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The therapeutic potential of the calpain family: new aspects

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The calpain family is a group of cysteine proteases unique in their dependency on calcium to attain functionally active forms. Calpains are involved in a wide range of cellular calcium-regulated functions, including signal transduction, cell proliferation and differentiation, and apoptosis. Moreover, altered calpain activity has been observed in several human diseases. Specific calpain inhibitors hold promise for the treatment of neuromuscular and neurodegenerative diseases in which calpains have been shown to be upregulated (e.g. Parkinson's disease and Duchenne muscular dystrophy). Conversely, calpain activators could be a useful approach for those diseases where reduced calpain activity has been observed, such as type 2 diabetes or metabolic syndrome.

The calpain family comprises a heterogeneous group of cysteine proteases with a broad expression pattern that includes multiple isoforms that are both ubiquitous and tissue specific. Calpains are involved in a variety of calcium-regulated cellular processes, such as signal transduction, cell proliferation and differentiation, apoptosis, membrane fusion and platelet activation. This wide variety of physiological functions in which calpains are involved determines their important pathological role in a host of human diseases (Table 1). Deregulation of their activity has been involved in neurological disorders (Alzheimer's, Huntington's and Parkinson's diseases and multiple sclerosis), ischemic and traumatic brain injury, cancer, muscular dystrophy, cataracts, strokes and diabetes.

In this review, we have focused initially on the protein structure of the different calpains, as well as the human diseases linked to each of them. In the second section, we have highlighted the therapeutic potential of calpain inhibitors and activators that have been gleaned from *in vitro* and *in vivo* investigations.

The calpain family

The common structural and functional element for this protein family is the conserved proteolytic domain II, which bears the catalytic triad sequence Cys-His-Arg (Figure 1). The location of these aminoacids dictates that they will only configure an active catalytic pocket when calcium is present. The domain responsible for Ca^{2+}-binding is the fourth domain, with its five sets of EF-hand sequences. The EF-hand domain consists of a 12-residue loop flanked on both sides by a 12-residue α-helical domain. Therefore, calpains that do not possess domain IV were initially thought to be non-calcium-dependent. However, the observation that there was limited structural changes to domain IV in the presence of Ca^{2+} in those calpains requiring Ca^{2+} to become activated led researchers to identify domains I to III as functional Ca^{2+}-binding domains, being the Ca^{2+} ions bind to the catalytic domain a crucial step for the formation of the active molecule [1–3]. Domain IV is frequently referred to as a calmodulin-like domain, owing to its structural homology with calmodulin. To explore the relationships between each calpain family member and human diseases, proteins included in this family have been subdivided into three different groups (classical, atypical and other EF-calpains) depending on the protein structure and the presence or absence of independent regulatory subunits.

The classical calpains

The classical μ-calpain (calpain 1) and m-calpain (calpain 2) are the most studied members of the family. Both calpains are proteins of ~80 kDa, each of them acting as heterodimers interacting with a small regulatory subunit of 30 kDa. This regulatory subunit possesses an additional set of five EF-hand motifs (domain VI) that makes contact with domain IV of calpain 1 and calpain 2 to form the functional protein [4]. The regulatory subunit is encoded by

