaluate the calcium sensitivity of cytosolic and mitochondrial calpain-13. After separating cytosolic and mitochondrial fractions, Ca^{2+} at a final concentration of 1 mM was added to each cytosolic and mitochondrial fraction sample, followed by incubation at 37 °C for 0–60 min. Additionally, calpeptin was used to inhibit calpain-13, and the samples were preincubated as described above. Next, Ca^{2+} at a final concentration of 1 mM was added to the sample, which was then incubated at 37 °C for 60 min. After stopping the reaction using a chelating agent, the mixture was boiled at 90 °C for 3 min. Proteolysis of calpain-13 was examined using SDS-PAGE and western blot analysis.

2.6. Establishment of cerebral I/R mouse model

Bilateral common carotid artery occlusion was performed according to the method described by Wahul et al. [18]. Male mice were intraperitoneally injected with a triple anesthetic (0.3 mg/kg medetomidine, Kyoritsu, Tokyo, Japan; 4 mg/kg midazolam, Sandoz, Tokyo, Japan; and 5 mg/kg butorphanol, Meiji Seika Pharma, Tokyo, Japan). After ensuring that the anesthesia had set in, the neck was incised and the bilateral common carotid artery was exposed. The bilateral common carotid arteries were occluded with clips for 15 min, and reperfusion was conducted for 3, 6, 12, and 24 h. Then, the incisions were occluded using soft tissue bonding adhesive (Aron Alpha A, 34,164,100, Sankyo, Tokyo, Japan). After the reperfusion, the mice were euthanized by cervical dislocation, and their brains were sampled. Sham control mice were prepared by exposing the bilateral common carotid arteries. The evaluation of brain tissue samples was performed using SDS-PAGE and western blot analysis.

2.7. Western blot analysis

Western blot analysis was performed as previously described by Ozaki et al. [10]. The samples were separated via SDS-PAGE and transferred to Immobilon®-P PVDF membranes (IPVH00010; EMD Millipore, Burlington, MA, USA). The membranes were then blocked with blocking buffer [10 mM phosphate buffer pH 7.4, 0.14 M NaCl, 1 % or 5 % skim milk containing 0.05 % Tween 20 (TW-PBS)] for 1 h at room temperature (approximately at 25 °C). The membranes were first incubated with primary antibodies at 4 °C overnight and then incubated with secondary antibodies at 4 °C overnight. The antibodies were diluted in blocking buffer as follows: anti-β-actin (1:2000), anti-AK2 (1:2000), anti-calpain-13 (1:1000), anti-COX IV (1:2000), horseradish peroxidase-conjugated polyclonal goat anti-rabbit immunoglobulins (1:10,000), and horseradish peroxidase-conjugated polyclonal rabbit anti-mouse immunoglobulins (1:10,000). Immunopositive signals were developed with ECL solutions (A solution: 100 mM Tris-HCl, pH 8.5, 0.4 mM p-coumaric acid, and 5 mM luminol; B solution: 0.04 % H_2O_2 and 100 mM Tris-HCl, pH 8.5). Images were captured using a ChemiDoc XRS Plus luminescent image analyzer (1708265J1PC, Bio-Rad, Hercules, CA, USA).

2.8. Amino acid sequence

The sequences of mouse calpains-1 (UniProt ID: O35350) and -13 (UniProt ID: Q3UW68) were obtained from the UniProt Knowledgebase. The sequences were compared using CLUSTALW and visualized using ESPript 3.0 [19]. The result was modified with domain information. The

sequence of mouse calpain-13 was analyzed using MitoFates (https://mitf.cbrc.pj.aist.go.jp/MitoFates/cgi-bin/top.cgi) [20] for prediction of the mitochondrial transfer sequence.

2.9. Prediction of calpain-13 structure

The predicted structure of mouse calpains-1 (AF-O35350-F1) and -13 (AF-Q3UW68–F1) were obtained from AlphaFold Protein Structure Database [21,22]. The predictions were visualized using EzMol [23].

2.10. Statistical analysis

The expression of the proteolytic form of mitochondrial calpain-13 was quantified using image lab software 6.0 (Bio-Rad). Each value was corrected using the values of expression of the loading control. The corrected value was analyzed using Statistical Package for the Social

