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The Critical Role of Calpain in Cell Proliferation

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Abstract

Calpain is a conserved family of calcium-dependent, cytosolic, neutral cysteine proteases. The best characterized members of the family are the ubiquitously expressed calpain 1 and calpain 2. They perform controlled proteolysis of their target proteins. The regulation of these enzymes includes autolysis, calcium, phosphorylation as a posttranslational modification, and binding of calpastatin, phospholipids or activator proteins, respectively. Calpain are implicated in many physiological and pathological processes. They have significant role in the cell proliferation, differentiation and migration in a variety of mammalian cell types, contributing to the development of angiogenesis, vascular remodeling, and cancer. Therefore the knowledge of the precise mechanism of calpain signaling could provide therapeutic approaches in these processes.

Keywords: Calpain; Proliferation; Survival; Migration; Apoptosis

Introduction

Calpain was first described as a neutral, calcium-activated proteinase in the soluble fraction of rat brain [1]. It accomplishes its proteolytic activity in the cytoplasm, not in the lysosomes at a neutral pH. Calpain was named calcium-activated neutral protease (CANP) after purification from chicken skeletal muscle in 1978 [2]. In 1984, Ohno et al. [3] found that the calpain is a fusion gene product with a combination of papain-like cysteine protease and calmodulin-like calcium-binding domains. The members of calpain superfamily can be found in many different species from Homo sapiens to the lower organism including invertebrates, plants, fungi, yeasts and bacteria. In mammalian cells, there are 15 genes encoding the large catalytic subunits, and two genes encoding small regulatory subunits (Table 1) [4,5]. They can be classified according to their localization (ubiquitous or tissue-specific). Several calpain isoforms are ubiquitously expressed in the cytosol (calpain 1, 2, 5, 7, 10, 13, 14, 15 and 16). The others show tissue-specific expression pattern (calpain 3, 6, 8, 9, 11 and 12). For example, calpain 3 is skeletal muscle specific [6], calpain 8 is specific to stomach smooth muscle [7], and the digestive track contains calpain 9 [8,9]. Based on their domain structure calpain can be divided into two classes (typical or atypical). Typical calpain (1, 2, 3, 8, 9, 11, 12, 13 and 14) have four domains in their 80 kDa large subunit as well as encode calmodulin-like EF-hands in domain IV. In contrast, atypical calpain (5, 6, 7, 10, 15 and 16) do not have EF-hands in domain IV and in some domains have been deleted or replaced [5,10]. The calpain 1, 2 and 9 form heterodimer with the 30 kDa subunit, while the other typical calpain (calpain 3, 8, 11, 12 and 14) do not dimerize with the small regulatory subunit, although they have domain IV. The atypical calpain are unsuitable for dimerization due to lack of domain IV.

Table 1: The members of calpain superfamily.

Calpain isoform	Calpain gene	Other names	Expression pattern	Classification
Calpain 1	*CAPN1*	μ-calpain; CAPN1	ubiquitous	typical
Calpain 2	*CAPN2*	m-calpain; CAPN2	ubiquitous	typical
Calpain 3	*CAPN3*	nCL-1; p94; LGMD2; LGMD2A	skeletal muscle, lens, retina	typical
Calpain 5	*CAPN5*	nCL-3; HTRA3	ubiquitous	atypical
Calpain 6	*CAPN6*	Calpamodulin, CAPNX	placenta	atypical
Calpain 7	*CAPN7*	PALBH	ubiquitous	atypical
Calpain 8	*CAPN8*	nCL-2	stomach	typical
Calpain 9	*CAPN9*	nCL-4	digestive track	typical
Calpain 10	*CAPN10*	CAPN10	ubiquitous	atypical
Calpain 11	*CAPN11*	CAPN11	testis	typical
Calpain 12	*CAPN12*	CAPN12	hair follicle	typical
Calpain 13	*CAPN13*	CAPN13	ubiquitous	typical
Calpain 14	*CAPN14*	CAPN14	ubiquitous	typical
Calpain 15	*CAPN15*	SOLH; CAPN15	ubiquitous	atypical
Calpain 16	*CAPN16*	CAPN16	ubiquitous	atypical
Small subunit 1	*CAPNS1*	CAPN4; CAPNS1; CSS1	ubiquitous	-
Small subunit 2	*CAPNS2*	CAPNS2; CSS2	ubiquitous	-

Structure

The most intensely studied members of the calpain superfamily are the mammalian ubiquitous calpain 1 and 2. They were named according to their Ca^{2+} requirement for activation *in vitro*. Calpain 1 and 2 need micro-molar and milli-molar Ca^{2+} concentrations, respectively, for their proteolytic activity [11,12]. They are heterodimers and consist of a distinct 80 kDa large catalytic subunit (encoded by *CAPN1* and *CAPN2*, respectively) and a common 30 kDa small subunit (encoded by *CAPNS1*) that regulates calpain activity [13,14]. The catalytic subunit can be further divided into four functional domains (DI-DIV), while the small subunit comprises two domains (DV-DVI) (Figure 1) [15,16]. Domain I is the N-terminal region of the large subunit that can interact with the DVI of the small subunit and stabilize the protein. It contains 19 amino acid residues that can be cleaved by autolysis during activation. The protease domain (DII) contains the active site of catalytic triad (Cys105, His262 and Asn286). Like other cysteine proteases, it has two subdomains, IIa and IIb. In the inactive state (in the absence of Ca^{2+}), the Cys105 residue is located on the subdomain IIa and the His262/

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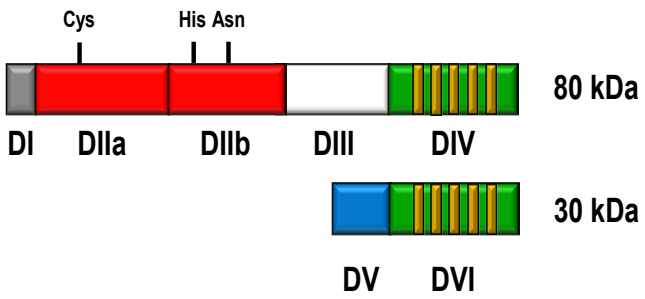

Figure 1: Schematic structure of typical calpains. The 80 kDa large catalytic subunit of calpain consists of four domains (DI-DIV). The 30 kDa small regulatory subunit comprises two domains (DV-DVI). The catalytic triad residues (Cys, His and Asn) are located within the DII protease domain. The five EF-hand motifs are represented by gold bars in DIV and DVI.