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TABLE 1

The calpain family

Calpain	Other names	Chromosome	Expression	Isoforms	Crystal structure	KO mouse phenotype	Known targets	Diseases
CAPN1	μ-calpain	11q13.1	Ubiquitous	-	Catalytic domain	Hematopoietic homeostasis alterations (MGI:88263)[a]	Multiples[b]	Huntington's disease, cataracts, stroke, muscular dystrophy, traumatic brain injury, spinal cord injury, alzheimer, cancer, multiple sclerosis, Lou Gehrig's disease, osteopenia
CAPN2	m-calpain	1q42.11	Ubiquitous	-	Complete	-	Multiples[c]	Cataracts, muscular dystrophy, stroke, spinal cord injury, traumatic brain injury, Alzheimer, Parkinson, atherosclerosis, multiple sclerosis, Lou Gehrig's disease, cancer, psoriasis
CAPN3	p94, nCL-1	15q15.1	Skeletal muscle, lens, retina	Yes (8)	-	Muscular dystrophy. Transmission distortion (MGI:107437)	Calpastatin	Limb girdle muscular dystrophy 2A (LGMD2A), cataracts
CAPN4	CAPNS1, CSS1	19q13.12	Ubiquitous	-	Domain VI	Embryonic lethality. Cardiovascular and erythropoyesis alterations (MGI:88266)	-	-
CAPN5	hTRA-3, nCL-3	11q13.5	Ubiquitous (high in colon, small intestine and testis)	-	-	Usually normal, occasionally reduced body weight (one allele). Embryonic lethality (another allele) (MGI:1100859)	Huntingtin	Huntington's disease polycystic ovarian syndrome, metabolic syndrome
CAPN6	CAPNX	Xq23	Placenta	-	-	-	-	-
CAPN7	PalBH	3p25.1	Ubiquitous	-	-	Postnatal lethality Decreased body weight (MGI:1338030)	Huntingtin	Huntington's disease
CAPN8	nCL-2	1q32-q41	Stomach mucosa	Yes (2)	-	Unknown (MGI:2181366)	-	-
CAPN9	nCL-4	1q42.2	Digestive track	Yes (2)	Catalytic domain	-	-	Gastric cancer
CAPN10	CAPN8	2q37.3	Ubiquitous and tissue-specific	Yes (8)	-	-	Huntingtin Crystallin(?)	Huntington's disease, cataracts (?), diabetes mellitus, atherosclerosis, metabolic syndrome
CAPN11	-	6p21.1	Testis	-	-	-	-	-
CAPN12	-	19q13.2	Ubiquitous (high in hair follicle)	-	-	-	-	Alzheimer (?)
CAPN13	-	2p23.1	Testis and lung	-	-	-	-	-
CAPN14	-	2p23.1	Ubiquitous	Yes (2)	-	-	-	-
CAPN15	Sol H	16p13.3	Ubiquitous	-	-	-	-	CATM (hereditary cataract with microphthalmia) (?)
CAPNS2	CAPN14 CSS2	16q12.2	Lens	-	Domain VI	-	-	-

[a] MGI: Mouse Genome Informatics database.

[b] Filamin, Talin, Pyk2, FAK, GFA, PKCα, Spectrin, Cadherin, Tau, Bcl-2 family, Caspase 12, Fodrin, MAP-2, Huntingtin, IκBα, Integrin, IGFBPs, ICA512, AQP2, XIAP, RhoA, Preselin, PTP-1B, Cortactin, Caldesmon, Calponin.

[c] Crystallin, Filamin A, Tau, Tali, Paxilin, IκBα, Fodrin, Pyk2, PKCα, δ, GAP-43, FAK, pp60src, p53, Cyclin E, Preselinin, Cortactin, Caldesmon, Calponin, Catenin, RNase L, CaMK.

