Arch Iran Med. April 2018;21(4):170-179

http www.aimjournal.ir

ARCHIVES OF IRANIAN MEDICINE

Review Article

Open Access

Calpains: Diverse Functions but Enigmatic

Masoumeh Hosseini, MSc[1]; Hossein Najmabadi, PhD[1*]; Kimia Kahrizi, MD[1*]

[1]Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

Abstract

Calpains are a group of non-lysosomal Ca^{2+}-dependent cysteine proteases with numerous substrates. Calpains have been identified in almost all eukaryotes and bacteria but not in archaebacteria. In the human genome, this group of enzymes has 15 isoforms and is present ubiquitously and demonstrates tissue-specific patterns of expression. Calpains are involved in different physiological and pathological processes such as cell proliferation, migration, invasion, apoptosis and signal transduction and their roles in various disorders have been reported. In this review, functions of calpains, their substrates, their mechanism of regulation and their involvement in diseases have been summarized.

Keywords: Calpains, Disorders, Physiological processes, Pathological processes

Cite this article as: Hosseini M, Najmabadi H, Kahrizi K. Calpains: diverse functions but enigmatic. Arch Iran Med. 2018;21(4):170–179.

Received: October 23, 2017, Accepted: January 10, 2018, ePublished: April 1, 2018

Introduction

Calpains are a well-conserved group of cysteine proteases that are involved in some Ca^{2+}-dependent cellular processes including cell proliferation, migration and differentiation, signal transduction and cell death.[1-3] Recent studies have provided increasing evidence of the role of calpains in neuronal migration and differentiation, synaptic plasticity, and neuroprotection.[4,5] Calpain homologs are present in a wide range of species including *Drosophila melanogaster*, *Caenorhabditis elegans*, several fungi, plants and some bacteria but there is no homolog in archaea.[6] These enzymes have 15 isoforms and are present in mammals ubiquitously and in a tissue-specific pattern (Figure 1). Based on different motif(s) in the C-terminal domain of calpain proteins, they are classified into 2 groups: classic (typical) and non-classic (atypical) calpains (Figure 2). Classic calpains include CAPN1, 2, 3, 8, 9, 11, 12, 13, and 14 composed of a large catalytic subunit (80 kDa) and a small regulatory subunit (28 kDa). The large subunit exhibits an anchor α-helix at the amino terminal, protease core 1 and 2 domains also identified as CysPc domain, a calpain-type β-sandwich (CBSW; previously referred to as C2-domain-like domain) and a penta EF hand domain [PEF(L)], L referring to a large subunit. Glycine rich (GR) and PEF(S) (S referring to a small subunit) are components of the small subunit (CAPNS1 or CAPN4).[7]

Non-classic calpains consist of CAPN5, 6, 7, 10, 13, 15, and 16, in which the PEF domain is replaced by another motif(s) in this group. The protease domain (domain II) and catalytically active sites, including Cys105, His262 and Asn286, are conserved in the calpain superfamily. Upon binding of Ca^{2+}, the N-terminal α-helix is autolysed in typical calpains1, 2 and 9, after its activation by Ca^{2+}, and this event leads to a decrease in Ca^{2+} requirement.[8-12] It is not clear whether autolysis occurs in other typical or atypical calpains.[11]

Mammalian calpains are also categorized into 2 classes based on their expression in different organ(s). Calpains 1, 2, 5, 7, 10, 13, and 15 are expressed ubiquitously whereas calpain 3 (p94) is expressed in skeletal muscle, calpain 6 is expressed in placenta, calpain 8/9 (nCL-2/nCL-4) is expressed in the gastrointestinal tract, calpain 11 is expressed in testis and calpain 12 is expressed in hair follicles. Defects in some of the ubiquitous calpains may induce lethality, which is observed in $Capn2^{-/-}$ and $Capn4^{-/-}$ mice highlighting the fundamental roles of these genes during development, while defects in organ-specific calpains result in tissue-specific phenotypes such as muscular dystrophy due to mutations in the *CAPN3* gene. In spite of the embryonic lethality of calpain-2 knock-out mice, calpain-1 knock-out mice did not exhibit lethality although they revealed abnormality in cerebellar development and ataxia.[7,12-15] Although calpain-1 and calpain-2 are the most widely studied calpains, little is known about other human calpains, their mechanism of action and their roles in physiological and pathological conditions.[16]

The precise number of known proteins cleaved by different calpains in mammals is unknown although it is

***Corresponding Authors:** Kimia Kahrizi MD, Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran. Email: kahrizi@yahoo.com; Hossein Najmabadi, Email: hnajm12@yahoo.com

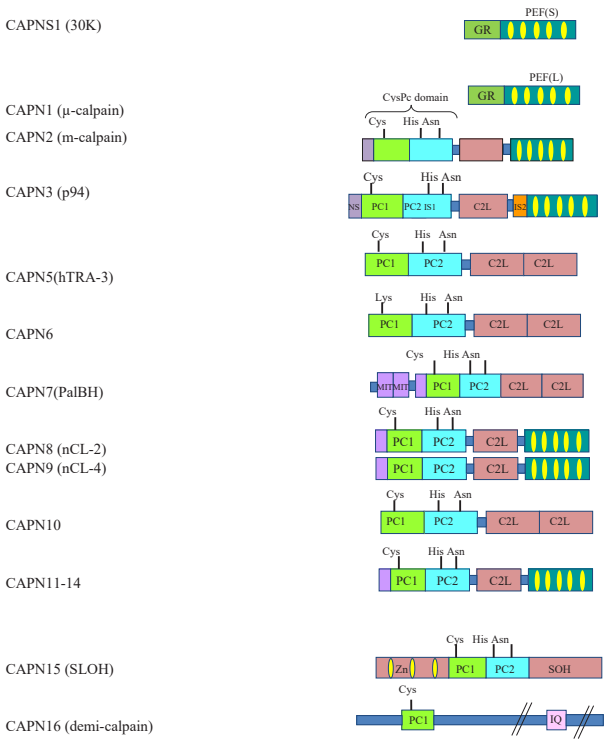

Figure 1. Human Calpains.

speculated that more than 1000 proteins are modulated by calpains.[17]

Calpains are recognized as intracellular 'modulator' proteases which act in the process of proteolysis rather than protein degradation, while 'eraser' proteases such as caspases and ubiquitin-proteasome and autophagy systems degrade their substrate proteins and exclude them. Another issue is that calpains recognize their substrates themselves, whereas in autophagy and proteasome systems, the substrate should be tagged by other systems such as autophagosome formation and ubiquitination, respectively. Calpains may modify the substrate at a limited number of sites, and after that, the modified protein acquires novel or additional functions.[1,10,18]

Substrate Specificities of Calpains

Calpains have been suggested to control cell migration and invasion through their ability to regulate adhesion and proteolysis of cytoskeletal proteins.[19-21] Calpain proteolyses the actin associated protein cortactin, which is a regulator of membrane protrusion.[20] In cell migration and invasion processes, calpains modulate talin, focal adhesion kinase (FAK) and paxillin; these proteins are present in focal adhesion and regulate adhesion turnover and cell migration. Calpain induces an increase in cell migration and invasion which is consistent with upregulation of calpains in some types of cancer.[20,22] The cytoplasmic tails of integrin and actinin are involved in cell spreading and are regulated by calpain proteolysis.[20]

Protein kinase C (PKC), CaMKII, Bid, Bax23, ERK1/2 and Cdk5/p35 are other calpain substrates that are protein kinases and regulate the proteolysis of cytoskeletal proteins.[23] P35 is converted to p25 by calpain, and p25 activates cyclin dependent kinase 5 (CDK5) itself, which may play a role in memory formation. Calpain also cleaves dysferlin, a protein involved in LGMD2B. One interesting calpain substrate is spectrin (spectrin-α is also known as α-fodrin).[24,25] Spectrin is proteolysed by calpains in neuronal cells and is associated with neuronal apoptosis.[19,23,24,26]

