Implications of Calpains in Health and Diseases

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The number of mammalian calpain protease family members has grown as many as 15 till recent count. Although initially described as a cytosolic protease, calpains have now been found in almost all subcellular locations i.e., from mitochondria to endoplasmic reticulum and from caveolae to Golgi bodies. Importantly, some calpains do not possess the 28 kDa regulatory subunit and have only the 80 kDa catalytic subunit. In some instances, the 80 kDa subunit by itself confers the calpain proteolytic activity. Calpains have been shown to be involved in a number of physiological processes such as cell cycle progression, remodeling of cytoskeletal-cell membrane attachments, signal transduction, gene expression and apoptosis. Recent studies have linked calpain deficiencies or its over production with a variety of diseases, such as muscular dystrophies, gastropathy, diabetes, Alzheimer’s and Parkinson’s diseases, atherosclerosis and pulmonary hypertension. Herein, we present a brief overview on some implications of calpains on human health and some diseases.

Keywords: Calpains, Calpastatin, Calcium, Gene expression, Apoptosis, Diseases.

Introduction
Calpains are a family of calcium-dependent cysteine proteases. They are also known as calcium activated neutral protease (CANP) and are found almost in all eukaryotes and also in a few prokaryotes. Calpains have limited proteolytic activity, which modulate both structure and functions of their substrates. They are, therefore, called modulator proteases. In addition, calpains participate in a variety of cellular processes, such as cytoskeleton cleavage and subsequent membrane attachments, reproductive processes, different signal transduction pathways and apoptosis. In human genome, 15 genes – CANP1, CANP2 etc. encode calpain-like protease domain (Table 1). In mammals, the two best characterized members of the calpain family — μ-calpain (calpain 1 or μM Ca\(^{2+}\) requiring calpain) and m-calpain (calpain-2 or mM Ca\(^{2+}\) requiring calpain) are ubiquitously expressed. Both calpains are heterodimers containing an identical 28 kDa small or regulatory subunits and an 80 kDa large or catalytic subunit that shares 55-65% sequence homology between the two proteases.

Several studies using different calpain inhibitors have indicated that calpain activity is required for the cell cycle progression. In the nucleus, μ-calpain plays a key role in the G-1 to S transition. Calpain inhibitors have been shown to completely block the proliferation of WI-38 fibroblasts at the late G-1 stage, suggesting that calpain has potent mitogenic effects. Biochemical and genetic studies have linked calpain deficiencies to a variety of diseases, such as muscular dystrophy, gastropathy and diabetes, whereas calpain overactivation contributes to many pathological consequences, such as myocardial infarction, pulmonary hypertension and neurodegenerative diseases. The acinar cell actin cytoskeleton cleavage by m-calpain during oxidative stress has been considered as the cellular target of the protease in pancreatitis. Oxidative stress-induced generation of free radicals has been implicated in the causation of cataract and compounds that scavenge free radicals can ameliorate the disease progression. An increase in free radical generation could cause inactivation of lens Ca\(^{2+}\)ATPase, leading to Ca\(^{2+}\) accumulation. This increase in Ca\(^{2+}\) can cause activation of calpain-mediated proteolysis in the lens, resulting in lens opacification. It has also been proposed that calpain...
can be used as a marker for the early detection of colorectal adenocarcinoma\(^\text{12}\). This has implications in monitoring colorectal cancer in the early stages of the cancer progression\(^\text{12}\).

**Regulation of calpain with calpastatin**

Regulation of calpain is important for normal cellular homeostasis. The activity of calpain, as an intracellular protease is regulated by various mechanisms and factors such as (i) phospholipid binding, (ii) Acyl CoA binding, (iii) phosphorylation by growth factors and kinases, (iv) intracellular Ca\(^{2+}\) concentration, and (v) autolysis of the N-terminal end of the 80 kDa subunit. Additionally, the activity of calpain is tightly regulated by its endogenous inhibitor calpastatin by forming a calpain-calpastatin complex in the presence of Ca\(^{2+}\). The inhibitory mechanism of calpain by its endogenous inhibitor calpastatin reflects...
Physiological functions of calpains