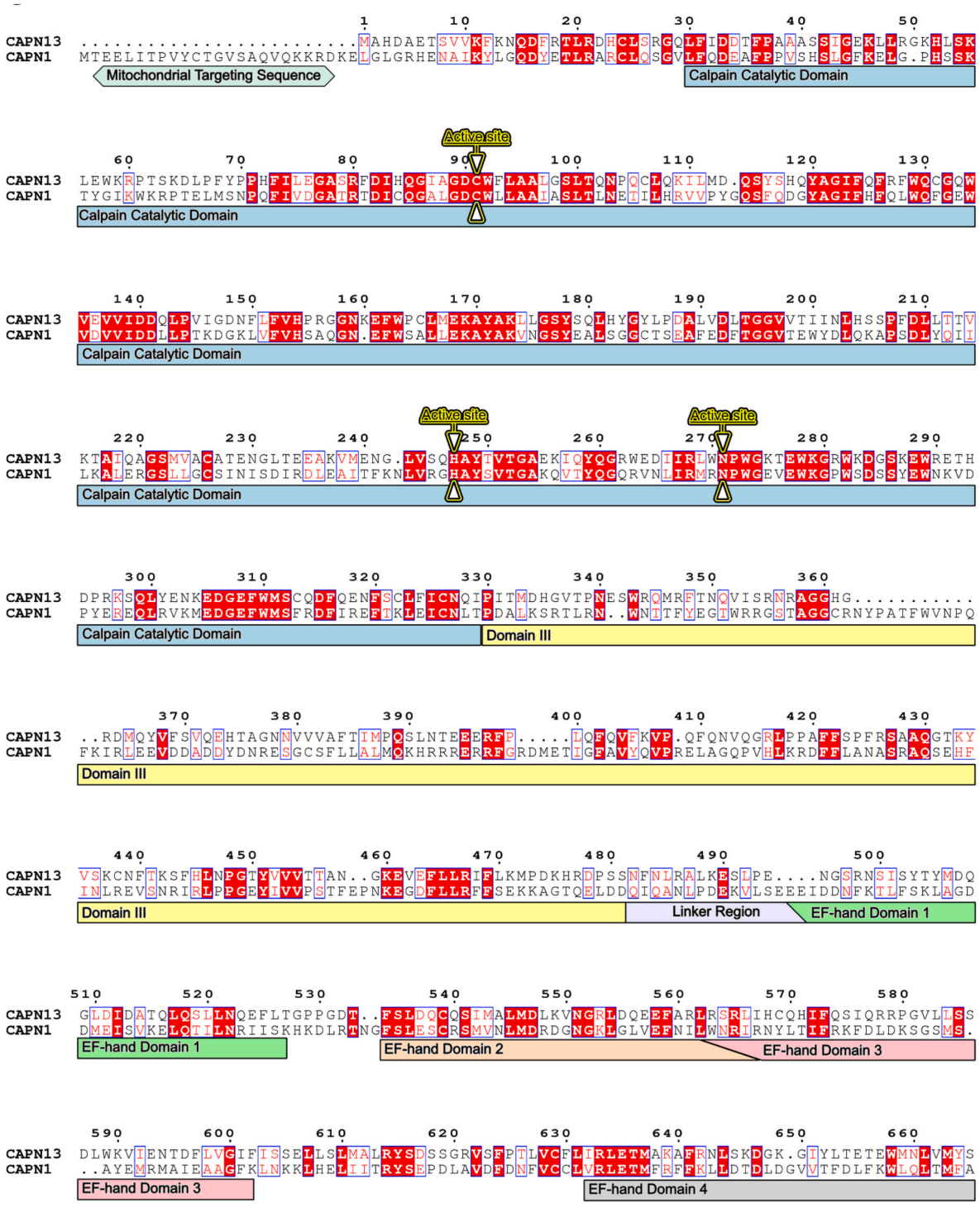

Fig. 1. Comparison of amino acid sequences between mouse calpain-1 (CAPN1) and -13 (CAPN13). The domains of CAPN1 are shown under the amino acid sequences. White characters in the red box indicate the completely conserved residues, whereas red characters in the blue box indicate similar residues. White arrowheads indicate the three conserved residues in the active site. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Science 28.0 (IBM Corp., Armonk, NY, USA). Following pre-analysis using the Shapiro–Wilk test for normality and Levene's test for homogeneity of variance, an unpaired *t*-test was adapted for analyzing the significance between sham control and I/R groups.

3. Results

3.1. Alignment of calpain-1 and calpain-13 in amino acid sequences

To predict the enzymatic properties of calpain-13, we compared the amino acid sequences between mouse calpains-1 and -13 (Fig. 1). Three residues composing the catalytic triad (cysteine, histidine, asparagine) were completely conserved. Though calpain-1 has a mitochondrial targeting sequence in the N-terminus [24], calpain-13 lacks the sequences.