Calpain modulates the activity of some proteins that are involved in neuronal plasticity, neurodegeneration and cognition. Axon outgrowth and guidance are Ca^{2+}-dependent processes and are mediated by a calpain which regulates the proteolysis of talin and FAK.[23,27] Among other substrates for calpain is drebrin, a neuron-specific F-actin binding protein. Drebrin controls microtubule entry into dendritic spines and a decrease in the level of this protein can lead to a decrease in dendritic spine microtubules.[25]

The role of calpain in modulating cytoskeletal proteins including actin is also an important step in long-term

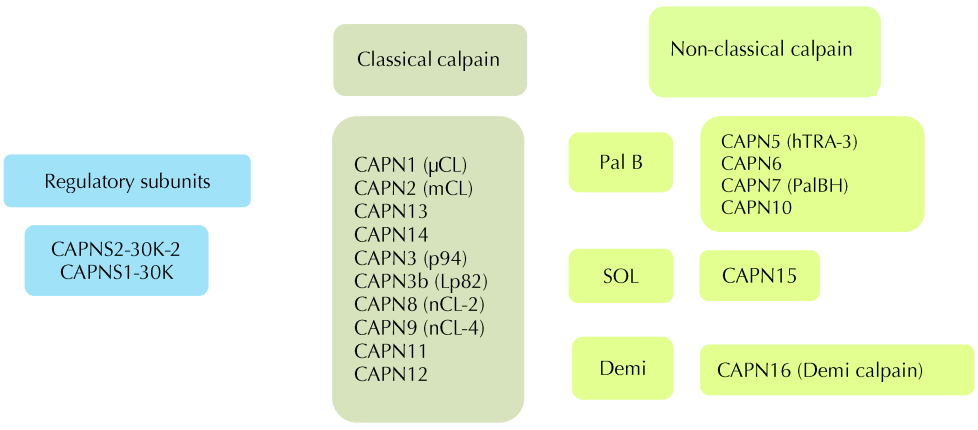

Figure 2. Calpain Classification.

potentiation (LTP) and synaptic plasticity. Calpain is activated by Ca^{+2} influx through glutamate receptors that result in truncation of spectrin and subsequently allows the disassembly and reassembly of actin filaments, thereby recruiting and regulating glutamate receptors.[5,19,27,28]

Calpain Inhibition and Induction

Under physiological conditions, these enzymes are regulated by Ca^{2+} and are involved in pathways which are regulated by this ion. The catalytic site in the protease core domain of calpain includes cysteine-105, histidine-262 and aspargine-286; this triad is conserved through the entire family with one exception, calpain-6. In this enzyme, the cysteine residue in catalytically active sites is replaced by lysine, leading to inactivation of the enzyme.[29,30] Ca^{2+} binding causes a conformational change in the protease core domain and brings PC1 and PC1 into close proximity producing an active catalytic site.[29]

Calpain inhibition is controlled by calpastatin (CAST), a specific inhibitor of classic calpains. Most of the calpain inhibitors are not specific to calpain and suppress other proteases.[10,18,24,31] Calpain-1 and calpain-2 are 2 major calpains recognized in the central nervous system (CNS)[5,13]; other calpain isoforms present in the brain include calpain-3, -5 and calpain-10.[32,33]

Role of Calpain in Synaptic Plasticity

Role of calpains in LTP, learning and memory was identified many years ago. Up to the present time, several studies have clarified participation of calpain-1 and -2 in neuronal migration and differentiation, synaptic plasticity, and neuroprotection.[5,19] LTP is a kind of synaptic plasticity, and translation initiation is an important step in strengthening of LTP.[16,19]

Extracellular signals that trigger calpain-1 and -2 activation include 17-β-estradiol (E2), brain derived neurotrophic factor (BDNF) and glutamate. Calpain-2 activation after LTP induction, which leads to consolidation of LTP, could take place through various intracellular signaling pathways, one of which is ERK signaling. ERK-mediated activation of calpain-2 initiates with binding of BDNF to tyrosine receptor kinase B (TrkB) receptor.[34] Activated calpain-2 inactivates Phosphatase and tensin homolog (PTEN) and stimulates Akt and phosphorylates mammalian target of rapamycin (mTOR). mTOR phosphorylation causes activation of different translation factors such as ribosomal protein S6 and eukaryotic initiation factor 4E which leads to synthesis of dendritic proteins.[5,19] 17-β-estradiol (E2) and positive modulators of AMPA receptors can also trigger the release of BDNF, stimulate calpain-2 through the ERK signaling pathway in dendritic spines and finally phosphorylate mTOR.[19]

On the other hand, activation of calpain-1 and -2 leads to cleavage of Poly(A)-binding protein interacting protein 2A (PAIP2A) and Cytoplasmic polyadenylation element-binding protein 3 (CPEB3). These proteins are repressors of translation initiation and elongation, respectively, and their cleavage by calpain after N-methyl-D-aspartate (NMDA) receptor stimulation initiates this inhibition.

The actin cytoskeleton is a key component of cell structure and plays a major role in various cellular functions including cell proliferation, migration, division and adhesion. In neuronal cells, actin filaments control dendritic spine growth and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking- 2 significant steps in synaptic plasticity.[7,19,27] AMPA receptors mediate the bulk of fast excitatory synaptic neurotransmission in the brain.[7,35] Calpains are among the most important genes involved in actin filament remodeling that occurs during LTP.[19] Calpain activation by Ca^{2+} influx through NMDA receptors leads to spectrin cleavage; spectrin as a cytoskeletal protein causes reorganization of actin filaments within dendritic spines required during LTP. Other proteins that are calpain targets and involved in the synaptic plasticity process include cortactin, talin, drebrin and Myristoylated alanine-rich C kinase substrate (MARCKS). MARCKS and drebrin manage the morphology and stability of dendritic spines.[7,19] Calpain regulates axon outgrowth and morphology via the proteolysis of talin and cortactin, respectively.[27]

Cortactin is necessary for dendritic spine remodeling during synaptic stimulation[36] and its binding to actin is regulated by phosphorylation. Phosphorylation modifies the susceptibility of cortactin to proteolysis by calpain. TrkB activation results in phosphorylation of cortactin at the Ser residue by ERK which associates this protein with F-actin, whereas its phosphorylation at the Tyr residue by Src dissociates cortactin from the actin cytoskeleton which subsequently causes its degradation by calpain and restrains membrane protrusion.[7,12,19,37]

Talin, an important calpain target, links integrin to the actin cytoskeleton and is involved in the assembly of adhesion complexes, and their truncation by calpain hampers adhesion signaling through integrin.[21,27,38] Calpain is also involved in RhoA synthesis and degradation; calpain-1 regulates RhoA degradation while calpain-2 stimulates RhoA synthesis.[39] Integrinβ3 cleavage by calpain results in cell shrinkage through triggering downstream RhoA signaling which is a major component in actin filament remodeling and cell migration. In addition, calpain indirectly controls RhoA signaling and axonal growth through p53 truncation.[7,12,19,40] Cofilin is a calpain substrate and downstream effector of RhoA. It is

another regulator of actin filament dynamics.[7,19,39]

Calpain and Diseases

In the human genome, several genes, such as *CANP1* and *CANP2*, encode calpain-like protease domain. According to the role of calpain in disease pathogenesis, calpain-involved disorders are classified into three groups: Type 1; disorders caused or worsened by calpain including cancer, neurodegenerative and cardiovascular disorders, cataracts and ischemia, and Type 2; including malaria, trypanosomiasis, schistosomiasis, candidiasis and periodontitis. In these disorders, parasites or pathogenic microorganisms use their host's calpains for infection. Type 3 includes calpainopathies caused by a deficiency in calpain genes. Examples include muscular dystrophies, vitreoretinopathy, gastric ulcer and esophagitis (Table 1).[24]