Asn286 residues are on the subdomain IIb. Thus, they are far from each other to form the catalytic site. Interactions between DI and DIIa as well as DIII and DIIb help maintain this inactive structure. It has been proposed that calcium-induced conformational changes lead to realign the key residues and create the functional active site [17]. Domain III has a β-sandwich structure which is similar to the C2 domain. C2 domain can be found in proteinkinase C and phospholipase C, which plays a role in the calcium and phospholipids binding of the proteins [18]. This domain can bind phospholipids in calcium dependent manner and may have a role in the activation of the enzyme [19]. Domain IV and domain VI are the C-terminal ends of the catalytic and regulatory subunits, respectively, and both of them contain five EF-hand motifs. The first four EF-hands are responsible for the calcium binding and show structural similarity to the calmodulin. The fifth EF-hand contribute to the heterodimerization of the subunits [20-22]. Domain V is the amino-terminal end of the regulatory subunit and contains clusters of glycine residues. Because of its hydrophobic feature it has an important role in the membrane anchoring [23]. Recently, a second regulatory subunit has been described, but its physiological function is not revealed yet [24].

Biological functions

Calpain, as regulatory proteases, perform limited proteolysis of their target proteins [25]. Calpain have a wide spectrum of substrates, including membrane (e.g. receptors, transporters) and cytoskeletal (e.g. talin, spectrin, vinculin) proteins, transcriptional factors (e.g. p53) as well as enzymes (e.g. phosphatases, caspases, PKC and phospolipase C) [4,10]. The members of this family are involved in various physiological processes such as cell proliferation, cell migration, cell cycle progress, apoptosis, cytoskeletal remodeling and signal transduction [26-30]. Mutations or over-activation of calpain can contribute to number of pathological phenomenon including multiple sclerosis, stroke, gastric cancer, type 2 diabetes mellitus, Alzheimer's disease, cataract, and muscular dystrophies [31-33]. Due to the inadequate specificity of the recently used calpain inhibitors their exact physiological roles remain elusive [34,35]. Homozygous disruption of *CAPN2* or *CAPNS1* causes embryonic lethality in mice emphasizing their important role in the embryonic development [36,37]. The *CAPN1* knockout mice are viable, but show impaired platelet function [38].

Regulation and activation

The micro-molar and milli-molar Ca^{2+} concentrations required for calpain 1 and 2 *in vitro* activation are far above the Ca^{2+} level available in the cytoplasm. Therefore numerous factors have been proposed that can reduce this enormous calcium requirement including autoproteolytic cleavage of the N-terminal tail, phospholipid binding, posttranslational modification (e.g. phosphorylation), endogenous inhibitor calpastatin and binding to regulator proteins.

The native calpain exists as an inactive proenzyme in the cytosol and cuts its N-terminal part of both subunits upon activation causing the dissociation of the catalytic and regulatory subunits most of the cases [12,39-41]. The autolysis of the large and small subunits occurs rapidly in two and three stages, respectively [42-44]. During this process the enzyme becomes active and has a lower Ca^{2+} requirement for its activation [45-47].

Several phospholipids (e.g. phosphatidylinositol, phosphatidylinositol 4,5-bisphosphate) have important roles in the regulation of the calpain by reducing the Ca^{2+} concentration required for their *in vitro* activation [19,48,49]. Increase in intracellular Ca^{2+} level can evoke the translocation of the calpain from the cytosol to the plasma membrane, where it can interact with phospholipids and become catalytically active [40]. Two phospholipids binding sites have been identified in the N-terminal region of the small subunit and the domain III of the large subunit, respectively [19,50].

Phosphorylation also takes part in controlling calpain activity. Earlier report suggests that the calpain could not be phosphorylated *in vivo* [51], but nine and eight phosphorylated residues were later identified in calpain 1 and 2, respectively [4]. This posttranslational modification is a double-edged sword because it can up-regulate and down-regulate calpain activity. Calpain 2 can be directly phosphorylated at Ser50 by the extracellular signal-regulated protein kinase (ERK) after epidermal growth factor (EGF) stimulation causing the activation of the enzyme independently of calcium [52,53]. In contrast, phosphorylation of the calpain 2 at Ser369/370 by protein kinase A (PKA) blocks the EGFR-stimulated calpain activation by freezing the enzyme in an inactive conformation [54,55]. Interestingly, the muscle-specific calpain 3 (p94) has a glutamic acid at Ser50 site, in which the negative charge mimics the effect of phosphorylation, and does not need increased intracellular calcium level for its activation [56]. Calpain 1 does not contain phosphorylatable amino acid residue at this position and it was revealed that it can be activated by cytokines in calcium flux dependent manner [57]. Nicotine induces phosphorylation of calpain 1 and 2 by protein kinase Cι which is associated with elevated activation and secretion of the enzymes [58]. Recent observation indicates that the closely related calpain homologue, calpain B can also be regulated by EGF-stimulated phosphorylation in fruit flies [59], which further emphasizes the regulatory role of this posttranslational modification in the calpain system. More recently it was found that the ERK- and PKA kinases-mediated phosphorylation of the calpain 2 regulates the distribution of the enzyme between cytoplasm and plasma membrane, indirectly contributing to its activation [60].

Calpastatin, the ubiquitously expressed endogenous calpain inhibitor, blocks both calpain 1 and 2 with similar efficiency and does not inhibit any other protease [61-63]. Its polypeptide chain composed of an N-terminal L domain and four repetitive inhibitory domains (I–IV). The L domain plays a role in the association of Ca^{2+} channels [64] and it does not have inhibitory effect [65]. Each inhibitory domain can bind and inhibit one calpain molecule independently [66]. The inhibitory domains can be further subdivided into three subdomains. The A and C subdomains are responsible for binding to the domain IV and domain VI of the calpain in a calcium dependent manner, respectively. The B subdomain binds to the domain II and blocks the catalytic side of the

protease [67-69]. Calpastatin can interact with both native and autolyzed form of calpain, although its affinity to the autolyzed calpain is higher [70,71]. Phosphorylation of calpastatin by PKA can modify its subcellular localization and inhibitory specificity [72].

Several proteins were identified as calpain activators which can reduce the calcium requirement for calpain activation and facilitates the autolysis [73,74]. Some of them are calpain 1 specific [75,76], while others are specific for calpain 2 [77-79]. But their precise role is poorly investigated.