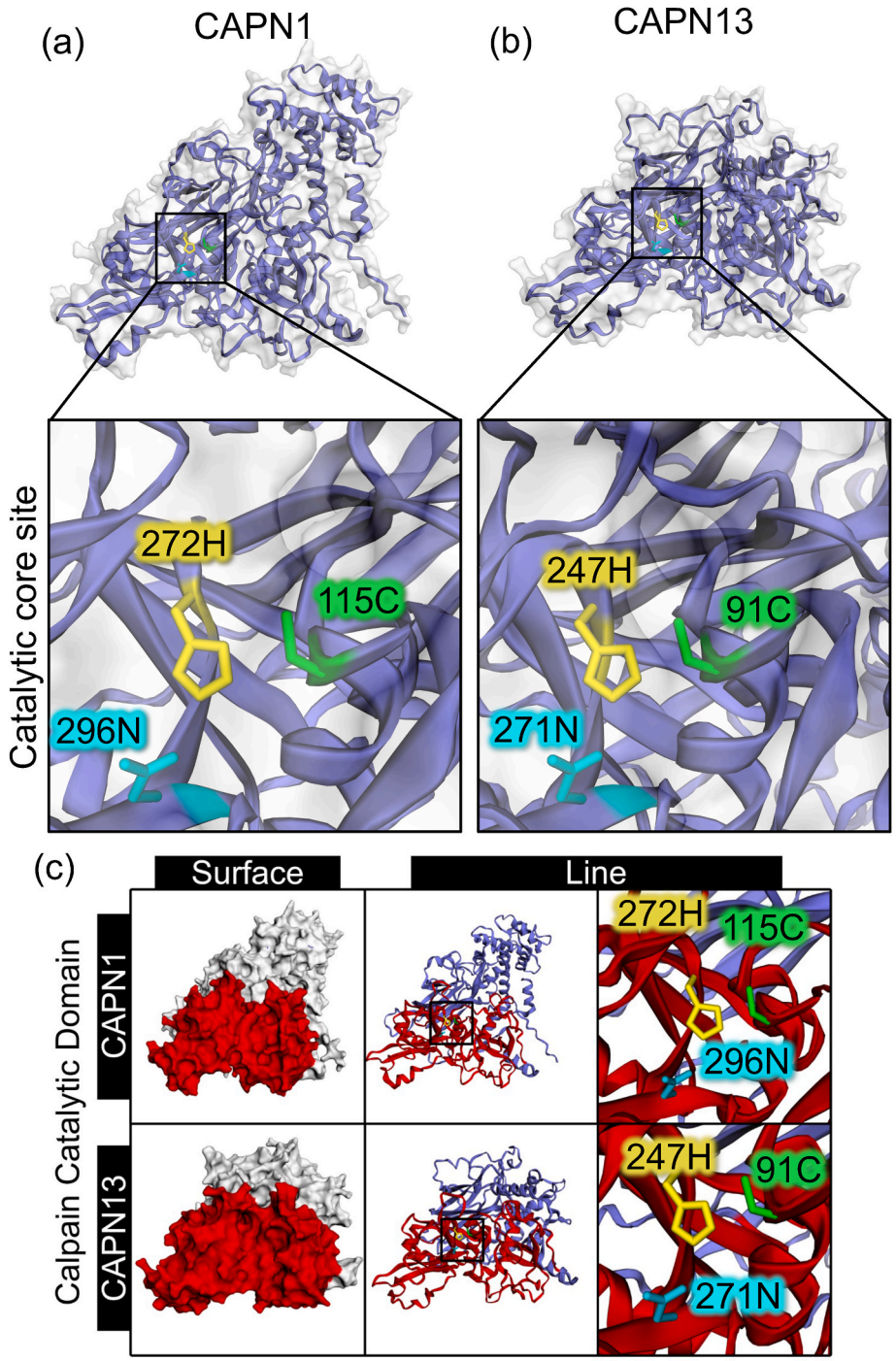

Fig. 2. Structural analysis of the catalytic core site and EF-hand domains of mouse calpains-1 and -13. (a and b) The predicted structure of mouse calpain-1 (CAPN1) and -13 (CAPN13). The catalytic triad is preserved in both calpains. (c) The calpain catalytic core site of calpains-1 and -13 is in the inner region of the structure. The red color indicates the calpain catalytic domain. Green, cysteine (C); yellow, histidine (H); blue, asparagine (N). (d and e) The EF-hand domain is in a similar position in calpain-1 and -13. The red color indicates the EF-hand domain 2 from 534F to 569C (d) and domain 4 from 632I to 665S (e) in calpain-13. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

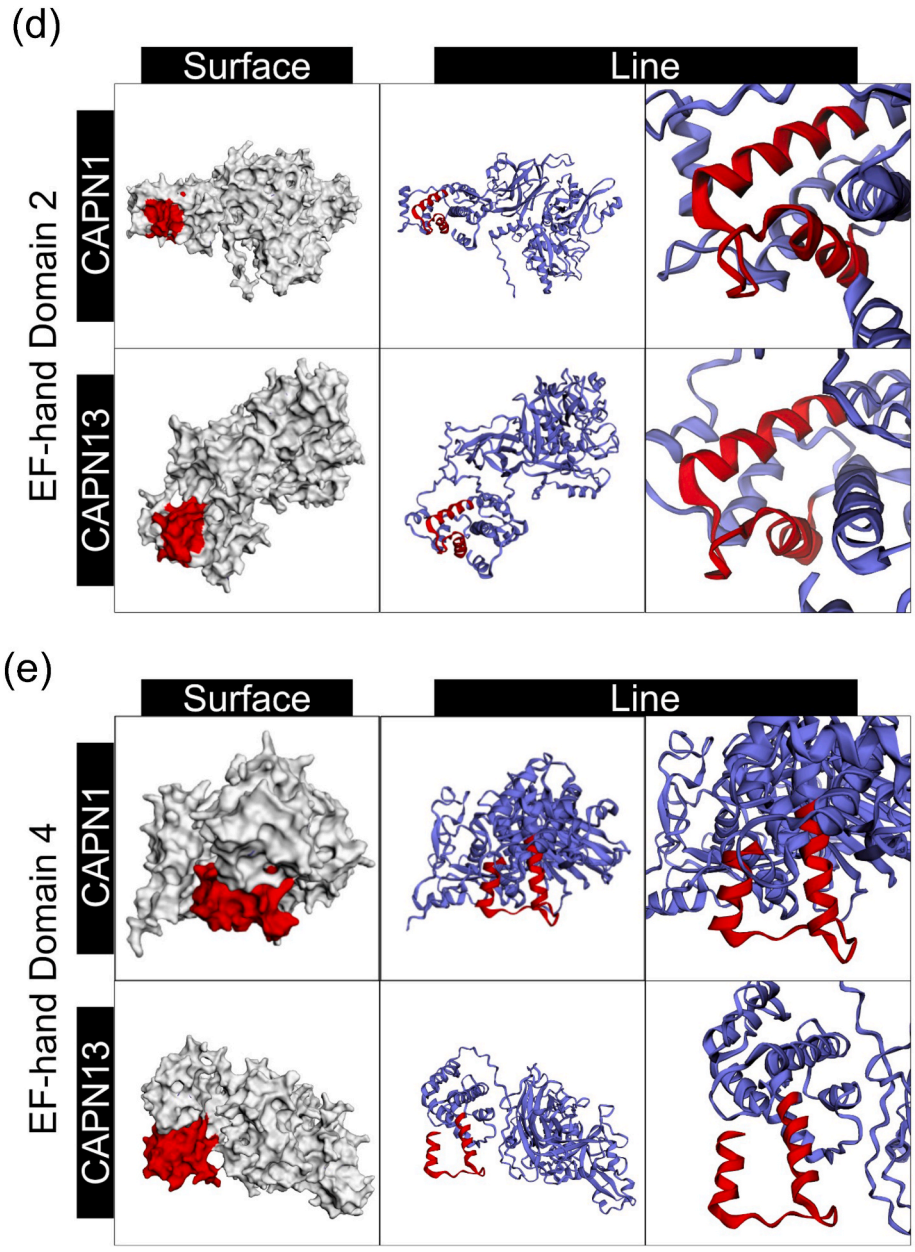

Fig. 2. (*continued*).