Neurodegenerative Disorders

In some cell types during aging process, level of modified or abnormal proteins increase as a result of improper and inefficient activity of protease, while in other cell types, aging happens due to increased activity of proteases; cataract and Alzheimer disease (AD) are examples of elevated calpain activity and degenerative aging.[41] Concentration of intracellular Ca^{2+} is precisely monitored, as increase in intracellular Ca^{2+} level lead to accumulation of protein and nucleic acid deposition and disruption of phospholipid membrane integrity. Impaired Ca signaling has been involved in pathogenesis of neurodegenerative disorders such as AD, Parkinson disease (PD) and motor neuron diseases.[42]

Under physiologic condition, calpain has a neuroprotective role, although its over-expression results in neurologic dysfunction such as AD. Calpain mediate proteolysis of disease associated proteins tau kinase, cylin dependent kinase 5 and glycogen kinase synthase 3.[43] AD phathogenesis is triggered by deposition of extracellular β-amyloid (Aβ), hyper-phosphorylated tau and formation of intraneuronal neurofibrillary tangles which are associated with abnormal synaptic plasticity and memory. Accumulation of Aβ induces increase in intracellular Ca^{2+} concentration, elevated Ca^{2+} subsequently lead to stimulation of Ca^{2+}-regulated proteins including calpains, calcium/calmodulin-dependent protein kinase (CAMKK2), calcineurin. Increased activity of calpain has been reported in end stage AD patient's brain compared

Table 1. Calpain Family Members and Their Mechanistic Linkage to Some Diseases and Other Pathological Conditions

Gene	Disease		Effect	Reference
Calpain-1 and -2	Alzheimer disease			42-44, 89, 90
	Amyotrophic lateral sclerosis (ALS)			49, 50
	Spinocerebellar ataxia type 3			51, 52
	Parkinson disease			91
	Huntington's diseases			47, 92
	Spastic paraplegia 76			53
	Cataract			93, 94
	Cardiovascular disorders			81, 87, 88
	Muscular dystrophy			95
	Brain ischemia			55
	Cancers	Breast cancer	Overexpression	96, 97
		Colorectal cancer	Overexpression	98, 99
		Lung cancer	Phosphorylations and secretion	100
		Renal carcinoma	Overexpression	101
		Rhabdomyosarcoma		102
Calpain-3	Limb-girdle muscular dystrophy type 2A			74-76, 78, 103
	Cancers	Melanoma	Overexpression	104
		Urothelial cancer	overexpression of active form	105
Calpain-5	Autosomal dominant neovascular inflammatory vitreoretinopathy			106
Calpain-6	Cancers	Uterine cancer	Overexpression	18
		Cervical carcinomas	Overexpression	18
Calpain-8 and -9	Gastric ulcer			10
	Cancers	Gastric cancer	Low/absent expression	107, 108
Calpain-10	Type 2 diabetes			81-83
	Polycystic ovary syndrome			109
	Metabolic syndrome			110
	Cancers	Laryngeal cancer	Influence of CAPN10variants on cancer susceptibility/prognosis low expression	111
		Colorectal cancer		112
		Esophageal cancer		113
Calpain-14	Eosinophilicoesophagitis			114

to controls and inhibition of calpain ameliorates dysfunction in synaptic plasticity and memory.[43-45]

Upregulated expression of astrocytic calpain-10 due to disrupted Ca^{2+} signaling and its relation to Alzheimer-type pathology has been reported, although the exact role of calpain-10 in pathology of the disease remains to be elucidated.[42]

Huntington disease (HD) is a neurodegenerative disorder caused by polyglutamine tract expansion near the N-terminus of huntingtin (Htt). Studies suggest that mutant Htt fragments can aggregate and cause cell death in HD. Mutant huntingtin (mHtt) contains several caspase and calpain cleavage sites that generate N-terminal fragments that are more toxic than mHtt. Cleavage of Htt in human HD tissue is mediated partly by the calpains. μ-, m- and -5, -7 and -10 calpains have increased levels and are activated in HD tissue in transgenic mouse model.[46,47]

Calpains especially calpain-1 plays an essential role in pathogenesis of PD. Pathological activation of calpain due to disruption of Ca^{2+} homeostasis lead to abnormal proteolysis of alpha-synculin (αSyn), truncated form of αSyn aggregate in nervous system which consequently, result in synaptic impairment.[48] Due to dysregulation of Ca^{2+} homeostasis, over-activated calpains cleave C terminus of transactive response DNA-binding protein 43 (TDP-43). The generated abnormal TDP-43 fragments are accumulation-prone and have been present in the motor neurons of patients with amyotrophic lateral sclerosis (ALS).[49,50]

Calpain and caspase dependent cleavage of mutant ataxin-3, the mutated protein in Spinocerebellar ataxia type 3 (SCA3), generate truncated form of ataxin-3. These toxic fragments predispose to aggregate in CNS to a higher extent than full-length ataxin-3. In calpastation knockout mice model, increased calpain activity was observed, which result in enhanced proteolytic cleavage and more severe phenotype in these mice implicating the crucial role of calpastatin in SCA3 pathogenesis.[51,52]

Homozygous mutations in *CAPN1* gene are involved in autosomal recessive hereditary spastic paraplegia (HSP), spastic ataxia, or both.[13,53,54] In three models of calpain1 deficiency in *C. elegans*, *Drosophila melanogaster* and *Danio rerio*, neuronal and axonal dysfunction, locomotor impairments and disrupted branchiomotor neuron migration were observed, respectively.[53]

Calpains are also associated with a number of neurodegenerative pathologies cerebral ischemia and traumatic brain injuries (TBI). Activation of calpain family of protease has been observed during and after brain ischemia. Brain ischemia and traumatic brain injury lead to glucose starvation in neuronal cells, increase in Ca^{2+} level, calpain activation and postischemicneurodegeneration.[32,33,55]

Macrophages have long been known as a crucial component of host defence against microbial infections. Inhibitions of PAR1 or μ-calpain in macrophages make them resistant to Leishmania infection, indicating that they may prove to be therapeutically effective drug targets against leishmaniasis.[56] The calpain inhibitor MDL28170 is found to be capable of significantly reducing the viability of blood stream trypomastigotes in Chagas disease patients, which making the calpain inhibitor useful to treat Chagas disease.[57] The cytotoxic effect of *Streptococcus pyogenes* on macrophages is mediated via subsequent activation of μ-calpain and activation of an inflammatory programmed cell death pathway.[58]

Myopathies

In Duchene muscular dystrophy (DMD), loss of functional dystrophin causes loss of Ca^{2+} homeostasis and activation of ubiquitous calpain. Increased calpain activity worsens DMD symptoms since several muscle proteins are calpain substrates. In a dystrophin-deficient DMD mouse model, increased calpain activation was modified by calpastatin (CAST) overexpression, although recent studies have demonstrated that calpain inhibition did not improve muscle histology and function.[8,24,59,60]

Ophthalmic Diseases

Ischemic injury to the retina is associated with calpain-1 and -2 activity. Overactivity of calpain leads to α-spectrin proteolysis, retinal cell degeneration and vision loss. Calpain overactivity also causes pathological neovascularization which results in defective vascular flow and retinal damage.[45,61,62]

Cancer

Due to the diverse roles of conventional calpains in cellular processes including apoptosis, cell proliferation and migration, these genes are also implicated in cancer pathogenesis and possible molecular mechanisms critical for cancer formation, progression, and growth such as cell transformation, cell survival/apoptosis, migration, invasion and angiogenesis.[18,63]