Over 100 proteins are known to be cleaved by calpains. They can be placed in one of four general categories: (i) cytoskeletal proteins, especially those involved in interaction of cytoskeletal and/plasma membrane, (ii) kinases and phosphatases, (iii) membrane–associated proteins, including some receptors and ion channel proteins, and (iv) some transcription factors such as c-fos, c-jun and p53. Calpains play an important role in normal vascular remodeling. CAPN4−/− fibroblasts, for instance, have no calpain activity and do not generate calpain-specific degradation products of cytoskeletal/membrane attachment proteins, spectrin and talin. This results in abnormal cytoskeleton formation with loss of central stress fibers, which display delayed retraction of membrane protrusions and have a decreased number of focal adhesions. Calpain in reproductive system

Sperm-egg interaction and calpain

Calpains mediate many specific Ca2+-dependent reactions, including cell fusion which has been demonstrated in several somatic mammalian cells. Spermatozoa require absolute Ca2+ for penetration of oocyte and maintain calpain activity. Sperm calpain is associated with the cell fusion process that takes place during penetration of the oocytes.

Sperm-egg interactions elicit a series of cellular events within mammalian eggs, leading to their activation. The initial event is a rapid elevation in intracellular Ca2+ concentration ([Ca2+]i), followed by [Ca2+] oscillations. It has been suggested that initial [Ca2+]i rise trigger completion of the second meiotic division. Calpain plays important role in meiosis of xenopus eggs, starfish oocytes and rat eggs presumably via cytoskeletal remodeling.
Freshly ejaculated mammalian sperm are unable to fertilize mature oocytes unless they go through a process called capacitation. Capacitation occurs in the female reproductive tract and involves reorganization of plasma membrane, an increase in protein tyrosine phosphorylation and hyperpolarization of plasma membrane (PM) potential ($E_m$). During capacitation, there is an increase in the intracellular concentration of $Ca^{2+}$ and cAMP. It is also associated with the appearance of increased motility of the sperm cells. Both capacitation and acrosome reaction require $Ca^{2+}$ influx to activate several indispensable signal pathways. Calpain plays an important role in the turnover of different molecules related to cell adhesion and motility by cleaving many but specific adhesion and cytoskeletal proteins; for example, it cleaves spectrin, but does not alter actin, utropin and filamin-1. In other cells, calpain might have other non-cytoskeletal substrates associated with PM, such as $Ca^{2+}$ channels or receptors. Different membrane domains are established in both acrosome and flagella of mammalian sperm, which display distinct biochemical and physiological functions; however, little is known about the role of the cytoskeleton in the establishment of these membrane domains.

**Polycystic ovary syndrome**

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age which is associated with several heterogeneous clinical features. The most common features of PCOS are irregular menstrual cycles, signs of excess androgen (hirsutism, acne and alopecia) and often obesity. Despite its prevalence, little is known about the etiology of PCOS, but there is increasing evidence for an important genetic involvement. Women affected by PCOS often show abnormalities of glucose metabolism and lipid profile and have an increase risk of type-2 diabetes and cardiovascular disease overtime. There are strong evidences that cardiovascular disease, PCOS and metabolic syndrome share genetic factors. A significant association between calpain-5 (CAPN-5) and calpain-10 (CAPN-10) genes with the clinical characteristics associated with PCOS have now been well established. This genetic association could be of relevance to the clinical management of PCOS patients and the increase of genetic risk to cardiovascular diseases in PCOS women.

**Placental development**

The downstream events that follow m-calpain activation appear to involve inactivation of caspase-3, -8 and -9. Caspase-8 is known to contribute to the fusion of cytotrophoblasts into syncytiotrophoblasts without cell death. Calpain inhibitors can suppress human chorionic gonadotropin $\beta$-subunit (hcg-$\beta$) secretion in placental culture. On the contrary, upregulation of $\mu$-calpain is suggested to have considerable link to recurrent miscarriage. Considering these results, control of calpain activation seems to be a key for proper embryonic development. It has been suggested that calpain can act as a target for prevention and treatment of pre-eclampsia and other maternal embryonic disorders associated with placental cell death.