MitoFate [20] indicated that the sequence was unlikely to exist in the amino acid sequence of calpain-13.

3.2. Structural prediction of calpain-13 compared with calpain-1

In the predicted conformation of mouse calpain-13, the ordinarily catalytic triad was present in calpain-13 as well as mouse calpain-1 (Fig. 2a and b, Table 1). Additionally, calpain-1 and -13 showed a calpain catalytic domain, which internally contains the catalytic triad (Fig. 2c). In addition to the calpain catalytic domain, we compared the EF-hand domains, which interact with Ca^{2+} [25]. According to the UniProt online database, calpain-13 has two EF-hand domains; thus, the EF-hand domains 2 and 4 (Fig. 2e and d) were compared. Both domains of mouse calpain-13 showed two alpha-helix structures and a linker region, similar to that observed in mouse calpain-1.

Table 1
Predicted distance between each residue in the ordinarily catalytic site of calpain-1 and -13.

	Residue 1	Residue 2	Distance (Å)	Area (Å^2)
CAPN1	272H	115C	5.21	18.335
	272H	296N	7.04	
	115C	296N	8.85	
CAPN13	247H	91C	5.17	18.276
	247H	271N	7.07	
	91C	271N	8.76	

3.3. Ca^{2+}-and time-dependency of cytosolic and mitochondrial calpain-13

Cytosolic and mitochondrial calpain-13 were evaluated using a proteolysis assay followed by western blot analysis to reveal the minimum Ca^{2+} concentrations essential for their activation. The proteolytic products of neither cytosolic nor mitochondrial calpain-13 were detected at 1 mM Ca^{2+} *in vitro* (Fig. 3a and b). To investigate the calcium sensitivity of cytosolic and mitochondrial calpain-13, we performed a