Oncogenic Transformation

Calpain activation is a key step in cell transformation stimulated by common oncoproteins. During pathological processes, increased calpain activity and calpastatin inhibition are induced through v-Src oncoprotein induction and are augmented by other oncoproteins such as v-Myc, v-Jun, k-Ras and v-Fos during cell transformation. Calpain overactivity leads

to decreased adhesiveness and increased migration of transformed cells through proteolysis of cytoskeletal proteins including spectrin, paxillin, talin and FAK.[63,64]

Cell survival and Apoptosis

Programmed cell death is one of the critical cell processes and is crucial for organism homeostasis. Various proteases including caspase and calpain are involved in apoptosis. Calpain1 and 2 regulate caspases via activation of caspase-3, -7 and -12 and inactivation of caspase-8 and -9.[11,18] P53 is an important modulator of equilibration between cell proliferation-DNA repair and cell death, and calpain mediates its processing and accordingly regulates cell fate.[65]Another regulator of apoptosis is Bcl-2 family members whose effects upstream of caspases and based on their homo- or heterodimerization have pro- or anti-apoptotic functions. Conventional calpains cleave a few members of the Bcl-2 proteins, including Bax, Bcl-xL, Bid, and Bak. Calpain intervention normally induces an apoptotic cascade.[18,66]

Migration and Invasion

Another crucial step in the invasion of malignant cells, which in the case of distant invasion leads to metastasis, is migration capability. Under physiologic conditions, calpain2 regulates the migration of different cell types by control of growth factors including EGF, PDGF, and VEGF.[63] Calpain plays an important role in the mesenchymal mechanism of tumor cell migration. In this process, adhesion is mediated by integrin, a proteolytic substrate of calpain. Other proteolytic targets of calpain associated with migration/invasion of tumor cells include talin, paxillin, FAK, vimentin,[68] vinculin,[69] cortactin,[70] fodrin and protein tyrosine phosphatase23 (PTPN23).[71] Calpain involvement in the expression and secretion of matrix metalloproteinase (MMP) has been identified; MMP is responsible for the degradation and remodeling of the extracellular matrix and subsequent tumor invasion.[18]

Angiogenesis

Calpain regulates angiogenesis by controlling the migration of endothelial cells. Vascular endothelial growth factor (VEGF) is one of the crucial activators of angiogenesis. Calpain activation is induced by VEGF. Under physiologic conditions, calpain activation leads to production of NO- a robust angiogenic factor. Calpain2 mediates VEGF-induced activation of PI3K/AMPK/Akt and subsequent phosphorylation of eNOS and production of NO in endothelial cells, while under pathological conditions, tumor cell overgrowth leads to hypoxia which stimulates calpain over-activity and expression in endothelial cells.[18,63,72] Hypoxia, which is a result of rapid proliferation of cancer cells and is required for survival and growth of these cells. Hypoxia stimulates calpain-dependent cleavage of FLNA which increases nuclear localization of hypoxia-inducible factor-1α (HIF-1α). HIF-1α is upregulated in cancer and forms a heterodimer with HIF-1β (HIF-1α/HIF-1β) which stimulates expression of VEGF.[72,73] Other growth factors implicated in angiogenesis include epidermal growth factor (EGF) and fibroblast growth factor (FGF).[63]

Calpainopathies

Mutations in calpain genes result in a group of diseases defined as calpainopathy. *CAPN3*, previously called p94 or calpain3, is mainly expressed in skeletal muscles although its expression has also been detected in other tissues. Mutations in this gene are responsible for limb-girdle muscular dystrophy 2A (LGMD2A), which is the most common type of LGMD. In most forms of LGMD, mutations in genes coding for structural proteins are the seminal event; however, *CAPN3* mutations were the first type of muscular dystrophy with mutations in a proteolytic enzyme rather than a structural protein.[74-76] CAPN3 has diverse substrates; however, it does not possess a consensus cleavage site. This protein has both proteolytic and non-proteolytic functions and is linked with sarcomere remodeling.[74-77]

In CAPN3 knock-out (CAPN3-KO) mice, diminished well-organized sarcomeres, decreased muscle mass, disrupted structure of myofibers and myopathy were observed.[74,75,78] Ca^{2+}-mediated signals are required for upregulation of adaptive response genes in skeletal muscle. In CAPN3 KO mice, a decrease in Ca^{2+} efflux and attenuation of CaMKII signaling occur and the muscle adaptation response is compromised due to a decrease in PGC1 level, a coactivator of transcription factors that regulate adaptation response. Decreased PGC1 level is a result of attenuation in the CaMKII signaling pathway and the subsequent decrease in p38 MAPK.[74,75,78]

Other Calpainopathies

Pathogenic homozygous mutations in *CAPN1* result in calpain-1 loss of function and are linked to spastic paraplegia 76 (SPG76).[53] Abnormal cerebellar development has been reported in $Capn1^{-/-}$ mice which led to ataxia phenotype and spinocerebellar ataxia was observed in dogs and humans with mutations in *CAPN1* gene. The underlying etiology includes defective development of cerebellar granule cells (CGCs) and impaired synaptic transmission.[79]

Single nucleotide polymorphisms (SNPs) in calpain8 and 9 (called calpain-8/9 or G-calpain) are thought to disrupt the proteolytic activity of the calpain 8/9 complex and are related to susceptibility to gastric

Table 2. Common Polymorphisms of *CAPN10* Gene Associated With T2DM

Phenotype	dbSNP		Mutation
Susceptibility to noninsulin-dependent Diabetes Mellitus	*SNP-43*	*rs3792267*	*CAPN10, IVS3, g.240591757G>A, c.471-176G>A*
	InDel-19	*rs3842570*	*CAPN10, IVS6, g.240594876_240594877insCGGGAGGAGGG TGATGATTCTGTCCCAGGAGC,c.998-148_998-147insCGGGA GGAGGGTGATGATTCTGTCCCAGGAGC*
	SNP-63	*rs5030952*	*CAPN10, IVS13, g.240603286C>T*
Susceptibility to noninsulin-dependent Diabetes Mellitus and Polycystic ovary syndrome	*SNP-44*	*rs2975760*	*CAPN10, IVS3, g.240603286C>T, c.471-187T>C*

injury; moreover, Capn8$^{-/-}$ and Capn9$^{-/-}$ mice showed susceptibility to gastric ulcers induced by ethanol which suggests that G-calpain is implicated in gastric mucosal defense.[24,80]

Numerous studies have identified an association between *CAPN10* gene polymorphism and type 2 diabetes mellitus (T2DM) in several populations although there is debate among the different studies and the underlying mechanism remains to be fully elucidated. These polymorphisms including SNP-43 (rs3792267), SNP-44 (rs2975760), SNP-63(rs5030952) and InDel-19 (rs3842570) are intronic variants (Table 2) and thus do not interrupt protein translation. They probably influence gene expression or transcriptional regulation of *CAPN10*.[81-84]

In the Iranian population, SNP-43, SNP-19 and -63 of CAPN10 have been studied and no association was observed between the genotypes and T2D.[85,86]

Calpains are among the proteases implicated in the pathogenesis of atherosclerosis, coronary heart disease, and obesity/insulin-associated heart disease as well as hypertensive heart disease. Uncontrolled activation of calpain and caspases results in stimulation of signaling pathways such as NF-κB and apoptosis pathways. NF-κB is a key player in inflammatory diseases and is involved in atherosclerotic plaque formation. The NF-κB signaling pathway is regulated by calpain through IκB cleavage without influencing its phosphorylation.[87,88]

Endothelial dysfunction is the first event implicated in cardiovascular disorder in diabetes. Calpain may be involved in insulin-regulated pathways and cardiac hypertrophy associated with Insulin dependent diabetes mellitus (IDDM). Vascular calpain is activated by hyperglycemia through the PKC signaling cascade which stimulates endothelial dysfunction.[88]

Calpain is involved in the development of hypertension-induced cardiac hypertrophy. Calpain regulates transcription factors including NF-κB, GATA binding protein4 (GATA4) and nuclear factor of activated T cells (NFAT) implicated in hypertensive heart disease.[87]

Future Directions

More research linking biochemistry, molecular biology and structural biology is needed to gain further insight into the basic mechanism for the involvement of both the large and small subunits of calpains responsible for its activation and regulation in different cells and subcellular locations. Although some information based on biochemical and genetic studies has indicated links between some forms of calpain overproduction/deficiency in the etiology of some diseases, more research is needed to better understand the concerted roles of serine proteases, calpains and caspases in a variety of physiological and pathological phenomena in health and diseases.