**Calpain in signal transduction pathways**

Calpain cleavage of PKC produces constitutively active enzyme, suggesting that calpains are involved in a variety of signal transduction processes. In vitro studies have indicated that many of the kinases (e.g. PP60, c-Src), phosphatases (e.g. protein tyrosine phosphatase PTP-IB) and cytoskeletal proteins (e.g., spectrin, talin, filamin, paxillin, vinculin) involved in signal transduction pathways are cleaved by calpains. Calpain cleavage of $\beta$-integrin occurs at several sites in COOH-terminal specifically in regions flanking the conserved NPXY/NXXY motifs of the cytoplasmic domain. These motifs are involved in binding of integrins to the cytoplasmic proteins, talin and filamin. Cleavage of different signaling components thus plays an important role in functioning of different signal transduction pathways in health and diseases.

Several studies have demonstrated that $\mu$-calpain acts as a signal transducer at a site upstream of both Rac1 and RhoA. RhoA activity is dynamic during transmembrane signaling. In the early stages of integrin-mediated cell spreading, activity of RhoA is down regulated, while at later stages, RhoA activity is increased and focal adhesions and stress fibers are formed. Downregulation of RhoA is caused by specific cleavage of RhoA to produce a 20 kDa fragment and that inhibits integrin–induced stress fiber formation and focal adhesions. The cleavage of RhoA by $\mu$-calpain to a 20 kDa fragment is shown to be inhibited by calpeptin, a synthetic inhibitor of calpain, but not by inhibitors of caspases. Glading et al. reported that ERK kinase phosphorylates $m$-calpain on Ser-50 and phosphorylated $m$-calpain is found to be fully active at $Ca^{2+}$ concentration below 1 $\mu$M, indicating that...
phosphorylation helps enable calpain to become active at physiological Ca\(^{2+}\) concentrations. Thus, calpain is activated in a number of signal transduction processes. It seems conceivable that \(\mu\)- and m-calpains are associated with different signaling pathways, depending on the signals that the cells receive.

**Calpain in gene expression and apoptosis**

The concept of involvement of calpains in regulating gene expression is based on their ability to cleave several transcription factors such as c-jun, c-fos and p\(^{53}\), which are expressed as sequence specific activators of apoptosis\(^{37,28}\). Recent evidence suggests that c-Fos, c-Jun and p53 can be degraded via different proteolytic pathways and one of these pathways may involve calpains. It is suggested that proteolytic cleavage of p\(^{53}\) by calpain plays a key role in regulating stability of the transcription factor\(^{38}\). For example, overexpression of calpastatin in NIH 3T3 cells decreases, while introduction of anti-sense calpastatin increases the rate of degradation of p\(^{53}\) in the cells\(^1\). The factors regulating participation of calpain in degradation of transcription factors are currently not clear.

The 80 kDa large along with the 28 kDa small subunits of m-calpain have been shown to be present in the cytosolic side of the ER membrane\(^{29}\). The 80 kDa, but not 28 kDa, subunit of m-calpain is present in the lumen of ER, which confers its catalytic activity\(^ {29}\). The ER has dualistic role in cells as both m-calpain storage centre and a processing organelle for folding and transport of proteins\(^{30}\). The association of calpain with ER places it in the vicinity of a large number of its potential substrates\(^31\). Under ischemia-reperfusion induced increase in [Ca\(^{2+}\)]\(_{i}\), the ER membrane m-calpain activity increases and that cleaves the membrane associated Na\(^+\)/Ca\(^{2+}\) exchanger-1 (NCX-1), which subsequently causes further increase in cytosolic Ca\(^{2+}\) level in the cells\(^{29}\). On the other hand, increase in 80 kDa ER luminal m-calpain cleaves IP\(_3\)R1, the ER Ca\(^{2+}\) release channel, leading to deregulation of ER Ca\(^{2+}\) level due to inability of Ca\(^{2+}\) release from the IP\(_3\) sensitive pool of ER to the cytosol\(^ {32}\), which initiates ER-mediated apoptosis leading to cell death\(^ {32}\).