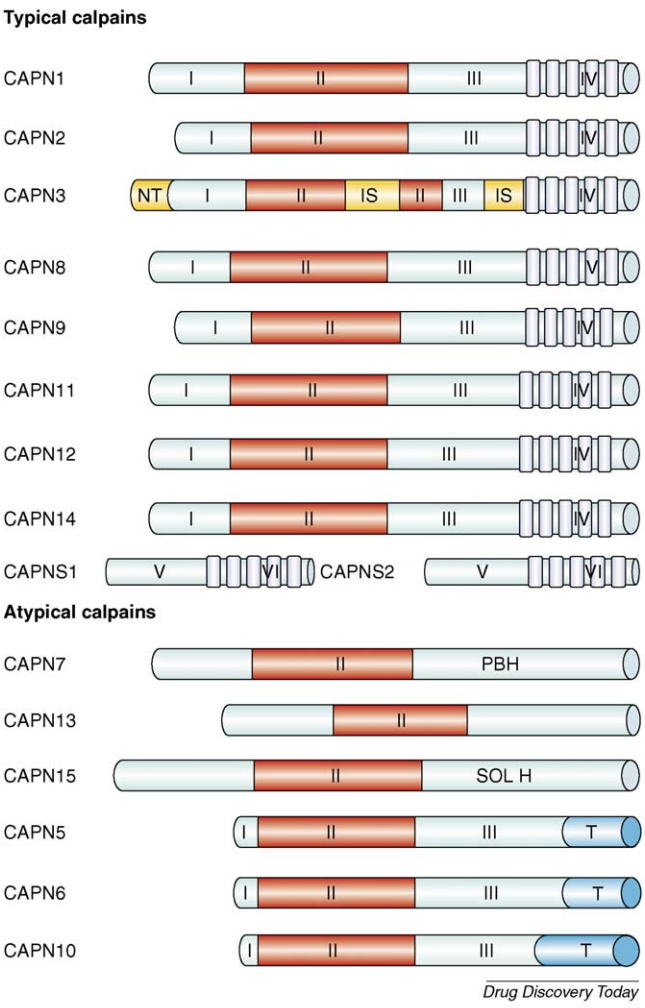

FIGURE 1

Schematic representation of the calpain family members. The human calpain family has a common proteolytic domain II. This domain bears the catalytic triad sequence Cys-His-Arg, except for CAPN6, in which Cys residue is replaced by Lys, probably abrogating its catalytic activity. Typical calpains are characterized by the presence of five sets of calcium-binding domains denominated EF-hand (domains IV and VI). In the atypical calpain subfamily, these EF-hand domains are not present and homology with typical domain is lost beyond domain II. Abbreviations: NT, nuclear translocation domain; IS, insertion sequences; PBH, Pal B homologous domain; SOL H, domain homologue to SOL protein from *Drosophila melanogaster*. Adapted, with permission, from Ref. [20].

the *CAPNS1* (calpain 4) gene, although the *CAPNS2* gene also encodes a small polypeptide, whose function is unclear, with a homology of ~63% with the product of the *CAPNS1* gene [5].

In vitro studies showed that calpain 1 and calpain 2 differ in the calcium concentration that they each require to become activated: μ-calpain requires a Ca^{2+} concentration in the micromolar range, whereas m-calpain calcium requires it in the millimolar range. The Ca^{2+} levels needed to achieve the functional active forms *in vivo* are reduced by binding to phospholipid membrane [5].

Alzheimer's disease (AD) is one of the conditions with which classical calpains have been most commonly associated. These calpains are thought to be involved in the molecular events that lead to the hyperphosphorylation of tau, the main protein found in the neurofibrillary tangles (NFTs) observed in the brains of those patients suffering from AD [6]. In addition, the proteolytic action of calpains over tau and other neurofilament proteins is related to the necrotic cell death observed in AD [7]. One of these cytoeskeletal proteins cleaved by calpains is αII-spectrin, which is very abundant in neurons; the action of calpains over this protein generates unique breakdown products (BDPs) used as markers of calpain activity *in vivo*. Increased levels of spectrin BDPs have been observed in hypoxic and ischemic injury of the brain, heart and kidney, allowing the identification of calpain activation as an early and causal event in the degeneration of these cellular types [8].

Another neurological disorder linked to calpain activity is Huntington's disease (HD) – calpains, and also proteosome and caspases, have been shown to cleave the htt protein at multiple sites, generating toxic protein fragments that finally lead to neuronal loss [9]. In Parkinson's disease (PD), α-synuclein, the major component of fibrillary neuronal inclusions called Lewy bodies (LBs), is also a substrate for calpains [10]. In addition, overexpression of m-calpain has been detected in brain of patients with PD [11]. The role of calpain activity in demyelinating diseases is mediated by the cleavage of myelin, as well as other cytoskeletal proteins, which are also important pathological mechanisms in brain and spinal cord traumatic injury [12].

Increased calpain activity has also been detected in Duchenne and Becker, two muscle-wasting neuromuscular disorders related to the absence of dystrophin, leading to increased Ca^{2+} levels in the muscle. This increase leads to calpain overactivation, which contributes to muscle wasting by the cleaveage of myofibrilar or cytoskeletal proteins, such as titin, nebulin, troponins and tropomyosin, but have little or no effect on myosin and actin, the two major structural proteins in the skeletal muscle [5].