Authors' Contribution

MH wrote the manuscript with support from KK and HN. KK and HN had also contribution to the revision of the manuscript..

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

Not applicable.

Acknowledgment

This research was supported by the Iranian National Science Foundation (grant no. S 92038458 & 92035782).

References

1. Sorimachi H, Hata S, Ono Y. Calpain chronicle--an enzyme family under multidisciplinary characterization. Proc Jpn Acad Ser B Phys Biol Sci. 2011;87(6):287-327.
2. Sorimachi H, Hata S, Ono Y. Impact of genetic insights into calpain biology. J Biochem. 2011;150(1):23-37. doi: 10.1093/jb/mvr070.
3. Suzuki K, Hata S, Kawabata Y, Sorimachi H. Structure, activation, and biology of calpain. Diabetes. 2004;53 Suppl 1:S12-8.
4. Baudry M, Bi X. Learning and memory: an emergent property of cell motility. Neurobiol Learn Mem. 2013;104:64-72. doi: 10.1016/j.nlm.2013.04.012.
5. Baudry M, Bi X. Calpain-1 and Calpain-2: The Yin and Yang of Synaptic Plasticity and Neurodegeneration. Trends Neurosci. 2016;39(4):235-45. doi: 10.1016/j.tins.2016.01.007.
6. Rawlings ND. Bacterial calpains and the evolution of the calpain (C2) family of peptidases. Biol Direct. 2015;10:66. doi: 10.1186/s13062-015-0095-0.
7. Baudry M, Chou MM, Bi X. Targeting calpain in synaptic plasticity. Expert Opin Ther Targets. 2013;17(5):579-92. doi: 10.1517/14728222.2013.766169.
8. Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. Physiol Rev. 2003;83(3):731-801. doi: 10.1152/physrev.00029.2002.
9. Sorimachi H, Hata S, Ono Y. Expanding members and roles of

the calpain superfamily and their genetically modified animals. Exp Anim. 2010;59(5):549-66.
10. Ono Y, Sorimachi H. Calpains: an elaborate proteolytic system. Biochim Biophys Acta. 2012;1824(1):224-36. doi: 10.1016/j.bbapap.2011.08.005.
11. Smith MA, Schnellmann RG. Calpains, mitochondria, and apoptosis. Cardiovasc Res. 2012;96(1):32-7. doi: 10.1093/cvr/cvs163.
12. Zadran S, Bi X, Baudry M. Regulation of calpain-2 in neurons: implications for synaptic plasticity. Mol Neurobiol. 2010;42(2):143-50. doi: 10.1007/s12035-010-8145-1.
13. Wang Y, Hersheson J, Lopez D, Hammer M, Liu Y, Lee KH, et al. Defects in the CAPN1 Gene Result in Alterations in Cerebellar Development and Cerebellar Ataxia in Mice and Humans. Cell Rep. 2016;16(1):79-91. doi: 10.1016/j.celrep.2016.05.044.
14. Lopez D, Pinto V, Wang Y, Liu Y, Seinfeld J, Bi X, et al. Calpain-1 Knockout in Mice Causes Degeneration of Cerebellar Granule Cells and Ataxia. Faseb j. 2015;29(1):727-8. doi: 10.1096/fasebj.29.1_supplement.727.8.
15. Dutt P, Croall DE, Arthur JS, Veyra TD, Williams K, Elce JS, et al. m-Calpain is required for preimplantation embryonic development in mice. BMC Dev Biol. 2006;6:3. doi: 10.1186/1471-213x-6-3.
16. Liu J, Liu MC, Wang KK. Calpain in the CNS: from synaptic function to neurotoxicity. Sci Signal. 2008;1(14):re1. doi: 10.1126/stke.114re1.
17. Piatkov KI, Oh JH, Liu Y, Varshavsky A. Calpain-generated natural protein fragments as short-lived substrates of the N-end rule pathway. Proc Natl Acad Sci U S A. 2014;111(9):E817-26. doi: 10.1073/pnas.1401639111.
18. Moretti D, Del Bello B, Allavena G, Maellaro E. Calpains and cancer: friends or enemies? Arch Biochem Biophys. 2014;564:26-36. doi: 10.1016/j.abb.2014.09.018.
19. BrizV, Baudry M. Calpains: Master Regulators of Synaptic Plasticity. Neuroscientist.2016.doi:10.1177/1073858416649178.
20. Cortesio CL, Boateng LR, Piazza TM, Bennin DA, Huttenlocher A. Calpain-mediated proteolysis of paxillin negatively regulates focal adhesion dynamics and cell migration. J Biol Chem. 2011;286(12):9998-10006. doi: 10.1074/jbc.M110.187294.
21. Franco SJ, Huttenlocher A. Regulating cell migration: calpains make the cut. J Cell Sci. 2005;118(Pt 17):3829-38. doi: 10.1242/jcs.02562.
22. Chan KT, Bennin DA, Huttenlocher A. Regulation of adhesion dynamics by calpain-mediated proteolysis of focal adhesion kinase (FAK). J Biol Chem. 2010;285(15):11418-26. doi: 10.1074/jbc.M109.090746.
23. Siklos M, BenAissa M, Thatcher GR. Cysteine proteases as therapeutic targets: does selectivity matter? A systematic review of calpain and cathepsin inhibitors. Acta Pharm Sin B. 2015;5(6):506-19. doi: 10.1016/j.apsb.2015.08.001.
24. Ono Y, Saido TC, Sorimachi H. Calpain research for drug discovery: challenges and potential. Nat Rev Drug Discov. 2016;15(12):854-76. doi: 10.1038/nrd.2016.212.
25. Seinfeld J, Baudry N, Xu X, Bi X, Baudry M. Differential Activation of Calpain-1 and Calpain-2 following Kainate-Induced Seizure Activity in Rats and Mice. eNeuro. 2016;3(4). doi: 10.1523/eneuro.0088-15.2016.