Mitochondria are well known for their involvement in cellular Ca\(^{2+}\) homeostasis, signaling, apoptosis and cell death\(^ {33-35}\). They sequester considerable amounts of Ca\(^{2+}\) and participate in regulating amplitude and shape of cytosolic Ca\(^{2+}\) transients\(^ {15}\). Excessive Ca\(^{2+}\) accumulation in mitochondria due to inhibition of Na\(^+\)/Ca\(^{2+}\) exchanger can trigger apoptosis and cell death\(^ {35}\). \(\mu\)-Calpain is integrally embedded to the outer surface of inner mitochondrial membrane\(^ {36}\). Calpain-mediated cleavage of apoptosis inducing factor (AIF) and its subsequent release from mitochondria during an increase in mitochondrial membrane permeability transition (MMPT) has been suggested to be an important event in apoptosis\(^ {36}\). Both precursor and mature forms of recombinant AIF have been demonstrated to be cleaved by \(\mu\)-calpain near its amino terminus\(^ {36}\). \(\mu\)-Calpain also cleaves p\(^ {21}\) Bax to the p\(^ {18}\) form, which enhances cell death initiated at the mitochondrial level\(^ {37}\). Thus, \(\mu\)-calpain localized in the inner mitochondrial membrane offers a potential candidate for caspase independent apoptotic pathway\(^{36}\).

Recent studies on overexpression of endogenous calpain inhibitor calpastatin have suggested that calpains are involved in some types of apoptosis\(^ {1,38}\). Anti-IgM induced apoptosis in an immature B-cell line is blocked completely by overexpression of calpastatin, but apoptosis induced by actinomycin D in the same cells is not impeded by calpastatin overexpression\(^ {39}\), clearly indicating that the role of calpain in apoptosis is cell and signal-specific. It has been shown that \(\mu\)-calpain cleaves caspase-7,-8 and -9 to inactivate them\(^ {40}\). On the other hand, m-calpain cleaves procaspase 12 to generate an active caspase, which in turn proteolytically process the loop region of Bcl-xl to change an antiapoptotic molecule into a proapoptotic molecule\(^ {41}\). In some instances, calpain may act as a negative regulator of apoptosis by inactivating upstream caspases. In contrast, Knepper et al\(^ {22}\) have demonstrated that caspases, calpains and proteosome function synergistically to complete apoptosis in senescent neutrophils and that both calpains and proteosome act downstream from the caspases. Whereas apoptosis in human platelets mediated by caspase-9, caspase-3, apoptotic peptidase activating factor-1 (APAF-1) and cytochrome c are regulated by \(\mu\)-calpain\(^1\).

DNA damage is an important initiator of neuronal apoptosis. An important component of DNA damage-induced cell death is the tumor suppressor p\(^ {53}\), which is elevated early and prior to death commitment and p\(^ {53}\) deficiency prevents neuronal death evoked by DNA damage\(^ {42,43}\). p\(^ {53}\) has also an absolute requirement for activation of the distal death effector pathway involving Bax translocation,
cytochrome c release and caspase activation.\textsuperscript{43,44} The signal(s) which imparts p\textsuperscript{57} stability/activity is not clearly understood. It is likely that multiple signals, such as NF-\textsuperscript{κB} and phosphatidyl inositol-3-kinase relating the calpain regulates p\textsuperscript{53} activation and it will be interesting to explore how these signals coordinate the p\textsuperscript{53} pathway.\textsuperscript{45,53}

Pathological implications of calpains

Ischemia reperfusion injury

Under pathological conditions in which Ca\textsuperscript{2+} homeostasis is lost, as occurs during ischemia-reperfusion, the tight control of the calpain is perturbed, causing its aberrant activation.\textsuperscript{46} Using specific substrate and inhibitors of calpains in different models of ischaemia-reperfusion, it has been suggested that activation of the enzyme occurs prior to membrane damage. This indicates that activation of calpain is an initial event and that eventually plays an important role in reperfusion-induced contractile dysfunction and cell death due to proteolysis of a wide variety of proteins.\textsuperscript{46,47} Oxidative processes have the ability to influence \(\mu\)-calpain activity. It has been demonstrated that cysteine present in the active site of \(\mu\)-calpain forms a disulfide bond, which could be a part of the unique oxidation mechanism for \(\mu\)-calpain activation during ischemia-reperfusion induced oxidative stress.\textsuperscript{48}