Calpain in cell proliferation

Calpain 1 and calpain 2 are the best characterized typical calpain isoforms which have been shown to be involved in many basic cellular processes such as cell proliferation and differentiation in various mammalian cell types. The role of calpain in cell proliferation was revealed in some earlier experiments using calpain inhibitors. Calpain inhibitor calpeptin (benzyloxycarbonyl-Leu-nLeu-H) and other thiol protease inhibitors were shown to reversibly inhibit the PDGF-BB- as well as serum-induced bovine aortic smooth muscle cell (BASMC) cycle progression *in vitro* [80]. Ariyoshi et al. [81] found that the cell-permeable calpeptin and its analogue (benzyloxycarbonyl-Leu-Met-H), as well as antisense oligonucleotide against calpain 2 (m-calpain) block the proliferation of vascular smooth muscle cell (VSMC) in dose-dependent manner supporting the fact that the calpain activity is involved in this process. Similar findings were observed in other types of cells. Culturing of the Chinese hamster ovary cell line (CHO) in the presence of cell-permeant calpain inhibitor ZLLY-CHN2 (benzyloxycarbonyl-Leu-Leu- Tyr diazomethyl ketone) causes not only a decrease in calpain 1 (μ-calpain) protein level but it also diminishes the growth rate of the cells [82]. Depletion of the calpain small subunit (calpain 4) using specific antisense oligonucleotide inhibits the proliferation of WI-38 human fibroblasts and HeLa cells [83]. Zhang et al. [84] also reported that the calpain-selective inhibitor ZLLY-CHN2 blocks the serum-stimulated growth and cell cycle progression into S-phase in WI-38 human fibroblasts cells. It was shown that overexpression of calpastatin in CHO cell lines significantly decreases the growth of isolated colonies [85]. The pro-proliferative effect of calpain was also revealed in osteoblast cells. It was reported that cell-permeable calpain inhibitor attenuates the proliferation of MC3T3-E1 preosteoblasts cells [86,87], while specific disruption of calpain 4 results in impaired proliferation and differentiation of osteoblastic cells [88]. Taken together, these observations strongly indicate the role of calpain in the proliferation of different mammalian cells.

Several mechanisms are responsible for calpain-mediated cell proliferation. Carragher [89] reported that the calpain-calpastatin system contributes to the v-Src induced cell cycle progression, cell transformation and motility. They found that v-Src activation increases the calpain 2 protein level which is associated with degradation of calpastatin and focal adhesion kinase (FAK). The elevated calpain activity causes disassembly of focal adhesion complexes and initiation of cell motility. Calpain also induces hyperphosphorylation of the retinoblastoma protein (pRb) and increases the level of cell cycle proteins (cyclins D, A and cyclin-dependent kinase 2) facilitating the progression of transformed cells through the G1 stage of the cell cycle. Inhibition of calpain activity by overexpression of calpastatin or using calpain inhibitors attenuates the effect of calpain on the cell proliferation and motility. Other report has indicated that calpain is involved in mitosis. Knockdown of calpain 2 expression, but not calpain 1, using specific siRNA and/or blocking the calpain activity by specific inhibitors cause abnormal mitosis which is accompanied by chromosome misalignment. Moreover, calpain inhibition delays the prometaphase events and suppresses the generation of polar ejection force on chromosomes [90]. Flow-cytometric analysis of calpain inhibition revealed that calpain activity is not only required to promote the cell cycle at the restriction, G1 checkpoint, but also has significant role in the S and G2M phase progression [27]. Furthermore, Ho et al. [91] reported that calpain 2 mediates the proliferation, migration, and tumorigenesis of mammary cancer cells via Akt-FoxO-$p27^{Kip1}$ signaling pathway. Silencing of the calpain 2 isoform results in increases in protein phosphatase 2A (PP2A) level and reduction of Akt activation. Impaired Akt activity contributes to the activation and nuclear translocation of FoxO3a transcription factor which is associated with elevated expression level of cyclin-dependent kinase inhibitor $p27^{Kip1}$ and a reduction in the breast carcinoma cell proliferation. Finally, our recent study provides strong evidence that calpain governs proliferation and collagen synthesis of pulmonary artery smooth muscle cells (PASMCs) induced by EGF and PDGF. This effect is contributed to calpain-mediated cleavage and activation of intracellular transforming growth factor-β1 (TGF-β1) in PASMCs. Incubation of the PASMCs with EGF and PDGF significantly increases the calpain activity, cell proliferation and collagen synthesis. The effects of the growth factors are attenuated using specific calpain inhibitor MDL28170 or siRNAs against calpain 1 and calpain 2. More importantly we found that conditional knockout of calpain 4 and calpain inhibitor MDL28170 prevent the progression of pulmonary vascular remodeling induced by hypoxia and monocrotaline [92].

Growing body of evidence demonstrates that calpain-mediated cell proliferation is associated to other complex processes such as cell migration, differentiation (e.g. angiogenesis) and survival. Several studies emphasize the role of calpain in regulating the cell migration. It was revealed that calpain mediates the cleavage of focal adhesion kinase ($pp125^{FAK}$), paxillin, and talin, contributing to the focal adhesion disassembly and cell motility. Calpain inhibition attenuates the proteolytic cleavage of the focal adhesion proteins, preventing the dissolution of the focal adhesion complex and cell migration [93,94]. Depletion of the calpain 1 and 2 isoforms using specific siRNAs showed that the calpain 2 activity is responsible for the limited proteolysis of several cytoskeletal and focal adhesion components such as FAK, paxillin, spectrin, and talin. It was also confirmed that calpain 2 isoform limits the membrane projections and transient membrane activity [95]. The same group demonstrated that silencing of calpain 2 not only diminishes the talin proteolysis but also reduced the disassembly rates of adhesion complexes [96]. Calpain is also implicated in the growth factor-induced cell migration. Glading et al. [52,97] found that inhibitions of either calpain activity or ERK signaling pathway by specific inhibitors (calpeptin, calpain inhibitor I or PD98059) abolish the EGF-induced de-adhesion and cell migration. They also determined that the EGF-mediated calpain activation occurs via ERK/MAP kinase dependent phosphorylation and independent of increased intracellular calcium level. Specific knockdown of calpain 2, but not calpain 1, reduces EGF-induced cell motility. Furthermore, it was confirmed that EGF-induced calpain activity requires plasma membrane-localized activation of EGFR and ERK [98]. Interferon inducible protein-10 (IP-10) abrogates the EGF-induced cell migration by blocking the calpain activity through cAMP-dependent PKA phosphorylation. Inhibition of the PKA activation prevents the inhibitory effect of IP-10 on the EGF-induced motility and calpain activation [55,99]. IP-10 also inhibits the VEGF-mediated calpain activation which is associated with reduced endothelial tube formation and cell motility. Down-regulation of the PKA protein level by specific siRNA or using PKA inhibitors reverses