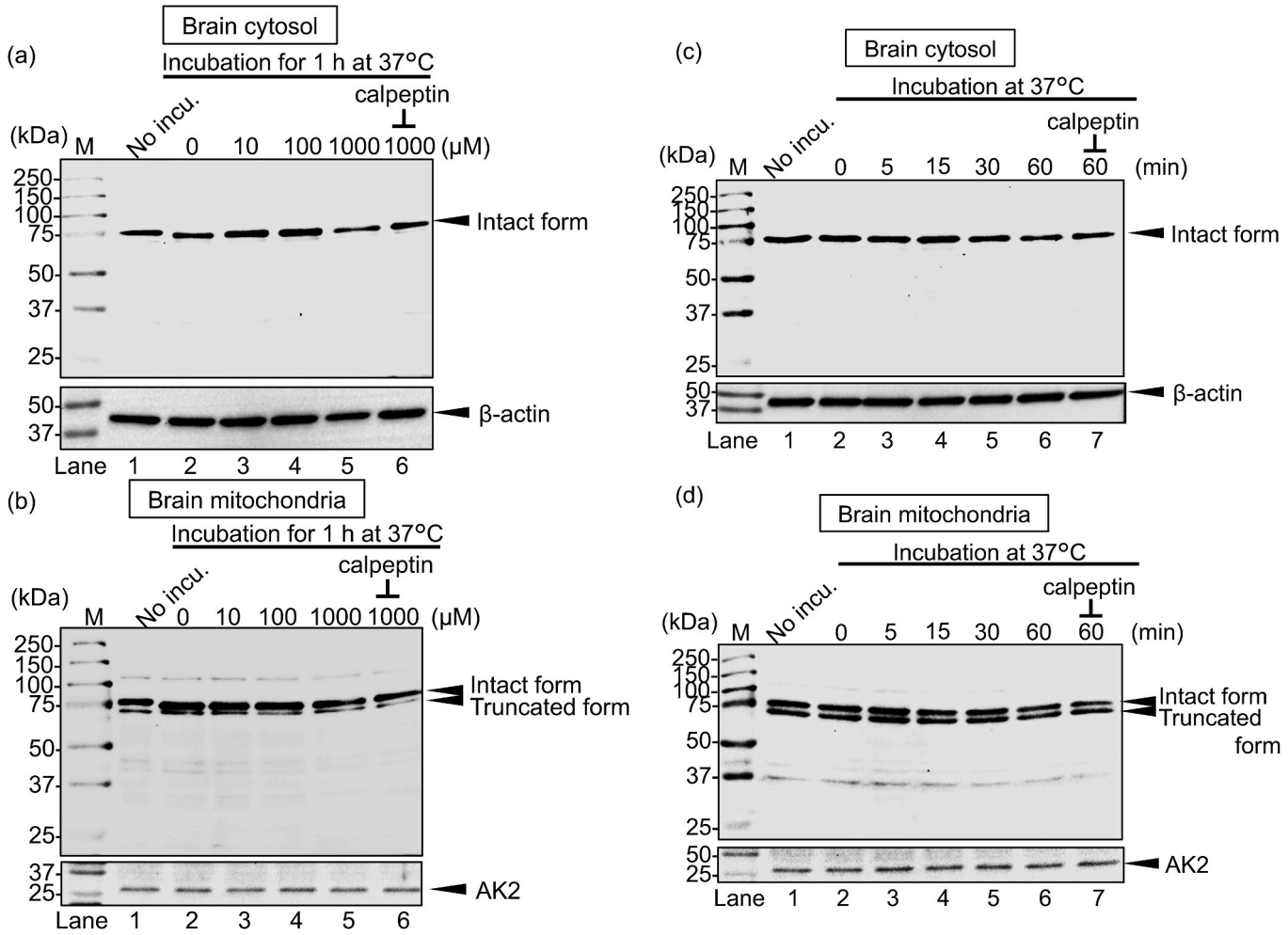

Fig. 3. *In vitro* calcium assay of cytosolic and mitochondrial calpain-13 in the mouse brain. (a and b) *In vitro* assay for Ca^{2+} concentration-dependency for calpain-13 in cytosol (a) and mitochondria (b). Each sample (cytosol, 20 μg/lane; mitochondria, 40 μg/lane) was incubated with 0–1000 μM Ca^{2+} concentrations for 60 min at 37 °C. (c and d) *In vitro* assay for time-dependency for calpain-13 in cytosol (c) and mitochondria (d). Each sample (cytosol, 20 μg/lane; mitochondria, 40 μg/lane) was incubated with 1 mM Ca^{2+} for 0–60 min at 37 °C. β-Actin and Adenylate kinase 2 (AK2) were used as the cytosolic and mitochondrial loading control. M, molecular weight marker. "No incu." indicates the untreated sample.

proteolytic assay followed by western blot analysis. No proteolytic fragments were observed with either cytosolic or mitochondrial calpain-13 60 min after Ca^{2+} addition (Fig. 3c and d).

3.4. Activation of cytosolic and mitochondrial calpain-13 in a cerebral I/R mouse model

To reveal the enzymatic properties of cytosolic and mitochondrial calpain-13 *in vivo*, western blot analysis was performed using samples from a cerebral I/R injury mouse model. Cytosolic calpain-13 showed no activation, regardless of reperfusion time (Fig. 4a). By contrast, the expression of the proteolytic products of mitochondrial calpain-13 significantly increased after 3–24 h of reperfusion (Fig. 4b and c).

4. Discussion

To our knowledge, this study is the first to reveal the enzymatic properties of calpain-13 in the mouse brain. Both cytosolic and mitochondrial calpain-13 were not proteolyzed at 1 mM Ca^{2+} *in vitro*; however, following cerebral I/R, mitochondrial calpain-13 produced its proteolytic products. These findings suggest that mitochondrial calpain-13 is involved in the responses associated with glutamate receptors and ER stress.

The amino acid sequences indicated that calpain-13 had a calpain catalytic triad (Fig. 1). Additionally, the predicted structure of calpain-13 showed that the residues formed a triangle similar to that observed in calpain-1 (Fig. 2a and b, Table 1). The positions of each triangle were embedded in the calpain catalytic domains (Fig. 2c). Considering that calpain-13 contained an EF-hand structure that interacted with Ca^{2+} (Fig. 2d and e), the reasonable hypothesis was that calpain-13 worked as a Ca^{2+}-activated protease, similar to calpain-1.

In general, calpain-1 receives calcium signals, followed by its autolysis and cleavage of substrates [26]. Hence, we performed an *in vitro* assay for cytosol and mitochondria. Contrary to our prediction, calpain-13 showed no proteolysis/autolysis *in vitro* (Fig. 3). This discordance between the prediction and *in vitro* results might be attributed to the *in vitro* environment. In particular, our *in vitro* system disrupted the mitochondrial structure. The association between calpain activation and membrane interaction has been explored previously [27,28]. Given the observation of proteolysis of mitochondrial calpain-13 *in vivo* (Fig. 4b), our *in vitro* results suggest that mitochondrial membrane structure contributes to the proteolysis of calpain-13.

The cause of cerebral ischemia is infarction due to an inadequate oxygen supply. Cerebral ischemia induces cytotoxicity owing to reactive oxygen and nitrogen species, which induces apoptosis and necrosis [29]. Reperfusion restores blood flow to ischemic tissues, damaging mitochondrial structure, enhancing cell death and tissue damage and involving the cytosolic and mitochondrial Ca^{2+} concentrations [30]. The