During reperfusion, calpains hydrolyze proteins from the sarcolemma and the cytoskeleton, including \(\alpha\)-fodrin and ankyrin. Alpha fodrin forms the backbone of the membrane cytoskeleton. Its degradation correlates with increased fragility of the membrane, thereby reducing the tolerance of the sarcolemma to acute cell swelling and contractile activation during reperfusion.\textsuperscript{46} Ankyrin has a central domain that binds to \(\alpha\)-fodrin and an N-terminal domain that interacts with several receptors, ion channels and the \(\alpha\) subunit of Na\textsuperscript{+}/K\textsuperscript{+} ATPase.\textsuperscript{46} Binding to ankyrin connects the Na\textsuperscript{+}/K\textsuperscript{+} ATPase to the fodrin based membrane cytoskeleton and determines its specific localization in the membrane and its function.\textsuperscript{46} During reperfusion, calpain degradation of fodrin and ankyrin not only cause sarcolemmal fragility, but also detach the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase from its anchorage to the fodrin based membrane skeleton, which results in impairment of cytosolic Na\textsuperscript{+} concentration and subsequently Ca\textsuperscript{2+} influx via reverse mode Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger leading to further activation of calpain.\textsuperscript{46} The activated calpain, for example, by cleaving Bid into an active form induces the release of cytochrome c and other proapoptotic factors from mitochondria and endoplasmic reticulum, respectively.\textsuperscript{47}

Neurodegeneration

Neuronal cell death after traumatic brain injury, Alzheimer’s disease (AD) and ischemic stroke may, in part, be mediated through the ER stress-associated unfolded protein responses (UPR). UPR results in induction of molecular chaperone GRP-78 and the ER resident caspase-12, whose activation has been suggested to be mediated by calpain.\textsuperscript{49} Calpain activation plays an important role in amyloid precursor protein processing and associated plaque formation by regulating amyloid precursor protein (APP)-cleaving enzyme-1 (BACE 1) with an increase in \(\beta\)-amyloid (A\(\beta\)) production.\textsuperscript{50} Prolonged activation of glutamate receptors lead to excitotoxic damage of brain. Several processes such as ROS production and activation of calpains contribute to glutamate-induced brain damage. NADPH oxidase (NOX) is stimulated by glutamate in neurons. Neuronal damage involves ROS production by NOX which, in turn, contributes to calpain activation.\textsuperscript{51,52}

Neurodegenerative disorders are becoming increasingly prevalent. Disorders such as cerebral ischemia, AD, Parkinson’s disease (PD), Huntington’s disease (HD), multiple sclerosis and amyotrophic lateral sclerosis (ALS) manifest in various stages of adulthood and involve neuronal dysfunction and ultimate death of neurons. While the etiology underlying each disorder varies, the pathologic mechanisms, at least in part, converge on impaired intracellular calcium homeostasis, leading to activation of calpains. Accordingly, calpain inhibition was determined to be neuroprotective in most models of neurodegeneration.\textsuperscript{53}

Alzheimer’s disease (AD)

Progressive dementia accompanied by neuronal death is the clinical signs of AD. The underlying neuronal histopathology consists of neurifibrillary tangles (NFTs) containing hyperphosphorylated aggregations of the microtubule associated protein tau and senile plaques made up of aggregated amyloid \(\beta\)-protein (A\(\beta\)) fibrils. A\(\beta\) is produced by the cleavage of integral membrane protein amyloid precursor protein (APP) by the action of \(\beta\) and \(\gamma\) secretase. Under physiological conditions, APP produces an \(\alpha\)-secretase, a product of unknown function. Mounting evidence has demonstrated that increased levels of soluble A\(\beta\) are
the primary cause of neuronal pathology in AD\textsuperscript{30,54}. Higher concentration of soluble Aβ results in elevation of intracellular Ca\textsuperscript{2+} levels via NMDA receptor activation. Calcium dysregulation, in turn, leads to increased calpain activation, which has been shown in post-mortem of human AD patient’s brain.