the IP-10 inhibition of VEGF-induced calpain activation [100]. The importance of calpain in the growth factor-mediated cell migration is also supported by other studies. The increases in the migration rate of myoblasts by insulin-like growth factor-1 (IGF-1), TGF-β1, and insulin are related to calpain. Incubation of cells with these growth factors elevates the expression level and activity of calpain 2. The calpain inhibitor, calpeptin, completely blocks the calpain activity diminishing the growth factors-induced cell migration [101,102]. It was also shown that incubation of pulmonary artery endothelial cells (PAECs) with cigarette smoke extract (CSE) causes dose-dependent inhibition of calpain activity and cell proliferation. Calpain inhibitor-1 which inhibits both calpain 1 and calpain 2 attenuates the angiogenesis and potentiates the inhibitory effect of CSE. Moreover, depletion of calpastatin with antisense oligodeoxyribonucleotides prevents CSE-induced decreases in calpain activity and angiogenesis. Collectively, these findings indicate that CSE induced inhibition of angiogenesis is mediated by calpain inhibition [103]. Mo et al. [104] revealed that calpain may have a role in the progression of angiogenesis under hypoxic condition. Overexpression of the hypoxia-inducible factor-1α (HIF-1α) in human umbilical vein endothelial cells (HUVECs) elevates the expression of VEGF, Na^+/H^+ exchanger-1 (NHE1) and calpain as well as endothelial cell proliferation, migration and tube formation. Specific siRNA-mediated depletion of NHE1 diminishes the HIF-1α-induced calpain 2 expression and activity as well as angiogenesis. They also found that VEGF prevents the inhibitory effect of siRNA against NHE1, while the calpain inhibitor ALLM abolishes the protective effect of the VEGF. These data suggest that the NHE1 and calpain 2 have a role in the hypoxia-induced cell proliferation. Incubation of human pulmonary microvascular endothelial cells (PMECs) with VEGF increases the activity and protein content of calpain 2. Inhibition of calpain activity using calpain 2 specific siRNA or by overexpression of calpastatin attenuates VEGF-induced increases in angiogenesis, supporting the mediator's role of calpain 2 in endothelial angiogenesis [105,106]. Youn et al. [107] found that calpain mediates the VEGF-induced endothelial nitric oxide (NO) production through the ezrin/calpain/PI3K/AMPK/AKT/eNOS axis contributing to the growth factor-stimulated angiogenesis. They claimed that VEGF provokes the membrane translocation and activation of calpain in ezrin dependent manner, causing PI3K/AMPK/AKT activation, eNOS phosphorylation at Ser1179 residue and NO production. A recent study shows that a novel calpain inhibitor, SNJ-1945, attenuates the VEGF-stimulated angiogenesis in human retinal microvascular endothelial cells (HRMECs). Treatment of HRMECs with VEGF causes significant increases mainly in calpain 2 activity as well as in endothelial tube formation and cell migration which are diminished in the presence of calpain inhibitor [108]. In addition, calpain plays an important role in the skin wound healing. Transgenic mice that overexpress calpastatin exhibit impaired wound healing and reduced cell proliferation in the epidermis and delayed re-epithelization. The calpain inhibition also decreases collagen I synthesis and blocks the myofibroblast differentiation and the scar formation [109]. Calpain-mediated cell proliferation is associated with cell survival. Depletion of the calpain 1 using specific siRNA causes significant reduction in the viability and proliferation of skeletal muscle satellite cells. Calpain 1 silencing enhances the expression of pro-apoptotic genes and reduces the genes for cell proliferation, differentiation, and migration. These results support that calpain 1 is implicated in controlling the proliferation and survival of satellite cells [110]. Calpain contributes the development of resistance to chemotherapic agents in tumor cell lines. Knockdown of calpain 2 by isoform specific siRNA or calpain inhibitor ALLN increase the protein level of the inhibitory subunit, IκBα, causing significant reduction in NF-κB activation. In addition, down-regulation of calpain 2 significantly resensitizes the anticancer drug resistant cell lines, indicating calpain may function as a resistance mediator during anticancer therapy [111].

Summary

In summary, convincing evidence highlights that the calpain system especially the ubiquitously expressed calpain 1 and 2 are involved in a broad range of physiological processes including cell growth and proliferation in many types of cells. Perturbed expression and activity of calpain 1 and/or 2 play a key role in tumor cell proliferation, migration and invasion as well as angiogenesis contributing to the development of different types of cancer [105,111-113]. Increased calpain activation also mediates pulmonary and systemic vascular remodeling in pulmonary hypertension and angiotensin II-induced hypertension [92,114,115]. On the other hand, calpain is implicated in other pathological states in which cell proliferation is not involved [31-33]. For instance, mutations in the calpain 3 encoding gene (*CAPN3*) are responsible for the limb-girdle muscular dystrophy 2A (LGMD2A) [116,117]. Polymorphism within the calpain 10 gene (*CAPN10*) is associated with type 2 diabetes mellitus [118,119]. Overexpression of calpain 6 has been shown in uterine cervical cancer [120], while down-regulation of calpain 9 is correlated with gastric cancer [9]. Inappropriate activation of calpain contributes to neurodegenerative diseases such as cerebral ischemia, multiple sclerosis, cataracts as well as Alzheimer's, Parkinson's, and Huntington's diseases [121]. Therefore, manipulating the proteolytic enzyme activity of calpain could provide useful therapies for the management of calpain-related diseases such as cancer, pathological angiogenesis, pulmonary hypertension, primary hypertension, muscular dystrophy, diabetes, and neurodegenerative disorders.