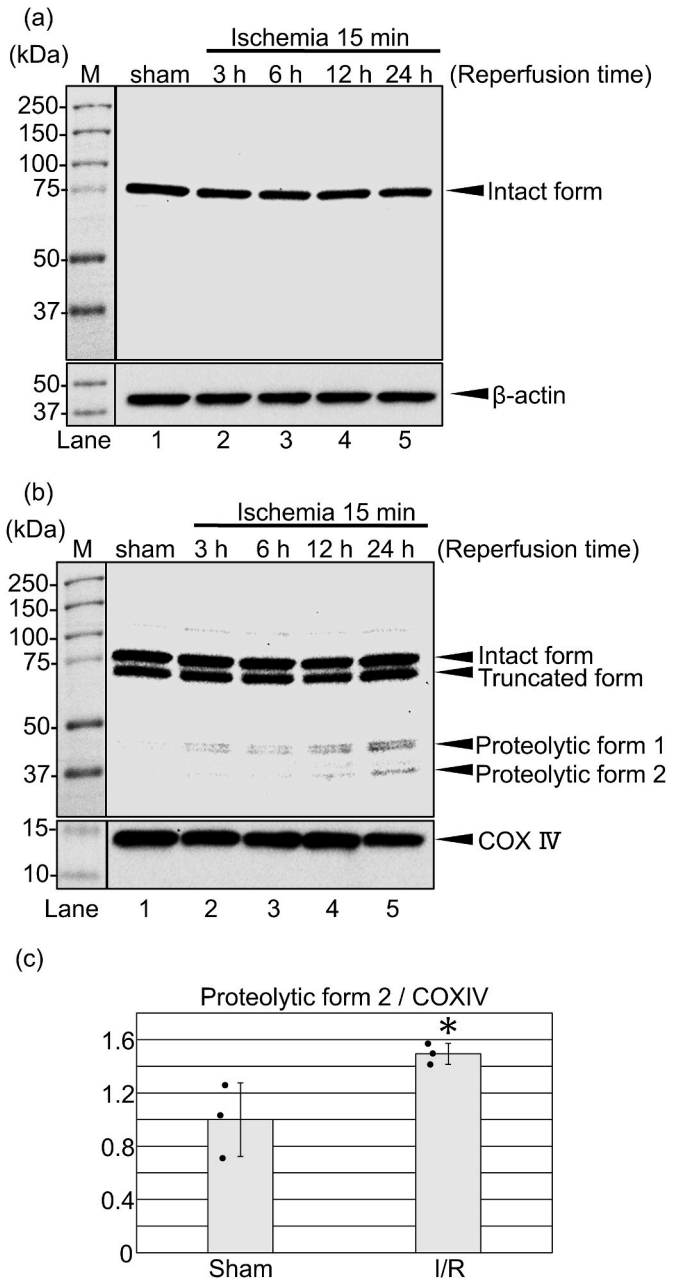

Fig. 4. *In vivo* proteolysis assay of cytosolic and mitochondrial calpain-13 using a cerebral I/R injury mouse model. (a) Western blot analysis of the proteolysis/autolysis products of cytosolic calpain-13 after 15 min of ischemia and 3–24 h of reperfusion (20 μg/lane). β-Actin was used as a cytosolic loading control. (b) Western blot analysis of the proteolysis/autolysis products of mitochondrial calpain-13 after 15 min of ischemia and 3–24 h of reperfusion (40 μg/lane). Cytochrome *c* oxidase subunit 4 (COX IV) was used as a mitochondrial loading control. M, molecular weight marker. (c) Statistical analysis of the proteolytic form of mitochondrial calpain-13 after 15 min of ischemia and 24 h of reperfusion (I/R). The expression is significantly increased in I/R mice compared to that in the sham control (*$p = 0.041$). Data indicate the average ± standard deviation of each group, and each dot represents the individual value. Detailed statistics are provided in Table S1.

Ca^{2+} elevation induces calpain activation [31]. In this study, no proteolysis of cytosolic calpain-13 was detected even 24 h after reperfusion *in vivo* (Fig. 4a). However, the proteolysis of mitochondrial calpain-13 was confirmed within 24 h after reperfusion (Fig. 4b and c). The results suggest an unknown regulatory factor depending on the localization of calpain-13.

The calculated molecular weight of intact calpain-13 is about 76 kDa. We were able to detect the intact calpain-13. Meanwhile, a truncated form (about 69 kDa) was consistently detected in the mitochondrial fraction (Figs. 3 and 4). The signal of this truncated form may be concealed from the cytosolic signal of calpain-13. Whether the truncated form of calpain-13 is a resident or activated form remains unclear. We previously reported that the intact and the truncated forms existed not in the mitochondrial soluble fraction but in the mitochondrial membrane fraction [9]. To examine the truncation of mitochondrial calpain-13, we suspected the elimination of mitochondrial transfer signals. The analysis using MitoFates [20] did not allow us to identify a mitochondrial transfer signal. However, these results did not disprove the notion that calpain-13 is transferred to the mitochondria followed by the cleavage of the signal sequence. To clarify this point, we should compare the structure and amino acid sequence between the two forms of calpain-13.

The findings of this study should be interpreted taking into account several limitations. First, proteolytic forms of mitochondrial calpain-13 have been identified in a cerebral I/R injury, but not *in vitro*, even at 1 mM Ca^{2+}. This might be because of the inability to completely reproduce *in vivo* conditions in an *in vitro* experimental system and the inability to completely inhibit the activation of calpain-13 at the time of sample collection. In particular, the presence of calpains in the membrane fraction depended on some detergents [32]. In this regard, we should aim to clarify the factors that activate calpain-13 in addition to Ca^{2+}. Second, the factor that leads to the proteolysis of calpain-13 remains unclear. The autolysis of calpains-1 and -2 have unique features; the autolysis product of calpain-1 reflects weak and sustained activity, while the autolysis product of calpain-2 is inactive [33]. However, autolysis of the calpains is the post-activation event [26,34,35]. Therefore, we proposed that the proteolysis product reflected calpain-13 activity similar to the autolysis products of other calpains. Future experiments will focus on repurifying calpain-13 to identify its substrate.

We showed that the calpain-13, which was predicted to possess enzymatic properties, was proteolyzed not *in vitro* but in the mitochondria of the I/R injured mouse brain. Following our previous study [36], we hope to use similar experimental methods to characterize the enzymatic properties of mitochondrial calpain-13 and elucidate its physiological functions in brain diseases in the future.

Data accessibility

The data from this study are available from the corresponding author, Taku Ozaki, upon reasonable request.

Funding

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Ethics approval

All animal experiments complied with the Animal Research: Reporting In Vivo Experiments guidelines and were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and the associated guidelines, EU Directive 2010/63/EU, for animal experiments. The animal experiments were approved by the Committee for the Ethics of Animal Experimentation of Iwate University (approval number: A202326) and were carried out according to the Guidelines for Animal Experimentation of Iwate University.