The proteolytic action of calpain 2 on lens crystallin proteins causes cataracts in humans. Although other calpains, such as calpain 10, have been located in the human lens, the increase in opacity of these ocular structures is mainly caused by the upregulation of m-calpain [13].

The disorders mentioned above are all accompanied by increases in intracellular calcium levels, often related to glutamate-mediated excitotoxicity, which leads to enhanced calpain activity [14]. Furthermore, altered calpain activity has also been observed in several types of cancer, including breast, ovarian, prostate, skin, brain or colorectal carcinomas, where calpain 1 and calpain 2 have been shown to be upregulated. Calpains can proteolytically degrade several substrates involved in cell adhesion, such as focal adhesion kinase (FAK), β-catenin, integrin or merlin proteins in addition to the product of several different oncogenes (c-Fos, c-Jun, v-Src, v-Jun, v-Myc, k-Ras and v-Fos) and tumour suppressor genes (p53) [5]. Several studies have provided evidence that μ-calpains and m-calpains are regulators of osteoclastogenesis, a process mediated via the activation of nuclear factor (NF) κB, a transcription factor involved in cell proliferation [15].

In addition to cleavage of cytoskeletal proteins that lead to programmed cell death, calpains are both positive and negative regulators of classical apoptosis. The pro-apoptotic action of calpains is exerted by proteolytic cleavage of caspases 3 and 12 and of Bax and Bid proteins. On the other hand, proteolysis of p53 and

caspases 7 and 9 inhibit apoptosis [5]. Apoptosis-related disorders in which calpains have been involved include ischemic injury of the brain, the heart and the kidneys [16].

Other EF-hand calpains

Calpains 3, 8, 9, 11, 12 and 13 have a similar structure to the classical calpains. However, they do not seem to form functional heterodimers with the regulatory subunit and only calpain 12 is ubiquitously distributed [17].

The skeletal muscle-specific calpain 3 has the peculiarity of bearing a nuclear translocation-like domain. This calpain is involved in limb girdle muscular dystrophy type 2A (LGMD2A), a recessive neuromuscular disorder characterized by a progressive symmetrical atrophy and weakness of the proximal limb muscles [18]. In rodents, two alternatively spliced isoforms of calpain 3 (Lp82 and Lp85) have been shown to be lens-specific and are involved in cataractogenesis in mice [19]. However, these proteins are not present in humans, so m-calpain is thought to be the main calpain involved in cataract formation as previously mentioned.

Calpain 8 is a stomach smooth-muscle-specific calpain [20]. Interestingly, calpain 8 gene is alternatively spliced to produce a protein isoform that lacks two-thirds of domain III and all of domain IV. The role of calpain 8 in human disease has not yet been established.

Calpain 9 is also tissue-specific, with an expression pattern restricted to the digestive tract [21]. This gene has been reported to be downregulated in gastric cancer [22]. The same situation has been recently reported to occur in the hypertensive target organ of the heart and kidney [23]. The calpain 9 protein is perhaps the most similar to the classical calpain in terms of its enzymatic properties and active form, because calpain 9 seems to interact with a 30kb subunit to achieve its functional conformation [24].

Calpain 12 is highly expressed in mouse hair follicles, although different expression levels have been detected in almost all human tissues [17]. The protein exists as three isoforms, two of which, including the most frequent isoform, lack the calmodulin-like domain (domain IV). The calpain 12 contained within the amyloid plaques of the Tg2576 mouse model of Alzheimer's disease have been modified by nitration, but the impact of such a modification in the pathogenesis of Alzheimer's disease remains unexplored [25].

The last proteins of this group, calpain 11 and calpain 13, are mainly detected in testis, although calpain 13 has also been detected in the lung [26,27]. Their functions and their eventual role in human diseases remain unexplored.

Atypical calpains

This subgroup of calpains includes calpains 5, 6, 7, 10, 13 and 15 and is characterized by the absence of the penta-EF-hand domain. The calpains of this subfamily can be further classified according to the protein organization beyond the proteolytic domain II and its homology with the *Caenorhabditis elegans* TRA-3 (TRAnsformer: XX animals transformed into males) calpain.