References

1. Guroff G (1964) A Neutral, Calcium-Activated Proteinase from the Soluble Fraction of Rat Brain. J Biol Chem 239: 149-155.
2. Ishiura S, Murofushi H, Suzuki K, Imahori K (1978) Studies of a calcium-activated neutral protease from chicken skeletal muscle. I. Purification and characterization. J Biochem 84: 225-230.
3. Ohno S, Emori Y, Imajoh S, Kawasaki H, Kisaragi M, et al. (1984) Evolutionary origin of a calcium-dependent protease by fusion of genes for a thiol protease and a calcium-binding protein? Nature 312: 566-570.
4. Goll DE, Thompson VF, Li H, Wei W, Cong J (2003) The calpain system. Physiol Rev 83: 731-801.
5. Sorimachi H, Hata S, Ono Y (2011) Calpain chronicle--an enzyme family under multidisciplinary characterization. Proc Jpn Acad Ser B Phys Biol Sci 87: 287-327.
6. Sorimachi H, Imajoh-Ohmi S, Emori Y, Kawasaki H, Ohno S, et al. (1989) Molecular cloning of a novel mammalian calcium-dependent protease distinct from both m- and mu-types. J Biol Chem 264: 20106-20111.
7. Sorimachi H, Ishiura S, Suzuki K (1993) A novel tissue-specific calpain species expressed predominantly in the stomach comprises two alternative splicing products with and without Ca(2+)-binding domain. J Biol Chem 268: 19476-19482.
8. Lee HJ, Sorimachi H, Jeong SY, Ishiura S, Suzuki K (1998) Molecular cloning and characterization of a novel tissue-specific calpain predominantly expressed in the digestive tract. Biol Chem 379: 175-183.
9. Yoshikawa Y, Mukai H, Hino F, Asada K, Kato I (2000) Isolation of two novel genes, down-regulated in gastric cancer. Jpn J Cancer Res 91: 459-463.
10. Suzuki K, Hata S, Kawabata Y, Sorimachi H (2004) Structure, activation, and biology of calpain. Diabetes 53: S12-S18.
11. Suzuki K (1991) Nomenclature of calcium dependent proteinase. Biomed Biochim Acta 50: 483-484.

12. Cong J, Goll DE, Peterson AM, Kapprell HP (1989) The role of autolysis in activity of the Ca^{2+}-dependent proteinases (mu-calpain and m-calpain). J Biol Chem 264: 10096-10103.

13. Suzuki K, Sorimachi H, Yoshizawa T, Kinbara K, Ishiura S (1995) Calpain: novel family members, activation, and physiologic function. Biol Chem Hoppe Seyler 376: 523-529.

14. Sorimachi H, Suzuki K (2001) The structure of calpain. J Biochem 129: 653-664.

15. Strobl S, Fernandez-Catalan C, Braun M, Huber R, Masumoto H, et al. (2000) The crystal structure of calcium-free human m-calpain suggests an electrostatic switch mechanism for activation by calcium. Proc Natl Acad Sci U S A 97: 588-592.

16. Hosfield CM, Elce JS, Davies PL, Jia Z (1999) Crystal structure of calpain reveals the structural basis for Ca(2+)-dependent protease activity and a novel mode of enzyme activation. EMBO J 18: 6880-6889.

17. Alexa A, Bozoky Z, Farkas A, Tompa P, Friedrich P (2004) Contribution of distinct structural elements to activation of calpain by Ca^{2+} ions. J Biol Chem 20118-20126.

18. Rizo J, Sudhof TC (1998) C2-domains, structure and function of a universal Ca^{2+}-binding domain. J Biol Chem 273: 15879-15882.

19. Tompa P, Emori Y, Sorimachi H, Suzuki K, Friedrich P (2001) Domain III of calpain is a ca^{2+}-regulated phospholipid-binding domain. Biochem Biophys Res Commun 280: 1333-1339.

20. Minami Y, Emori Y, Kawasaki H, Suzuki K (1987) E-F hand structure-domain of calcium-activated neutral protease (CANP) can bind Ca^{2+} ions. J Biochem 101: 889-895.

21. Imajoh S, Kawasaki H, Suzuki K (1987) The COOH-terminal E-F hand structure of calcium-activated neutral protease (CANP) is important for the association of subunits and resulting proteolytic activity. J Biochem 101: 447-452.

22. Blanchard H, Grochulski P, Li Y, Arthur JS, Davies PL, et al. (1997) Structure of a calpainCa(2+)-binding domain reveals a novel EF-hand and Ca(2+)-induced conformational changes. Nat Struct Biol 4: 532-538.

23. Imajoh S, Kawasaki H, Suzuki K (1986) The amino-terminal hydrophobic region of the small subunit of calcium-activated neutral protease (CANP) is essential for its activation by phosphatidylinositol. J Biochem 99: 1281-1284.

24. Schad E, Farkas A, Jekely G, Tompa P, Friedrich P (2002) A novel human small subunit of calpains. Biochem J 362: 383-388.

25. Friedrich P, Bozoky Z (2005) Digestive versus regulatory proteases: on calpain action in vivo. Biol Chem 386: 609-612.

26. Glading A, Lauffenburger DA, Wells A (2002) Cutting to the chase: calpain proteases in cell motility. Trends Cell Biol 12: 46-54.

27. Janossy J, Ubezio P, Apati A, Magocsi M, Tompa P, et al. (2004) Calpain as a multi-site regulator of cell cycle. Biochem Pharmacol 67: 1513-1521.

28. Sato K, Kawashima S (2001) Calpain function in the modulation of signal transduction molecules. Biol Chem 382: 743-751.

29. Sorimachi H, Ishiura S, Suzuki K (1997) Structure and physiological function of calpains. Biochem J 328: 721-732.

30. Carafoli E, Molinari M (1998) Calpain: a protease in search of a function? Biochem Biophys Res Commun 247: 193-203.

31. Huang Y, Wang KK (2001) Thecalpain family and human disease. Trends Mol Med 7: 355-362.

32. Branca D (2004) Calpain-related diseases. BiochemBiophys Res Commun 322: 1098-10104.

33. Zatz M, Starling A (2005) Calpains and disease. N Engl J Med 352: 2413-2423.

34. Donkor IO (2000) A survey of calpain inhibitors. Curr Med Chem 7: 1171-1188.

35. Donkor IO (2011) Calpain inhibitors: a survey of compounds reported in the patent and scientific literature. Expert Opin Ther Pat 21: 601-636.

36. Dutt P, Croall DE, Arthur JS, Veyra TD, Williams K, et al. (2006) m-Calpain is required for preimplantation embryonic development in mice. BMC Dev Biol 6: 3.