26. Czogalla A, Sikorski AF. Spectrin and calpain: a 'target' and a 'sniper' in the pathology of neuronal cells. Cell Mol Life Sci. 2005;62(17):1913-24. doi: 10.1007/s00018-005-5097-0.
27. Kerstein PC, Patel KM, Gomez TM. Calpain-Mediated Proteolysis of Talin and FAK Regulates Adhesion Dynamics Necessary for Axon Guidance. J Neurosci. 2017;37(6):1568-80. doi: 10.1523/jneurosci.2769-16.2016.
28. Baudry M, Chou MM, Bi X. Targeting calpain in synaptic plasticity. Expert Opin Ther Targets. 2013;17(5):579-92. doi: 10.1517/14728222.2013.766169.
29. Bose K. Proteases in Apoptosis: Pathways, Protocols and Translational Advances. Switzerland: Springer International Publishing; 2015.
30. Cagmat EB, Guingab-Cagmat JD, Vakulenko AV, Hayes RL, Anagli J. Potential use of Calpain inhibitors as brain injury therapy. In: Kobeissy FH, ed. Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. Boca Raton (FL): CRC Press/Taylor & Francis; 2015.
31. Ji J, Su L, Liu Z. Critical role of calpain in inflammation. Biomed Rep. 2016;5(6):647-52. doi: 10.3892/br.2016.785.
32. Ma M. Role of calpains in the injury-induced dysfunction and degeneration of the mammalian axon. Neurobiol Dis. 2013;60:61-79. doi: 10.1016/j.nbd.2013.08.010.
33. Bevers MB, Neumar RW. Mechanistic role of calpains in postischemic neurodegeneration. J Cereb Blood Flow Metab. 2008;28(4):655-73. doi: 10.1038/sj.jcbfm.9600595.
34. Zhu G, Liu Y, Wang Y, Bi X, Baudry M. Different patterns of electrical activity lead to long-term potentiation by activating different intracellular pathways. J Neurosci. 2015;35(2):621-33. doi: 10.1523/jneurosci.2193-14.2015.
35. Henley JM, Wilkinson KA. AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. Dialogues Clin Neurosci. 2013;15(1):11-27.
36. Hsueh YP. Neuron-specific regulation on F-actin cytoskeletons: The role of CTTNBP2 in dendritic spinogenesis and maintenance. Commun Integr Biol. 2012;5(4):334-6. doi: 10.4161/cib.20364.
37. Olson ML, Ingebretson AE, Harmelink KM. Hippocampal Cortactin Levels are Reduced Following Spatial Working Memory Formation, an Effect Blocked by Chronic Calpain Inhibition. Brain Sci. 2015;5(2):241-57. doi: 10.3390/brainsci5020241.
38. Kerstein PC, Jacques-Fricke BT, Rengifo J, Mogen BJ, Williams JC, Gottlieb PA, et al. Mechanosensitive TRPC1 channels promote calpain proteolysis of talin to regulate spinal axon outgrowth. J Neurosci. 2013;33(1):273-85. doi: 10.1523/jneurosci.2142-12.2013.
39. Briz V, Zhu G, Wang Y, Liu Y, Avetisyan M, Bi X, et al. Activity-dependent rapid local RhoA synthesis is required for hippocampal synaptic plasticity. J Neurosci. 2015;35(5):2269-82. doi: 10.1523/jneurosci.2302-14.2015.
40. Qin Q, Liao G, Baudry M, Bi X. Role of calpain-mediated p53 truncation in semaphorin 3A-induced axonal growth regulation. Proc Natl Acad Sci U S A. 2010;107(31):13883-7. doi: 10.1073/pnas.1008652107.
41. Covington MD, Arrington DD, Schnellmann RG. Calpain 10 is required for cell viability and is decreased in the aging kidney. Am J Physiol Renal Physiol. 2009;296(3):F478-86. doi: 10.1152/ajprenal.90477.2008.
42. Garwood C, Faizullabhoy A, Wharton SB, Ince PG, Heath PR, Shaw PJ, et al. Calcium dysregulation in relation to Alzheimer-type pathology in the ageing brain. Neuropathol Appl Neurobiol. 2013;39(7):788-99. doi: 10.1111/nan.12033.
43. Kurbatskaya K, Phillips EC, Croft CL, Dentoni G, Hughes MM, Wade MA, et al. Upregulation of calpain activity precedes tau phosphorylation and loss of synaptic proteins in Alzheimer's disease brain. Acta Neuropathol Commun. 2016;4:34. doi: 10.1186/s40478-016-0299-2.
44. Magi S, Castaldo P, Macri ML, Maiolino M, Matteucci A, Bastioli G, et al. Intracellular Calcium Dysregulation: Implications for Alzheimer's Disease. Biomed Res Int. 2016;2016:6701324. doi: 10.1155/2016/6701324.
45. Potz BA, Abid MR, Sellke FW. Role of Calpain in Pathogenesis of Human Disease Processes. J Nat Sci. 2016;2(9).
46. Gafni J, Ellerby LM. Calpain activation in Huntington's disease. J Neurosci. 2002;22(12):4842-9.
47. Gafni J, Hermel E, Young JE, Wellington CL, Hayden MR, Ellerby LM. Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. J Biol Chem. 2004;279(19):20211-20. doi: 10.1074/jbc.M401267200.
48. Diepenbroek M, Casadei N, Esmer H, Saido TC, Takano J, Kahle PJ, et al. Overexpression of the calpain-specific inhibitor calpastatin reduces human alpha-Synuclein processing, aggregation and synaptic impairment in [A30P]alphaSyn transgenic mice. Hum Mol Genet. 2014;23(15):3975-89. doi: 10.1093/hmg/ddu112.