CRediT authorship contribution statement

Yusaku Chukai: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Toru Sudo:** Validation, Investigation, Formal analysis, Data curation. **Tomokazu Fukuda:** Methodology.

Hiroshi Tomita: Methodology. **Eriko Sugano:** Methodology. **Taku Ozaki:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101768.

References

[1] D.E. Goll, V.F. Thompson, H. Li, W. Wei, J. Cong, The calpain system, Physiol. Rev. 83 (2003) 731–801, https://doi.org/10.1152/PHYSREV.00029.2002.
[2] M.A. Smith, R.G. Schnellmann, Calpains, mitochondria, and apoptosis, Cardiovasc. Res. 96 (2012) 32–37, https://doi.org/10.1093/CVR/CVS163.
[3] M. Hosseini, H. Najmabadi, K. Kahrizi, Calpains: diverse functions but enigmatic, arch. Iran, Méd. 21 (2018) 170–179.
[4] Y. Ono, H. Sorimachi, Calpains: an elaborate proteolytic system, Biochim. Biophys. Acta 1824 (2012) 224–236, https://doi.org/10.1016/J.BBAPAP.2011.08.005.
[5] T. Ozaki, H. Tomita, M. Tamai, S.I. Ishiguro, Characteristics of mitochondrial calpains, J. Biochem. 142 (2007) 365–376, https://doi.org/10.1093/JB/MVM143.
[6] T. Ozaki, T. Yamashita, S. Ishiguro, Mitochondrial m-calpain plays a role in the release of truncated apoptosis-inducing factor from the mitochondria, Biochim. Biophys. Acta 1793 (2009) 1848–1859, https://doi.org/10.1016/J.BBAMCR.2009.10.002.
[7] D.D. Arrington, T.R. Van Vleet, R.G. Schnellmann, Calpain 10: a mitochondrial calpain and its role in calcium-induced mitochondrial dysfunction, Am. J. Physiol. Cell Physiol. 291 (2006) C1159–C1171, https://doi.org/10.1152/AJPCELL.00207.2006.
[8] T. Iwamoto, E. Ishiyama, K. Ishida, T. Yamashita, H. Tomita, T. Ozaki, Presence of calpain-5 in mitochondria, Biochem. Biophys. Res. Commun. 504 (2018) 454–459, https://doi.org/10.1016/J.BBRC.2018.08.144.
[9] E. Funajima, G. Ito, E. Ishiyama, K. Ishida, T. Ozaki, Mitochondrial localization of calpain-13 in mouse brain, Biochem. Biophys. Res. Commun. 609 (2022) 149–155, https://doi.org/10.1016/J.BBRC.2022.04.002.
[10] M. Sugawara, T. Abe, S. Kasai, K. Itoh, T. Ozaki, Calpain-1 C2L domain peptide protects mouse hippocampus-derived neuronal HT22 cells against glutamate-induced oxytosis, Biochem. Biophys. Rep. 27 (2021) 101101, https://doi.org/10.1016/J.BBREP.2021.101101.
[11] Y. Chukai, T. Iwamoto, K. Itoh, H. Tomita, T. Ozaki, Characterization of mitochondrial calpain-5, Biochim. Biophys. Acta Mol. Cell Res. 1868 (2021) 118989, https://doi.org/10.1016/J.BBAMCR.2021.118989.
[12] Y. Chukai, G. Ito, M. Konno, Y. Sakata, T. Ozaki, Mitochondrial calpain-5 truncates caspase-4 during endoplasmic reticulum stress, Biochem. Biophys. Res. Commun. 608 (2022) 156–162, https://doi.org/10.1016/J.BBRC.2022.03.156.
[13] Y. Chukai, G. Ito, Y. Miki, K. Wakabayashi, K. Itoh, E. Sugano, H. Tomita, T. Fukuda, T. Ozaki, Role of calpain-5 in cerebral ischemia and reperfusion injury, Biochim. Biophys. Acta Gen. Subj. 1868 (2024) 130506, https://doi.org/10.1016/J.BBAGEN.2023.130506.
[14] M.H. Li, K. Inoue, H.F. Si, Z.G. Xiong, Calcium-permeable ion channels involved in glutamate receptor-independent ischemic brain injury, Acta Pharmacol. Sin. 32 (2011) 734–740, https://doi.org/10.1038/APS.2011.47.
[15] Y. Han, M. Yuan, Y.S. Guo, X.Y. Shen, Z.K. Gao, X. Bi, Mechanism of endoplasmic reticulum stress in cerebral ischemia, Front. Cell. Neurosci. 15 (2021) 704334, https://doi.org/10.3389/FNCEL.2021.704334.
[16] D. Bano, P. Nicotera, Ca2+ signals and neuronal death in brain ischemia, Stroke 38 (2007) 674–676, https://doi.org/10.1161/01.STR.0000256294.46009.29.
[17] K. Shintani-Ishida, K.I. Yoshida, Mitochondrial m-calpain opens the mitochondrial permeability transition pore in ischemia-reperfusion, Int. J. Cardiol. 197 (2015) 26–32, https://doi.org/10.1016/J.IJCARD.2015.06.010.
[18] A.B. Wahul, P.C. Joshi, A. Kumar, S. Chakravarty, Transient global cerebral ischemia differentially affects cortex, striatum and hippocampus in Bilateral Common Carotid Arterial occlusion (BCCAo) mouse model, J. Chem. Neuroanat. 92 (2018) 1–15, https://doi.org/10.1016/J.JCHEMNEU.2018.04.006.
[19] X. Robert, P. Gouet, Deciphering key features in protein structures with the new ENDscript server, Nucleic Acids Res. 42 (2014) W320–W324, https://doi.org/10.1093/NAR/GKU316.