37. Arthur JS, Elce JS, Hegadorn C, Williams K, Greer PA (2000) Disruption of the murine calpain small subunit gene, Capn4: calpain is essential for embryonic development but not for cell growth and division. Mol Cell Biol 20: 4474-4481.

38. Azam M, Andrabi SS, Sahr KE, Kamath L, Kuliopulos A, et al. (2001) Disruption of the mouse mu-calpain gene reveals an essential role in platelet function. Mol Cell Biol 21: 2213-2220.

39. Baki A, Tompa P, Alexa A, Molnar O, Friedrich P (1996) Autolysis parallels activation of mu-calpain. Biochem J 318: 897-901.

40. Suzuki K, Sorimachi H (1998) A novel aspect of calpain activation. FEBS Lett 433: 1-4.

41. Nakagawa K, Masumoto H, Sorimachi H, Suzuki K (2001) Dissociation of m-calpain subunits occurs after autolysis of the N-terminus of the catalytic subunit, and is not required for activation. J Biochem 130: 605-611.

42. Zimmerman UJ, Schlaepfer WW (1991) Two-stage autolysis of the catalytic subunit initiates activation of calpain I. Biochim Biophys Acta 1078: 192-198.

43. Brown N, Crawford C (1993) Structural modifications associated with the change in Ca^{2+} sensitivity on activation of m-calpain. FEBS Lett 322: 65-68.

44. McCelland P, Lash JA, Hathaway DR (1989) Identification of major autolytic cleavage sites in the regulatory subunit of vascular calpain II. A comparison of partial amino-terminal sequences to deduced sequence from complementary DNA. J Biol Chem 264: 17428-17431.

45. Hathaway DR, Werth DK, Haeberle JR (1982) Limited autolysis reduces the Ca^{2+} requirement of a smooth muscle Ca^{2+}-activated protease. J Biol Chem 257: 9072-9077.

46. Suzuki K, Tsuji S, Kubota S, Kimura Y, Imahori K (1981) Limited autolysis of Ca^{2+}-activated neutral protease (CANP) changes its sensitivity to Ca^{2+} ions. J Biochem 90: 275-278.

47. Imajoh S, Kawasaki H, Suzuki K (1986) Limited autolysis of calcium-activated neutral protease (CANP): reduction of the Ca^{2+}-requirement is due to the NH2-terminal processing of the large subunit. J Biochem 100: 633-642.

48. Arthur JS, Crawford C (1996) Investigation of the interaction of m-calpain with phospholipids: calpain-phospholipid interactions. Biochim Biophys Acta 1293: 201-206.

49. Melloni E, Michetti M, Salamino F, Minafra R, Pontremoli S (1996) Modulation of the calpainautoproteolysis by calpastatin and phospholipids. Biochem Biophys Res Commun 229: 193-197.

50. Crawford C, Brown NR, Willis AC (1990) Investigation of the structural basis of the interaction of calpain II with phospholipid and with carbohydrate. Biochem J 575-579.

51. Adachi Y, Kobayashi N, Murachi T, Hatanaka M (1986) Ca^{2+}-dependent cysteine proteinase, calpains I and II are not phosphorylated in vivo. Biochem Biophys Res Commun 136: 1090-1096.

52. Glading A, Bodnar RJ, Reynolds IJ, Shiraha H, Satish L, et al. (2004) Epidermal growth factor activates m-calpain (calpain II), at least in part, by extracellular signal-regulated kinase-mediated phosphorylation. Mol Cell Biol 24: 2499-2512.

53. Zadran S, Jourdi H, Rostamiani K, Qin Q, Bi X, et al. (2010) Brain-derived neurotrophic factor and epidermal growth factor activate neuronal m-calpain via mitogen-activated protein kinase-dependent phosphorylation. J Neurosci 30: 1086-1095.

54. Smith SD, Jia Z, Huynh KK, Wells A, Elce JS (2003) Glutamate substitutions at a PKA consensus site are consistent with inactivation of calpain by phosphorylation. FEBS Lett 542: 115-118.

55. Shiraha H, Glading A, Chou J, Jia Z, Wells A (2002) Activation of m-calpain (calpain II) by epidermal growth factor is limited by protein kinase A phosphorylation of m-calpain. Mol Cell Biol 22: 2716-2727.

56. Branca D, Gugliucci A, Bano D, Brini M, Carafoli E (1999) Expression, partial purification and functional properties of themuscle-specific calpain isoform p94. Eur J Biochem 265: 839-846.

57. Satish L, Blair HC, Glading A, Wells A (2005) Interferon-inducible protein 9 (CXCL11)-induced cell motility in keratinocytes requires calcium flux-dependent activation of mu-calpain. Mol Cell Biol 25: 1922-1941.

58. Xu L, Deng X (2006) Protein kinase Ciota promotes nicotine-induced migration and invasion of cancer cells via phosphorylation of micro- and m-calpains. J Biol Chem 281: 4457-4466.

59. Kovacs L, Alexa A, Klement E, Kokai E, Tantos A, et al. (2009) Regulation of calpain B from Drosophila melanogaster by phosphorylation. FEBS J 276: 4959-4972.

60. Leloup L, Shao H, Bae YH, Deasy B, Stolz D, et al. (2010) m-Calpain activation is regulated by its membrane localization and by its binding to phosphatidylinositol 4,5-bisphosphate. J Biol Chem 285: 33549-33566.

61. Suzuki K, Imajoh S, Emori Y, Kawasaki H, Minami Y, et al. (1987) Calcium-activated neutral protease and its endogenous inhibitor. Activation at the cell membrane and biological function. FEBS Lett 220: 271-277.

62. Mellgren RL, Nettey MS, Mericle MT, Renno W, Lane RD (1988) An improved purification procedure for calpastatin, the inhibitor protein specific for the intracellular calcium-dependent proteinases, calpains. Prep Biochem 18: 183-197.

63. Wendt A, Thompson VF, Goll DE (2004) Interaction of calpastatin with calpain: a review. Biol Chem 385: 465-472.

64. Hao LY, Kameyama A, Kuroki S, Takano J, Takano E, et al. (2000) Calpastatin domain L is involved in the regulation of L-type Ca^{2+} channels in guinea pig cardiac myocytes. Biochem Biophys Res Commun 279: 756-761.