Calpain has been hypothesized as a critical candidate α-secretase responsible for the normal processing of APP. The underlying role of calpain in AD pathology stems from the following: (i) Initially, a subclinical decrease in calpain activity alters APP processing toward the production of Aβ, and (ii) the subsequent rise in Aβ concentration could then result in NMDA-receptor activation, leading to hyperactivation of calpain and the negative consequences unmasked by persistent calpain activity. Calpain inhibitors can be used to provide therapeutic benefits for AD patients as they have been shown to improve memory and synaptic transmission in mouse model of AD\textsuperscript{30,54}.

Parkinson’s disease
Parkinson’s disease (PD) is associated with degeneration of dopaminergic neurons and inflammatory responses in the mid brain. Studies in rodent and cell culture models of PD suggest that treatment with calpain inhibitors (\textit{i.e.} calpeptin, MDL-28170) can prevent neuronal death and restore function\textsuperscript{55}. There is an increase in calpain expression in microglia, astrocytes and neurons in the spinal cord of 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine (MPTP)-treated (treatment mimics human PD model) mice as compared to controls. Neurons in the spinal cord and mid brain show apoptotic characteristics\textsuperscript{56}. At depleted levels of ATP, calpain degrades procaspase-9 to activate caspase-3 to initiate caspase-independent cell death which in turn starts dopaminergic neurodegeneration process\textsuperscript{57}.

Dysfunctional mitochondria increase cytosolic Ca\textsuperscript{2+}, thereby inducing calpain activation. Interestingly, its inhibition significantly attenuates the accumulation of α-secretase oligomers and contributes to an increase of insoluble α-synculin aggregates, known to be cytoprotective\textsuperscript{58}. Thus, calpain has putative role and calpain inhibitors provide therapeutic tool in PD.

Huntington’s disease
Huntington’s 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. Thus, to reduce toxicity, processing is then required for the degradation of these fragments\textsuperscript{59}. Proteolytic processing of mutant huntingtin (mHtt) is critical for mHtt toxicity and disease progression. Cleavage of Htt in human HD tissue is mediated partly by the calpains\textsuperscript{60}. μ-, m- and -5, -7 and -10 Calpains have increased levels and are activated in HD tissue in transgenic mouse model\textsuperscript{61}.

Ageing and meningiomas
Oxidative stress has long been implicated in ageing and such related processes. Ageing may also contribute to the occurrence of meningiomas, which is associated with wide set of tumors arising from meningines (the membranous layers surrounding the CNS). Recently, it has been reported that aside from neurofibromatosis type-2 (NF-2) gene mutations, the calpain-dependent proteolysis of the NF-2 gene product merlin might be closely related to the development of certain NF-2 associated tumors, which is more frequent in aged persons\textsuperscript{52}. Production of free radicals with ageing has been suggested to be one of the causes of calpain activation. During oxidative stress, μ-calpain activation associated with merlin proteolysis has been observed. The proteolyzed merlin fragments were translocated to the perinuclear cytoplasm or into the nucleus. It has been suggested that oxidative stress-induced μ-calpain activation causes proteolysis of merlin and vitiates cell adhesion and/or contact inhibition of messengial cells\textsuperscript{52,63}.

Diabetes and cardiac diseases
Diabetes mellitus is a major risk factor for cardiovascular diseases such as atherosclerosis and thrombosis. A number of studies support the concept that platelets contribute to the pathogenesis and progression of vascular complications of diabetes. μ-Calpain is expressed in platelets and is involved in physiological platelet activation. However, the inappropriate activation of calpain alters platelet function, partially degrades a spectrum of proteins and results in hyperaggregability. Along with that, changes in the activity of calpain have been shown to be associated with diabetes related pathways. Polymorphism of calpain genes have been shown to be associated with the development of type-2
diabetes\textsuperscript{64}, but their relevance to the diabetes related vascular complications is not fully clear.