[20] Y. Fukasawa, J. Tsuji, S.C. Fu, K. Tomii, P. Horton, K. Imai, MitoFates: improved prediction of mitochondrial targeting sequences and their cleavage sites, Mol. Cell. Proteomics 14 (2015) 1113–1126, https://doi.org/10.1074/MCP.M114.043083.
[21] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S.A. A. Kohl, A.J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A.W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, Highly accurate protein structure prediction with AlphaFold, Nature 596 (2021) 583–589, https://doi.org/10.1038/S41586-021-03819-2.
[22] M. Varadi, S. Anyango, M. Deshpande, S. Nair, C. Natassia, G. Yordanova, D. Yuan, O. Stroe, G. Wood, A. Laydon, A. Zídek, T. Green, K. Tunyasuvunakool, S. Petersen, J. Jumper, E. Clancy, R. Green, A. Vora, M. Lutfi, M. Figurnov, A. Cowie, N. Hobbs, P. Kohli, G. Kleywegt, E. Birney, D. Hassabis, S. Velankar, AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models, Nucleic Acids Res. 50 (2022) D439–D444, https://doi.org/10.1093/NAR/GKAB1061.
[23] C.R. Reynolds, S.A. Islam, M.J.E. Sternberg, EzMol: a web server wizard for the rapid visualization and image production of protein and nucleic acid structures, J. Mol. Biol. 430 (2018) 2244–2248, https://doi.org/10.1016/J.JMB.2018.01.013.
[24] R.K. Badugu, M. Garcia, V. Bondada, A. Joshi, J.W. Geddes, N terminus of calpain 1 is a mitochondrial targeting sequence, J. Biol. Chem. 283 (2008) 3409–3417, https://doi.org/10.1074/JBC.M706851200.
[25] M. Maki, Y. Kitaura, H. Satoh, S. Ohkouchi, H. Shibata, Structures, functions and molecular evolution of the penta-EF-hand Ca2+-binding proteins, Biochim. Biophys. Acta, Proteins Proteomics 1600 (2002) 51–60, https://doi.org/10.1016/S1570-9639(02)00444-2.
[26] A. Baki, P. Tompa, A. Alexa, O. Molnar, P. Friedrich, Autolysis parallels activation of mu-calpain, Biochem. J. 318 (1996) 897–901, https://doi.org/10.1042/BJ3180897.
[27] S. Gil-Parrado, O. Popp, T.A. Knoch, S. Zahler, F. Bestvater, M. Felgenträger, A. Holloschi, A. Fernández-Montalván, E.A. Auerswald, H. Fritz, P. Fuentes-Prior, W. Machleidt, E. Spiess, Subcellular localization and in vivo subunit interactions of ubiquitous mu-calpain, J. Biol. Chem. 278 (2003) 16336–16346, https://doi.org/10.1074/JBC.M208657200.
[28] V. Bondada, J. Gal, C. Mashburn, D.W. Rodgers, K.E. Larochelle, D.E. Croall, J. W. Geddes, The C2 domain of calpain 5 contributes to enzyme activation and membrane localization, Biochim. Biophys. Acta Mol. Cell Res. 1868 (2021) 119019, https://doi.org/10.1016/J.BBAMCR.2021.119019.
[29] K. Szydlowska, M. Tymianski, Calcium, ischemia and excitotoxicity, Cell Calcium 47 (2010) 122–129, https://doi.org/10.1016/J.CECA.2010.01.003.
[30] C.J.A. Ramachandra, S. Hernandez-Resendiz, G.E. Crespo-Avilan, Y.H. Lin, D. J. Hausenloy, Mitochondria in acute myocardial infarction and cardioprotection, EBioMedicine 57 (2020) 102884, https://doi.org/10.1016/J.EBIOM.2020.102884.
[31] N. Christiane, N. Heinz, Calpain system and its involvement in myocardial ischemia and reperfusion injury, World J. Cardiol. 6 (2014) 638–652, https://doi.org/10.4330/WJC.V6.I7.638.
[32] T. Ozaki, T. Yamashita, S. Ishiguro, Ca2+-induced release of mitochondrial m-calpain from outer membrane with binding of calpain small subunit and Grp75, Arch. Biochem. Biophys. 507 (2011) 254–261, https://doi.org/10.1016/J.ABB.2010.12.003.
[33] F. Shinkai-Ouchi, M. Shindo, N. Doi, S. Hata, Y. Ono, Calpain-2 participates in the process of calpain-1 inactivation, Biosci. Rep. 40 (2020), https://doi.org/10.1042/BSR20200552. BSR20200552.
[34] J.S. Elce, C. Hegadorn, J.S. Arthur, Autolysis, Ca2+ requirement, and heterodimer stability in m-calpain, J. Biol. Chem. 272 (1997) 11268–11275, https://doi.org/10.1074/jbc.272.17.11268.
[35] T. Ozaki, T. Yamashita, S. Ishiguro, ERp57-associated mitochondrial μ-calpain truncates apoptosis-inducing factor, Biochim. Biophys. Acta 1783 (2008) 1955–1963, https://doi.org/10.1016/j.bbamcr.2008.05.011.
[36] G. Ito, Y. Tatara, K. Itoh, M. Yamada, T. Yamashita, K. Sakamoto, T. Nozaki, K. Ishida, Y. Wake, T. Kaneko, T. Fukuda, E. Sugano, H. Tomita, T. Ozaki, Novel dicarbonyl metabolic pathway via mitochondrial ES1 possessing glyoxalase III activity, BBA Adv 3 (2023) 100092, https://doi.org/10.1016/J.BBADVA.2023.100092.