65. Takano E, Maki M, Mori H, Hatanaka M, Marti T, et al. (1988) Pig heart calpastatin: identification of repetitive domain structures and anomalous behavior in polyacrylamide gel electrophoresis. Biochem 27: 1964-1972.

66. Emori Y, Kawasaki H, Imajoh S, Minami Y, Suzuki K (1988) All four repeating domains of the endogenous inhibitor for calcium-dependent protease independently retain inhibitory activity. Expression of the cDNA fragments in Escherichia coli. J Biol Chem 263: 2364-2370.

67. Takano E, Ma H, Yang HQ, Maki M, Hatanaka M (1995) Preference of calcium-dependent interactions between calmodulin-like domains of calpain and calpastatin subdomains. FEBS Lett 362: 93-97.

68. Kawasaki H, Emori Y, Suzuki K (1993) Calpastatin has two distinct sites for interaction with calpain--effect of calpastatin fragments on the binding of calpain to membranes. Arch Biochem Biophys 305: 467-472.

69. Hanna RA, Campbell RL, Davies PL (2008) Calcium-bound structure of calpain and its mechanism of inhibition by calpastatin. Nature 456: 409-412.

70. Barnoy S, Zipser Y, Glaser T, Grimberg Y, Kosower NS (1999) Association of calpain (Ca(2+)-dependent thiol protease) with its endogenous inhibitor calpastatin in myoblasts. J Cell Biochem 74: 522-531.

71. De Tullio R, Passalacqua M, Averna M, Salamino F, Melloni E, et al. (1999) Changes in intracellular localization of calpastatin during calpain activation. Biochem J2: 467-472.

72. Averna M, de Tullio R, Passalacqua M, Salamino F, Pontremoli S, et al. (2001) Changes in intracellular calpastatin localization are mediated by reversible phosphorylation. Biochem J 354: 25-30.

73. Pontremoli S, Melloni E, Michetti M, Salamino F, Sparatore B, et al. (1988) An endogenous activator of the Ca^{2+}-dependent proteinase of human neutrophils that increases its affinity for Ca^{2+}. Proc Natl Acad Sci U S A 85: 1740-1743.

74. Salamino F, De Tullio R, Mengotti P, Viotti PL, Melloni E, et al. (1993) Site-directed activation of calpain is promoted by a membrane-associated natural activator protein. Biochem J 290: 191-197.

75. Melloni E, Michetti M, Salamino F, Pontremoli S (1998) Molecular and functional properties of a calpain activator protein specific for mu-isoforms. J Biol Chem 273: 12827-12831.

76. Melloni E, Michetti M, Salamino F, Sparatore B, Pontremoli S (1998) Mechanism of action of a new component of the Ca(2+)-dependent proteolytic system in rat brain: the calpain activator. Biochem Biophys Res Commun 249: 583-588.

77. Pontremoli S, Viotti PL, Michetti M, Sparatore B, Salamino F, et al. (1990) Identification of an endogenous activator of calpain in rat skeletal muscle. Biochem Biophys Res Commun 171: 569-574.

78. Michetti M, Viotti PL, Melloni E, Pontremoli S (1991) Mechanism of action of the calpain activator protein in rat skeletal muscle. Eur J Biochem 202: 1177-1180.

79. Melloni E, Averna M, Salamino F, Sparatore B, Minafra R, et al. (2000) Acyl-CoA-binding protein is a potent m-calpain activator. J Biol Chem 275: 82-86.

80. March KL, Wilensky RL, Roeske RW, Hathaway DR (1993) Effects of thiol protease inhibitors on cell cycle and proliferation of vascular smooth muscle cells in culture. Circ Res 72: 413-423.

81. Ariyoshi H, Okahara K, Sakon M, Kambayashi J, Kawashima S, et al. (1998) Possible involvement of m-calpain in vascular smooth muscle cell proliferation. Arterioscler Thromb Vasc Biol 18: 493-498.

82. Mellgren RL, Lu Q, Zhang W, Lakkis M, Shaw E, et al. (1996) Isolation of a Chinese hamster ovary cell clone possessing decreased mu-calpain content and a reduced proliferative growth rate. J Biol Chem 271: 15568-15574.

83. Zhang W, Lane RD, Mellgren RL (1996) The major calpainisozymes are long-lived proteins. Design of an antisense strategy for calpain depletion in cultured cells. J Biol Chem 271: 18825-18830.

84. Zhang W, Lu Q, Xie ZJ, Mellgren RL (1997) Inhibition of the growth of WI-38 fibroblasts by benzyloxycarbonyl-Leu-Leu-Tyr diazomethyl ketone: evidence that cleavage of p53 by a calpain-like protease is necessary for G1 to S-phase transition. Oncogene 14: 255-263.

85. Xu Y, Mellgren RL (2002) Calpain inhibition decreases the growth rate of mammalian cell colonies. J Biol Chem 277: 21474-21479.

86. Murray SS, Grisanti MS, Bentley GV, Kahn AJ, Urist MR, et al. (1997) Thecalpain-calpastatin system and cellular proliferation and differentiation in rodent osteoblastic cells. Exp Cell Res 233: 297-309.

87. Murray EJ, Grisanti MS, Bentley GV, Murray SS (1997) E64d, a membrane-permeable cysteine protease inhibitor, attenuates the effects of parathyroid hormone on osteoblasts *in vitro*. Metabolism 46: 1090-1094.

88. Shimada M, Greer PA, McMahon AP, Bouxsein ML, Schipani E (2008) *In vivo* targeted deletion of calpain small subunit, Capn4, in cells of the osteoblast lineage impairs cell proliferation, differentiation, and bone formation. J Biol Chem 283: 21002-21010.

89. Carragher NO, Westhoff MA, Riley D, Potter DA, Dutt P, et al. (2002) v-Src-induced modulation of the calpain-calpastatinproteolytic system regulates transformation. Mol Cell Biol 22: 257-269.

90. Honda S, Marumoto T, Hirota T, Nitta M, Arima Y, et al. (2004) Activation of m-calpain is required for chromosome alignment on the metaphase plate during mitosis. J Biol Chem 279: 10615-10623.

91. Ho WC, Pikor L, Gao Y, Elliott BE, Greer PA (2012) Calpain 2 regulates Akt-FoxO-p27(Kip1) protein signaling pathway in mammary carcinoma. J Biol Chem 287: 15458-15465.