Calpain 7 and calpain 15 are the more divergent members of the family because their homology with classical calpains is restricted to domain II. Calpain 7 is characterized by the presence of a large N-terminal domain and a PalB-homologous (PBH) C-terminal domain, so called owing to its similarity with the *Aspergillus nidulans* PalB protease [28]. The levels of this ubiquitous protein, along with other calpain family members, such as calpain 1, calpain 5 and calpain 10, are increased in Huntington's disease target-tissue culture and transgenic mouse models, suggesting that they might contribute to this neurological pathology [29].

Calpain 15 (also referred to as Sol H) is the vertebrate homologue of the calpain SOL from *Drosophila melanogaster* optic lobes and contains zinc finger-like motifs in the N-terminal region [30]. Calpain 15 is expressed at low levels in most tissues, except in lung and testis, where it has its highest expression rate. Although the genomic region that contains the calpain 15 gene has been implicated in a chromosomal translocation in a family affected by hereditary cataract with microphthalmia (CATM), its role in this disease has not yet been fully examined [31].

The calpain 5, 6 and 10 proteins retain domain III, but it is followed by the T-domain, a homologue of the one of *C. elegans* TRA-3 protein, the nematode orthologue of calpain 5 [32].

The calpain 6 gene is located at Xq23 in humans, being the only calpain encoded on a sex chromosome. Its expression pattern is mainly restricted to the placenta, with low expression levels in all adult tissues [32]. The calpain 6 protein lacks the cysteine residue of the catalytic core (domain II), which is replaced by a lysine. For this reason, this protein is not thought to be a functional protease. Calpain 6 has been shown to be upregulated during early myoblast differentiation in cell culture corresponding to upregulation of MyoD, supporting the hypothesis that calpain 6 is a downstream target of MyoD [33]. This calpain is also overexpressed in uterine leiomyomas, the most common tumour of the genitourinary system in women [34]. However, downregulation by >50% of calpain 6 (and calpain 9) has been reported to occur in Dahl salt-sensitive rat hearts during cardiac hypertrophy [23].

Of the atypical calpains, calpain 10 is, perhaps, the most extensively studied owing to the interest and expectation stemming from the first positional cloning of a gene for a multifactorial disorder, type 2 diabetes mellitus (T2DM) [35]. In this study, the common G allele at the UCSNP-43 polymorphism and the haplotype combination 112–121 (that comprises UCSNP-43, -19 and -63 polymorphisms at calpain 10 locus) were associated with an increased risk of diabetes. Since then, the role of calpain 10 has been examined in different ethnic groups with variable results. A meta analysis performed by Weedon *et al.* [36] confirmed the association of CAPN10 with T2DM.

Several continuous traits related to T2DM and cardiovascular diseases have been investigated at the calpain 10 locus. The association of calpain 10 gene variants with insulin levels is consistent with its regulatory role in pancreatic β-cell insulin secretion and with apoptotic mechanisms in these pancreatic cells that also involves the type 2 ryanodine receptor (RyR2) [37,38]. Cholesterol levels have been also associated with the calpain 10 gene in a combined sample of T2DM patients and control population from Japan and in women affected by polycystic ovary syndrome (PCOS), a common endocrine disorder in women of a reproductive age, characterized by hyperandrogenemia, chronic anovulation and increased prevalence of cardiovascular related disorders [39–42]. Recently, an association of calpain 10 with metabolic syndrome in type 2 diabetes has been described in Korean population [43].

As previously mentioned, the calpain 5 protein is the human orthologue of the nematode *C. elegans* TRA-3 protein [32]. TRA-3 is

involved in the sexual determination of the worm through the proteolytic cleavage of the cytoplasmic end of the TRA-2 transmembrane receptor, which is delivered to the nucleus where it enhances the feminizing activity of the transcription factor TRA-1 [44]. In humans, calpain 5 is ubiquitously distributed with the highest levels in the brain, lungs, liver and testis [45]. Its broad expression pattern suggested that it has a more general function in humans than in its nematode orthologue. In this way, calpain 5 gene variants have been associated with PCOS and several features of metabolic syndrome, including obesity, hypertension and dyslipidemia [46,47].