49. Akamatsu M, Yamashita T, Hirose N, Teramoto S, Kwak S. The AMPA receptor antagonist perampanel robustly rescues amyotrophic lateral sclerosis (ALS) pathology in sporadic ALS model mice. Sci Rep. 2016;6:28649. doi: 10.1038/srep28649.
50. Stifanese R, Averna M, De Tullio R, Pedrazzi M, Milanese M, Bonifacino T, et al. Role of calpain-1 in the early phase of experimental ALS. Arch Biochem Biophys. 2014;562:1-8. doi: 10.1016/j.abb.2014.08.006.
51. Evers MM, Toonen LJ, van Roon-Mom WM. Ataxin-3 protein and RNA toxicity in spinocerebellar ataxia type 3: current insights and emerging therapeutic strategies. Mol Neurobiol. 2014;49(3):1513-31. doi: 10.1007/s12035-013-8596-2.
52. Hubener J, Weber JJ, Richter C, Honold L, Weiss A, Murad F, et al. Calpain-mediated ataxin-3 cleavage in the molecular pathogenesis of spinocerebellar ataxia type 3 (SCA3). Hum Mol Genet. 2013;22(3):508-18. doi: 10.1093/hmg/dds449.
53. Gan-Or Z, Bouslam N, Birouk N, Lissouba A, Chambers DB, Veriepe J, et al. Mutations in CAPN1 Cause Autosomal-Recessive Hereditary Spastic Paraplegia. Am J Hum Genet. 2016;98(5):1038-46. doi: 10.1016/j.ajhg.2016.04.002.
54. Gan-Or Z, Rouleau GA. Calpain 1 in neurodegeneration: a therapeutic target? Lancet Neurol. 2016;15(11):1118. doi: 10.1016/s1474-4422(16)30175-2.
55. Frederick JR, Chen Z, Bevers MB, Ingleton LP, Ma M, Neumar RW. Neuroprotection with delayed calpain inhibition after transient forebrain ischemia. Crit Care Med. 2008;36(11 Suppl):S481-5.
56. Rana T, Misra S, Mittal MK, Farrow AL, Wilson KT, Linton MF, et al. Mechanism of down-regulation of RNA polymerase III-transcribed non-coding RNA genes in macrophages by Leishmania. J Biol Chem. 2011;286(8):6614-26. doi: 10.1074/jbc.M110.181735.
57. Ennes-Vidal V, Menna-Barreto RF, Santos AL, Branquinha MH, d'Avila-Levy CM. MDL28170, a calpain inhibitor, affects Trypanosoma cruzi metacyclogenesis, ultrastructure and attachment to Rhodnius prolixus midgut. PLoS One. 2011;6(4):e18371. doi: 10.1371/journal.pone.0018371.
58. Goldmann O, Sastalla I, Wos-Oxley M, Rohde M, Medina E. Streptococcus pyogenes induces oncosis in macrophages through the activation of an inflammatory programmed cell death pathway. Cell Microbiol. 2009;11(1):138-55. doi: 10.1111/j.1462-5822.2008.01245.x.
59. Tidball JG, Spencer MJ. Calpains and muscular dystrophies. Int J Biochem Cell Biol. 2000;32(1):1-5.
60. Briguet A, Erb M, Courdier-Fruh I, Barzaghi P, Santos G, Herzner H, et al. Effect of calpain and proteasome inhibition on Ca2+-dependent proteolysis and muscle histopathology in the mdx mouse. Faseb j. 2008;22(12):4190-200. doi: 10.1096/fj.07-099036.
61. Hoang MV, Smith LE, Senger DR. Calpain inhibitors reduce retinal hypoxia in ischemic retinopathy by improving neovascular architecture and functional perfusion. Biochim Biophys Acta. 2011;1812(4):549-57. doi: 10.1016/j.bbadis.2010.08.008.
62. Azuma M, Sakamoto-Mizutani K, Nakajima T, Kanaami-Daibo S, Tamada Y, Shearer TR. Involvement of calpain isoforms in retinal degeneration in WBN/Kob rats. Comp Med. 2004;54(5):533-42.
63. Leloup L, Wells A. Calpains as potential anti-cancer targets. Expert Opin Ther Targets. 2011;15(3):309-23. doi: 10.1517/14728222.2011.553611.
64. Carragher NO, Fonseca BD, Frame MC. Calpain activity is generally elevated during transformation but has oncogene-specific biological functions. Neoplasia. 2004;6(1):53-73.
65. Del Bello B, Moretti D, Gamberucci A, Maellaro E. Cross-talk between calpain and caspase-3/-7 in cisplatin-induced apoptosis of melanoma cells: a major role of calpain inhibition in cell death protection and p53 status. Oncogene. 2007;26(19):2717-26. doi: 10.1038/sj.onc.1210079.
66. Gao G, Dou QP. N-terminal cleavage of bax by calpain generates a potent proapoptotic 18-kDa fragment that promotes bcl-2-independent cytochrome C release and apoptotic cell death. J Cell Biochem. 2000;80(1):53-72.
67. Dave JM, Bayless KJ. Vimentin as an integral regulator of cell adhesion and endothelial sprouting. Microcirculation. 2014;21(4):333-44. doi: 10.1111/micc.12111.
68. Serrano K, Devine DV. Vinculin is proteolyzed by calpain during platelet aggregation: 95 kDa cleavage fragment associates with the platelet cytoskeleton. Cell Motil Cytoskeleton. 2004;58(4):242-52. doi: 10.1002/cm.20011.
69. Perrin BJ, Amann KJ, Huttenlocher A. Proteolysis of cortactin by calpain regulates membrane protrusion during cell migration. Mol Biol Cell. 2006;17(1):239-50. doi: 10.1091/mbc.E05-06-0488.
70. Castiglioni S, Maier JA. The tyrosine phosphatase HD-PTP (PTPN23) is degraded by calpains in a calcium-dependent manner. Biochem Biophys Res Commun. 2012;421(2):380-3. doi: 10.1016/j.bbrc.2012.04.024.
71. Manfredi LH, Lustrino D, Machado J, Silveira WA, Zanon NM, Navegantes LC, et al. Adrenodemedullation activates the Ca(2+)-dependent proteolysis in soleus muscles from rats exposed to cold. J Appl Physiol (1985). 2017;122(2):317-26. doi: 10.1152/japplphysiol.00198.2016.
72. Zhang Y, Liu NM, Wang Y, Youn JY, Cai H. Endothelial cell calpain as a critical modulator of angiogenesis. Biochim Biophys Acta. 2017;1863(6):1326-35. doi: 10.1016/j.bbadis.2017.03.021.
73. Zheng X, Zhou AX, Rouhi P, Uramoto H, Boren J, Cao Y, et al. Hypoxia-induced and calpain-dependent cleavage of filamin A regulates the hypoxic response. Proc Natl Acad Sci U S A. 2014;111(7):2560-5. doi: 10.1073/pnas.1320815111.
74. Kramerova I, Beckmann JS, Spencer MJ. Molecular and cellular basis of calpainopathy (limb girdle muscular dystrophy type 2A). Biochim Biophys Acta. 2007;1772(2):128-44. doi: 10.1016/j.bbadis.2006.07.002.
75. Kramerova I, Ermolova N, Eskin A, Hevener A, Quehenberger O, Armando AM, et al. Failure to up-regulate transcription of genes necessary for muscle adaptation underlies limb girdle muscular dystrophy 2A (calpainopathy). Hum Mol Genet. 2016;25(11):2194-207. doi: 10.1093/hmg/ddw086.
76. Ono Y, Ojima K, Shinkai-Ouchi F, Hata S, Sorimachi H. An eccentric calpain, CAPN3/p94/calpain-3. Biochimie. 2016;122:169-87. doi: 10.1016/j.biochi.2015.09.010.
77. Huang J, Zhu X. The molecular mechanisms of calpains action on skeletal muscle atrophy. Physiol Res. 2016;65(4):547-60.
78. Kramerova I, Kudryashova E, Ermolova N, Saenz A, Jaka O, Lopez de Munain A, et al. Impaired calcium calmodulin kinase signaling and muscle adaptation response in the absence of calpain 3. Hum Mol Genet. 2012;21(14):3193-204. doi: 10.1093/hmg/dds144.
79. Wang Y, Lopez D, Davey PG, Cameron DJ, Nguyen K, Tran J, et al. Calpain-1 and calpain-2 play opposite roles in retinal ganglion cell degeneration induced by retinal ischemia/reperfusion injury. Neurobiol Dis. 2016;93:121-8. doi: 10.1016/j.nbd.2016.05.007.
80. Hata S, Abe M, Suzuki H, Kitamura F, Toyama-Sorimachi N, Abe K, et al. Calpain 8/nCL-2 and calpain 9/nCL-4 constitute an active protease complex, G-calpain, involved in gastric mucosal defense. PLoS Genet. 2010;6(7):e1001040. doi: 10.1371/journal.pgen.1001040.
81. Pandurangan M, Hwang I, Orhirbat C, Jieun Y, Cho SH. The calpain system and diabetes. Pathophysiology. 2014;21(2):161-7. doi: 10.1016/j.pathophys.2014.01.003.
82. Saez ME, Gonzalez-Sanchez JL, Ramirez-Lorca R, Martinez-Larrad MT, Zabena C, Gonzalez A, et al. The CAPN10 gene is associated with insulin resistance phenotypes in the Spanish population. PLoS One. 2008;3(8):e2953. doi: 10.1371/journal.pone.0002953.
83. Panico P, Salazar AM, Burns AL, Ostrosky-Wegman P. Role of calpain-10 in the development of diabetes mellitus and its complications. Arch Med Res. 2014;45(2):103-15. doi: 10.1016/j.arcmed.2014.01.005.
84. Song Y, Niu T, Manson JE, Kwiatkowski DJ, Liu S. Are variants in the CAPN10 gene related to risk of type 2 diabetes? A quantitative assessment of population and family-based association studies.