92. Ma W, Han W, Greer PA, Tuder RM, Toque HA, et al. (2011) Calpain mediates pulmonary vascular remodeling in rodent models of pulmonary hypertension, and its inhibition attenuates pathologic features of disease. J Clin Invest 121: 4548-4566.

93. Carragher NO, Levkau B, Ross R, Raines EW (1999) Degraded collagen fragments promote rapid disassembly of smooth muscle focal adhesions that correlates with cleavage of pp125(FAK), paxillin, and talin. J Cell Biol 147: 619-630.

94. Carragher NO, Fincham VJ, Riley D, Frame MC (2001) Cleavage of focal adhesion kinase by different proteases during SRC-regulated transformation and apoptosis. Distinct roles for calpain and caspases. J Biol Chem 276: 4270-5275.

95. Franco S, Perrin B, Huttenlocher A (2004) Isoform specific function of calpain 2 in regulating membrane protrusion. Exp Cell Res 299: 179-187.

96. Franco SJ, Rodgers MA, Perrin BJ, Han J, Bennin DA, et al. (2004) Calpain-mediated proteolysis of talin regulates adhesion dynamics. Nat Cell Biol 6: 977-983.

97. Glading A, Chang P, Lauffenburger DA, Wells A (2000) Epidermal growth factor receptor activation of calpain is required for fibroblast motility and occurs via an ERK/MAP kinase signaling pathway. J Biol Chem 275: 2390-2398.

98. Glading A, Uberall F, Keyse SM, Lauffenburger DA, Wells A (2001) Membrane proximal ERK signaling is required for M-calpain activation downstream of epidermal growth factor receptor signaling. J Biol Chem 276: 23341-23348.

99. Shiraha H, Glading A, Gupta K, Wells A (1999) IP-10 inhibits epidermal growth factor-induced motility by decreasing epidermal growth factor receptor-mediated calpain activity. J Cell Biol 146: 243-254.

100. Bodnar RJ, Yates CC, Wells A (2006) IP-10 blocks vascular endothelial growth factor-induced endothelial cell motility and tube formation via inhibition of calpain. Circ Res 98: 617-625.

101. Leloup L, Mazeres G, Daury L, Cottin P, Brustis JJ (2006) Involvement of calpains in growth factor-mediated migration. Int J Biochem Cell Biol 38: 2049-2063.

102. Leloup L, Daury L, Mazeres G, Cottin P, Brustis JJ (2007) Involvement of the ERK/MAP kinase signalling pathway in milli-calpain activation and myogenic cell migration. Int J Biochem Cell Biol 39: 1177-1189.

103. Su Y, Cao W, Han Z, Block ER (2004) Cigarette smoke extract inhibits angiogenesis of pulmonary artery endothelial cells: the role of calpain. Am J Physiol Lung Cell Mol Physiol 287: L794-800.

104. Mo XG, Chen QW, Li XS, Zheng MM, Ke DZ, et al. (2011) Suppression of NHE1 by small interfering RNA inhibits HIF-1alpha-induced angiogenesis *in vitro* via modulation of calpain activity. Microvasc Res 81: 160-168.

105. Su Y, Cui Z, Li Z, Block ER (2006) Calpain-2 regulation of VEGF-mediated angiogenesis. FASEB J 20: 1443-1451.

106. Qiu K, Su Y, Block ER (2006) Use of recombinant calpain-2 siRNA adenovirus to assess calpain-2 modulation of lung endothelial cell migration and proliferation. Mol Cell Biochem 292: 69-78.

107. Youn JY, Wang T, Cai H (2009) Anezrin/calpain/PI3K/AMPK/eNOSs1179 signaling cascade mediating VEGF-dependent endothelial nitric oxide production. Circ Res 104: 50-59.

108. Ma H, Tochigi A, Shearer TR, Azuma M (2009) Calpain inhibitor SNJ-1945 attenuates events prior to angiogenesis in cultured human retinal endothelial cells. J Ocul Pharmacol Ther 25: 409-414.

109. Nassar D, Letavernier E, Baud L, Aractingi S, Khosrotehrani K (2012) Calpain activity is essential in skin wound healing and contributes to scar formation. PLoS One 7: e37084.

110. Van Ba H, Inho H (2013) Significant role of mu-calpain (CANP1) in proliferation/survival of bovine skeletal muscle satellite cells. *In Vitro* Cell Dev Biol Anim 49: 785-797.

111. Fenouille N, Grosso S, Yunchao S, Mary D, Pontier-Bres R, et al. (2012) Calpain 2-dependent IkappaBalpha degradation mediates CPT-11 secondary resistance in colorectal cancer xenografts. J Pathol 227: 118-129.

112. Leloup L, Wells A (2011) Calpains as potential anti-cancer targets. Expert Opin Ther Targets 15: 309-323.

113. Storr SJ, Carragher NO, Frame MC, Parr T, Martin SG (2011) Thecalpain system and cancer. Nat Rev Cancer 11: 364-374.

114. Letavernier E, Perez J, Bellocq A, Mesnard L, de Castro Keller A, et al. (2008) Targeting the calpain/calpastatin system as a new strategy to prevent cardiovascular remodeling in angiotensin II-induced hypertension. Circ Res 102: 720-728.

115. Scalia R, Gong Y, Berzins B, Freund B, Feather D, et al. (2011) A novel role for calpain in the endothelial dysfunction induced by activation of angiotensin II type receptor signaling. Circ Res 108: 1102-1111.

116. Richard I, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, et al. (1995) Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. Cell 81: 27-40.

117. Ono Y, Shimada H, Sorimachi H, Richard I, Saido TC, et al. (1998) Functional defects of a muscle-specific calpain, p94, caused by mutations associated with limb-girdle muscular dystrophy type 2A. J Biol Chem 273: 17073-17078.

118. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, et al. (2000) Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 26: 163-175.

119. Baier LJ, Permana PA, Yang X, Pratley RE, Hanson RL, et al. (2000) A calpain-gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. J Clin Invest 106: R69-R73.

120. Lee SJ, Kim BG, Choi YL, Lee JW (2008) Increased expression of calpain 6 during the progression of uterine cervical neoplasia: immunohistochemical analysis. Oncol Rep 19: 859-863.

121. Camins A, Verdaguer E, Folch J, Pallas M (2006) Involvement of calpain activation in neurodegenerative processes. CNS Drug Rev 12: 135-148.