Potential therapeutic value of calpain inhibitors and activators

Calpain inhibitors

As reviewed in previous sections, the upregulation of several members of the calpain family is involved in a diverse range of biological processes and diseases, which is the reason why this family of proteases has important therapeutic potential. A huge effort has been made in the field of research to develop a means of identifying selective calpain inhibitors because many of them also inhibit other cysteine proteases, serine proteases and even the proteosome. *In vivo*, the calpain activity is controlled by calpastatin, a protein encoded by the calpastatin gene; this peptide is the only specific inhibitor of the calpain family discovered to date. The specificity of calpastatin is determined by the simultaneous binding of three calpastatin subdomains to domains II, IV and VI of the heterodimeric calpains [48].

Synthetic calpain inhibitors can be subdivided in two groups: peptidic (see supplementary material, Box 1) and non-peptidic inhibitors (see supplementary material, Box 2). The former can be also subdivided into reversible (peptidyl aldehydes and peptidyl α-ketoamides) and irreversible inhibitors (peptidyl epoxides), but they all share the same mechanism of action in that they form a covalent bond between the thiol group (-SH) of the active site Cys and an electrophilic centre of the inhibitor. Reversible inhibitors are preferable, as a result of the ubiquitous distribution of most of the calpain isoforms and non-selective inhibition of them might have serious side effects. To improve cell permeability, N-terminal capping and esterification of the carboxyl group with lipophilic groups have been used in chemical syntheses [49,50]. Regarding selectivity, peptidyl epoxides and peptidyl aldehydes are relatively selective for cysteine proteases compared with serine proteases but not selective for calpains over other cysteine proteases [51]. Peptidyl ketoamides are the third generation of calpain inhibitors developed to improve potency, cell permeability and selectivity; morpholine derivates (i.e.AK275, AK295), benzodioxothianzines (derived from SJA-6017) and chromones are highly specific for calpains compared with other cysteine proteases [52].

The non-peptidic calpain inhibitors constitute the fourth generation of calpain inhibitors and have better stability, selectivity and pharmacological profiles than the peptidic inhibitors. This class of compounds are reversible non-competitive inhibitors that are not directed against the active site, but interact with calpain domains relevant for their activation (see Ref. [50] for a recent review). For example, the high specificity of α-mercaptoacrylic acids for calpains over other cysteine proteases is as a result of its binding to domain VI of the small subunit.

Neurodegenerative disorders are one of the fields in which calpain inhibitors have been extensively studied. In animal models, these compounds have shown to prevent neuronal death, improve neurological functioning and ameliorate motor disturbances in spinal cord injury [12], muscular dystrophies [53], Alzheimer's disease [6], traumatic brain injury and optic nerve degeneration [54,55]. Although the neuroprotective effects of calpain inhibitors have been clearly established, their use in chronic human disease is limited by their ability to cross the blood–brain barrier. Fusion of calpain inhibitors with molecules that have specific transporters in the brain is another strategy to overcome the blood–brain barrier and allow entry into the central nervous system. The combination of the calpain inhibitor leupeptin with taurine has been reported to facilitate access to the brain and spinal cord via the taurine transport system [56]. This strategy has been also employed to more efficiently incorporate the drug into the muscle fibres and, thus, reducing treatment dose. C101 is a leupeptin analogue covalently bound to L-aminocarnitine, which easly penetrates muscle cells via the high affinity OCTN2 carnitine transporter [57].

The use of calpain inhibitors to prevent ischemic apoptotic death in several cell types, such as the heart, brain, liver and renal cells, also shows promise because their use reduces the extent of the lesions and improve the recovery of normal tissue functions [50].

The synthetic calpain inhibitor SJA6017 and its derivates can ameliorate cataractogenesis and diminish lens opacity in sheep, rats and porcine cataract models [58,59]. However, this compound has poor oral bioavailability due to extensive metabolism of the aldehyde group, so high oral doses are required to exert its protective effect in a rat retinal ischemic model [60]. An hemiacetal derivative of this compound has proven to have better efficacy in the rat retinal ischemic model because it is neuroprotective at a five-times lower dose as a consequence of its metabolic stability and increased bioavailability [61]. The ketoamide derivate SNJ-2008 also has a good oral bioavailability and retinal efficacy [62].