Am J Hum Genet. 2004;74(2):208-22. doi: 10.1086/381400.
85. Maleki F, Haghani K, Shokouhi S, Mahmoodi K, Sayehmiri K, Mahdieh N, et al. A case-control study on the association of common variants of CAPN10 gene and the risk of type 2 diabetes in an Iranian population. Clin Lab. 2014;60(4):663-70.
86. Rezazadeh M, Sohrabifar N, Jafari H, Mohaddes Ardabili M, Gharesouran J, Sohrabifar N, et al. A study on association of polymorphisms in Calpain10 and TCF7L2 genes with type 2 diabetes mellitus. Genetika. 2015;47(1):161-70. doi: 10.2298/GENSR1501161R.
87. Hua Y, Nair S. Proteases in cardiometabolic diseases: Pathophysiology, molecular mechanisms and clinical applications. Biochim Biophys Acta. 2015;1852(2):195-208. doi: 10.1016/j.bbadis.2014.04.032.
88. Potz BA, Sabe AA, Abid MR, Sellke FW. Calpains and Coronary Vascular Disease. Circ J. 2016;80(1):4-10. doi: 10.1253/circj.CJ-15-0997.
89. Laske C, Stellos K, Kempter I, Stransky E, Maetzler W, Fleming I, et al. Increased cerebrospinal fluid calpain activity and microparticle levels in Alzheimer's disease. Alzheimers Dement. 2015;11(5):465-74. doi: 10.1016/j.jalz.2014.06.003.
90. Raynaud F, Marcilhac A. Implication of calpain in neuronal apoptosis. A possible regulation of Alzheimer's disease. Febs j. 2006;273(15):3437-43. doi: 10.1111/j.1742-4658.2006.05352.x.
91. Diepenbroek M, Casadei N, Esmer H, Saido TC, Takano J, Kahle PJ, et al. Overexpression of the calpain-specific inhibitor calpastatin reduces human alpha-Synuclein processing, aggregation and synaptic impairment in [A30P]alphaSyn transgenic mice. Hum Mol Genet. 2014;23(15):3975-89. doi: 10.1093/hmg/ddu112.
92. Weber JJ, Ortiz Rios MM, Riess O, Clemens LE, Nguyen HP. The calpain-suppressing effects of olesoxime in Huntington's disease. Rare Dis. 2016;4(1):e1153778. doi: 10.1080/21675511.2016.1153778.
93. David LL, Shearer TR. Purification of calpain II from rat lens and determination of endogenous substrates. Exp Eye Res. 1986;42(3):227-38.
94. Biswas S, Harris F, Dennison S, Singh J, Phoenix DA. Calpains: targets of cataract prevention? Trends Mol Med. 2004;10(2):78-84. doi: 10.1016/j.molmed.2003.12.007.
95. Hollinger K, Selsby JT. The physiological response of protease inhibition in dystrophic muscle. Acta Physiol (Oxf). 2013;208(3):234-44. doi: 10.1111/apha.12114.
96. Al-Bahlani SM, Al-Rashdi RM, Kumar S, Al-Sinawi SS, Al-Bahri MA, Shalaby AA. Calpain-1 Expression in Triple-Negative Breast Cancer: A Potential Prognostic Factor Independent of the Proliferative/Apoptotic Index. Biomed Res Int. 2017;2017:9290425. doi: 10.1155/2017/9290425.
97. Storr SJ, Zhang S, Perren T, Lansdown M, Fatayer H, Sharma N, et al. The calpain system is associated with survival of breast cancer patients with large but operable inflammatory and non-inflammatory tumours treated with neoadjuvant chemotherapy. Oncotarget. 2016;7(30):47927-37. doi: 10.18632/oncotarget.10066.
98. Lakshmikuttyamma A, Selvakumar P, Kanthan R, Kanthan SC, Sharma RK. Overexpression of m-calpain in human colorectal adenocarcinomas. Cancer Epidemiol Biomarkers Prev. 2004;13(10):1604-9.
99. Selvakumar P, Sharma RK. Involvement of Calpain in colorectal adenocarcinomas. Exp Ther Med. 2010;1(3):413-17. doi: 10.3892/etm_00000064.
100. Xu L, Deng X. Suppression of cancer cell migration and invasion by protein phosphatase 2A through dephosphorylation of mu- and m-calpains. J Biol Chem. 2006;281(46):35567-75. doi: 10.1074/jbc.M607702200.
101. Braun C, Engel M, Seifert M, Theisinger B, Seitz G, Zang KD, et al. Expression of calpain I messenger RNA in human renal cell carcinoma: correlation with lymph node metastasis and histological type. Int J Cancer. 1999;84(1):6-9.
102. Roumes H, Leloup L, Dargelos E, Brustis JJ, Daury L, Cottin P. Calpains: markers of tumor aggressiveness? Exp Cell Res. 2010;316(9):1587-99. doi: 10.1016/j.yexcr.2010.02.017.
103. Hauerslev S, Sveen ML, Duno M, Angelini C, Vissing J, Krag TO. Calpain 3 is important for muscle regeneration: evidence from patients with limb girdle muscular dystrophies. BMC Musculoskelet Disord. 2012;13:43. doi: 10.1186/1471-2474-13-43.
104. Moretti D, Del Bello B, Allavena G, Corti A, Signorini C, Maellaro E. Calpain-3 impairs cell proliferation and stimulates oxidative stress-mediated cell death in melanoma cells. PLoS One. 2015;10(2):e0117258. doi: 10.1371/journal.pone.0117258.
105. Roperto S, De Tullio R, Raso C, Stifanese R, Russo V, Gaspari M, et al. Calpain3 is expressed in a proteolitically active form in papillomavirus-associated urothelial tumors of the urinary bladder in cattle. PLoS One. 2010;5(4):e10299. doi: 10.1371/journal.pone.0010299.
106. Mahajan VB, Lin JH. Lymphocyte infiltration in CAPN5 autosomal dominant neovascular inflammatory vitreoretinopathy. Clin Ophthalmol. 2013;7:1339-45. doi: 10.2147/opth.s46450.
107. Peng P, Wu W, Zhao J, Song S, Wang X, Jia D, et al. Decreased expression of Calpain-9 predicts unfavorable prognosis in patients with gastric cancer. Sci Rep. 2016;6:29604. doi: 10.1038/srep29604.
108. Yoshikawa Y, Mukai H, Hino F, Asada K, Kato I. Isolation of two novel genes, down-regulated in gastric cancer. Jpn J Cancer Res. 2000;91(5):459-63.
109. Shen W, Li T, Hu Y, Liu H, Song M. Calpain-10 genetic polymorphisms and polycystic ovary syndrome risk: a meta-analysis and meta-regression. Gene. 2013;531(2):426-34. doi: 10.1016/j.gene.2013.08.072.
110. Kang ES, Nam M, Kim HJ, Kim HJ, Myoung SM, Rhee Y, et al. Haplotype combination of Calpain-10 gene polymorphism is associated with metabolic syndrome in type 2 diabetes. Diabetes Res Clin Pract. 2006;73(3):268-75. doi: 10.1016/j.diabres.2006.01.011.
111. Moreno-Luna R, Abrante A, Esteban F, Gonzalez-Moles MA, Delgado-Rodriguez M, Saez ME, et al. Calpain 10 gene and laryngeal cancer: a survival analysis. Head Neck. 2011;33(1):72-6. doi: 10.1002/hed.21404.
112. Hu XQ, Yuan P, Luan RS, Li XL, Liu WH, Feng F, et al. Calpain-10 SNP43 and SNP19 polymorphisms and colorectal cancer: a matched case-control study. Asian Pac J Cancer Prev. 2014;14(11):6673-80.
113. Chan D, Tsoi MY, Liu CD, Chan SH, Law SY, Chan KW, et al. Oncogene GAEC1 regulates CAPN10 expression which predicts survival in esophageal squamous cell carcinoma. World J Gastroenterol. 2013;19(18):2772-80. doi: 10.3748/wjg.v19.i18.2772.
114. Litosh VA, Rochman M, Rymer JK, Porollo A, Kottyan LC, Rothenberg ME. Calpain-14 and its association with eosinophilic esophagitis. J Allergy Clin Immunol. 2017;139(6):1762-71.e7. doi: 10.1016/j.jaci.2016.09.027.