Calpain-10 is resident in mitochondria and is implicated in type 2 diabetes. Glucose-induced loss of calpain-10 \textit{in vivo} results in apoptosis of renal cells and subsequently kidney failure through accumulation of mitochondrial calpain-10 substrates and mitochondrial dysfunction\textsuperscript{65}. Short-term exposure of mouse pancreatic islets to calpain inhibitors enhances glucose-induced insulin secretion, but reduces insulin-stimulated glucose uptake into adipocytes and skeletal muscle and glycogen synthesis rates in muscle\textsuperscript{66}. Genetic variants of calpain-10 gene are associated with elevated free fatty acids and insulin resistance. \textit{In vitro} studies have shown that free fatty acids activate some isoforms of PKC, which results in hyperphosphorylation of insulin receptors, leading to reduction in the kinase activity of insulin receptors and thus enhancing insulin resistance. Therefore, downregulation of some isoform-specific PKC activity appears to be an important factor for maintaining proper phosphorylation level of insulin receptors and subsequently insulin secretion\textsuperscript{67}.

Cardiac diseases, for example, myocardial infarction (MI) activates intracellular proteases that often trigger programmed cell death and contributes to maladaptive changes in myocardial structure and function\textsuperscript{68}. Studies have indicated that calpain contribute to MI-induced alterations in myocardial structure and function and that it could be a potential target in treating MI patients\textsuperscript{68}. \(\mu\)-Calpain has been suggested to play an important role in endothelial dysfunction induced by hyperglycemic activation of some protein kinase C sub-types\textsuperscript{69}. \(\mu\)-Calpain activation contributes to cardiomyocyte apoptosis induced by hyperglycemia. Targeted disruption of calpain reduces myocardial hypertrophy and fibrosis in mouse model of type-1 diabetes. Thus, calpain inhibition may provide a potential novel therapeutic strategy for reversing diabetic cardiomyopathy\textsuperscript{70}.

Glucocorticoid therapy has been demonstrated to preserve calpastatin level in myocardium and that may be correlated with a decrease in troponin I degradation\textsuperscript{71}. This suggests a possible role of calpain inhibition in preserving cardiopulmonary function, following ischemia and reperfusion. \textit{In vivo} calpain activity is tightly regulated by intracellular calcium and also by calpastatin levels. Calcium overload associated with ischemia activates calpain and reduces calpastatin levels. Administration of calpain inhibitors minimizes infarct size in ischemic hearts and enhances recovery after reperfusion\textsuperscript{72}. \(\mu\)-Calpain, cathepsins and matrix metalloprotease-9 act in concert to elicit ischemia-reperfusion induced injury in the heart\textsuperscript{73}. Therefore, these proteases appear to play important roles in ischemia-reperfusion induced injury to the heart\textsuperscript{73}.

### Pulmonary hypertension

Pulmonary artery hypertension (PAH) is a life-threatening disease caused by a variety of pathophysiological conditions, such as asthma, acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary disease (COPD). One of the important features of pulmonary hypertension is pulmonary vascular remodeling\textsuperscript{74}. Several growth factors, including epidermal growth factor (EGF), platelet derived growth factor (PDGF) and transforming growth factor-\(\beta\) (TGF-\(\beta\)) are involved in pulmonary vascular remodeling during pulmonary hypertension\textsuperscript{74}. However, our current knowledge of the downstream signaling cascades provides calpain as an interesting candidate for therapeutic target\textsuperscript{75}. In two rodent models of pulmonary hypertension using rats treated with calpain inhibitors has resulted in prevention of increased right ventricular systolic pressure, right ventricular hypertrophy, as well as decrease in collagen deposition and thickening of pulmonary arterioles in models of hypertension\textsuperscript{75}.

Pulmonary hypertension is thought to result from a combination of elevated endothelin-1 levels and direct ischemia reperfusion injury to the pulmonary endothelium, resulting in a marked decrease in endogenous nitric oxide production\textsuperscript{76,77}. A complex regulatory interaction exists between endothelin-1 and nitric oxide and that plays a major role in regulating pulmonary vascular resistance. Blockade of the endothelin-1 signaling pathway can prevent post cardiopulmonary bypass (CPB) pulmonary hypertension despite diminished nitric oxide generation\textsuperscript{78}. Decreased endothelin-1 levels in calpain- inhibitor treated animals are associated with lower pulmonary vascular resistance after CPB and deep hypothermic circulatory arrest. Similar to glucocorticoids, calpain inhibition decreases endothelin-1 levels, indicating a direct regulatory role of calpain on endothelin-1 level\textsuperscript{78}.