In the treatment of cancer, the calpain inhibitors can modify invasiveness of tumour cells and promote p53 dependent apoptosis in various tumour cell lines, including leukaemia, lymphoma, prostate adenocarcinoma and mammary cancer cells [63–65]. Calpain inhibitors have been successfully used in combination with chemotherapeutic agents to overcome acquired resistance in cancer treatment [66] and with radical inhibitors in inner ear disorders [67].

Finally, the inhibition of calpain activity in pathological situations could be useful for the treatment of pain and inflammation (acute and chronic) because calpains cleave different proteins involved in nociception and the inflammatory response [59–69].

Considering the volume of research into the subject, surprisingly, with the exception of classical calpains and some studies in CAPN3, CAPN9 and CAPN10, the expression levels of most calpain isoforms in health and disease is almost unknown. RNA interference assays could be a valuable tool to identify the specific target for drug development avoiding side effects derived from unspecific protease inhibition.

Calpain activators

Some of the pathologies related to the calpain family result from loss of protein function. This is the case for calpain 3 and LGMD2A and calpain 9 and gastric cancer. The metabolic disturbances

associated with the calpain 10 gene are also related to loss of functional variants [70]. In fact, exposure of mice pancreatic islet cells to calpain inhibitors (ALLN, ALLM, E64d, MDL18270 and PD147631) but not to other inhibitors of proteases (i.e. cathepsin B and proteosome) affects fuel sensing at mitochondria and insulin exocytosis [71]. In addition, a recent study has pointed out that transgenic mice overexpressing calpastatin have increased numbers of GLUT4 glucose transporters in the muscle that is not accompanied by an increase in insulin-stimulated glucose transport; perhaps due to a defect in the GLUT4 translocation [72]. This adverse metabolic profile associated with calpain activity inhibition could represent a problem for therapies using calpain inhibitors that must be carefully addressed.

The concentration of Ca^{2+} needed for calpains to achieve the conformational changes required for their activity *in vitro* is considerably higher than physiological Ca^{2+} concentrations. Experiments designed to identify a mechanism for reducing the Ca^{2+} requirements of calpains have demonstrated that certain phospholipids (phosphatidylinositol or phosphatidylinositol 4,5-bisphosphate) can reduce the Ca^{2+} concentration required for activation of μ-calpain and m-calpain [5].

Other calpain activators in mammals have been described. They include a heat-stable protein of ~40–50 kDa that has been identified in rat skeletal muscle and human neutrophils [73,74]. However, although this activator reduces the Ca^{2+} concentration required for m-calpain, it had no effect on μ-calpain. Conversely, a similar activator was purified from human erythrocytes that only reduced the Ca^{2+} requirements of μ-calpain [75]. However, the nature of these activator proteins has not been addressed.

The protein UK114 is a homodimer with a molecular mass of ~30 kDa that was isolated from bovine brain as a specific μ-calpain inhibitor [76]. In addition, the highly conserved protein, acyl-CoA-binding protein, is also a homodimer of ~20 kDa with a potent m-calpain-activating property [77].

The development of calpain activators has been limited by several factors. The most important is the lack of structural information of the activator molecule, its mechanism of action or both. As yet, no commercial activators have been synthesized. However, calpain activators are expected to have important secondary effects because calpain overactivation has been linked to several diseases.

Conclusion

Although research into the relationship between the calpain family and human diseases has accelerated over the last two decades, many issues have yet to be resolved before this target is fully validated:

(i) The identification of potential protein–protein interaction and protease targets of all calpain proteins.
(ii) The determination and/or confirmation of the involvement of each calpain in specific human pathologies.
(iii) The development of a set of calpain-specific activators or inhibitors for each family member.

Given the available data, the multiple and pleiotropic effects demonstrated by these proteins, their involvement in human diseases and the molecular characteristics of their mechanism of action, the development of calpain-related clinical drugs is warranted in the near future.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drudis.2006.08.009.

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