Calpain inhibition also preserves endothelial nitric oxide synthase (eNOS) levels, which helps to further attenuate post-reperfusion pulmonary hypertension.
Calpain inhibition has been shown to decrease leukocyte endothelial interactions through endothelial-NOS (eNOS)–dependent mechanism in mesenteric endothelial cells. Calpain may diminish eNOS activity by disrupting the association between the subunits of HSP-90, an important regulator of NOS activity. HSP-90 activates eNOS and calpain inhibition prevents HSP-90 degradation by calpain. Therefore, maintenance of HSP-90 by calpain inhibition represents a mechanism to preserve eNOS activity and counter vasoconstriction induced by endothelin-1.

Microbial infection

Macrophages have long been known as a crucial component of host defence against microbial infections. Leishmania deregulates the expression of several non-coding RNAs (ncRNAs e.g., AluRNA, B1RNA and signal recognition particle RNA) in macrophages thereby establish its infection in the phagolysosomes of these cells. The ncRNA genes are expressed by Leishmania infection in macrophages and require polymerase III for their expression. Leishmania promastigotes through their surface protease (gp63) activate the protease activated receptor-1 (PAR-1) in the macrophages, which play a crucial role in increasing [Ca2+]i, leading to activation of μ-calpain. The μ-calpain then degrades transcription factor IIIC110 and that in turn inhibits the expression of selected ncRNAs. Avirulent Leishmania not expressing surface gp63 fails to downregulate ncRNAs in the exposed macrophages. 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.

The calpain inhibitor MDL28170 is found to be capable of significantly reducing the viability of blood stream trypomastigotes in Chagas disease patients. Macrophages experimentally infected with Trypanasoma cruzi that are treated with the calpain inhibitor present a significant reduction in the percentage of infection. Thus, calpain inhibitor may prove useful to treat Chagas disease.

Macrophages exposed to Streptococcus pyogenes exhibit extensive cytoplasmic vacuolization, cellular and organelle swelling and rupture of the plasma membrane typical of oncosis. The cytotoxic effect of S. pyogenes on macrophages is mediated via activation of an inflammatory programmed cell death pathway, which involves loss of mitochondrial transmembrane potential (∆Ψm), opening of MMPT with subsequent activation of μ-calpain that leads to initiation of cell death processes.

Future directions

In general, both the 80 kDa catalytic and the 28 kDa regulatory subunits are required for calpain and to exhibit its activity. However, in some subcellular locations, for example, in the lumen of endoplasmic reticulum, only the 80 kDa subunit of calpain is present and that by itself confers its proteolytic activity. To explain this fact and to further gain an insight into the basic mechanism for the requirements of both the large and the small subunits of calpains for its activation and regulation in different cells and subcellular locations, more research linking biochemistry, molecular biology and structural biology is needed.

Given the potency of calpain, its activity must be tightly regulated. One of the mechanisms for calpain activation may be due to be concerted action linking other proteases, such as serine proteases, caspases and matrix metalloprotease. In many pathophysiological conditions, for example, ischemic injury to the heart, activation of matrix metalloprotease-9 (MMP-9) has been shown to be an initial event for m-calpain activation and that, if persistent, eventually disrupts the entire cardiac machinery. More research is needed to better understand the concerted roles of serine proteases, matrix metalloproteases, calpains and caspases in a variety of physiological and pathological phenomena in health and diseases.

Although some information based on biochemical and genetic studies have indicated links between some forms of calpain overproduction/deficiency in the etiology of some diseases. However, studies regarding the molecular targets identifying the mechanism(s) associated with the enzyme activation/inactivation underlying a variety of diseases are scanty and needs further exploration.

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Inconsistency in the text regarding references and citations, making it difficult to accurately extract the information. Further analysis or contextual information is required to provide a coherent representation of the text.
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