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Review

Calpains and cancer: Friends or enemies?

Daniele Moretti, Barbara Del Bello, Giulia Allavena, Emilia Maellaro *

Department of Molecular and Developmental Medicine, University of Siena, via A. Moro, I-53100 Siena, Italy
Istituto Toscano Tumori (ITT), via T. Alderotti 26/N, I-50139 Firenze, Italy

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ABSTRACT

Calpains are a complex family of ubiquitous or tissue-specific cysteine proteases that proteolyze a variety of substrates (leading to their degradation or functional modulation) and are implicated in several pathophysiological phenomena. In tumor cell biology, calpains are implicated in a triple way: they are involved in different processes crucial for tumor progression, including cell proliferation, apoptotic cell death, survival mechanisms, migration and invasiveness; they have aberrant expression in several human cancers; a variety of anticancer drugs induce cytotoxicity through activation of calpains or the latter can influence response to therapy. This review covers established and recent literature showing these diverse aspects in tumor cells.

Introduction

The calpain story started in 1964, when a calcium-activated proteinase was identified in rat brain [1]. An increasing number of genetic, biochemical and functional studies have since revealed the complexity of this proteolytic system [2,3]. This long story is also a big story, because since the current term "calpain" was introduced, more than 7000 items dealing with calpains in a variety of topics of cell biology and medicine have been listed in PubMed.

Calpains (EC 3.4.22.17; Clan CA; Family C2,) constitute a superfamily of intracellular cysteine proteases, evolutionarily well-conserved from bacteria to mammals. On the basis of their domains structure, a complex classification of calpains and calpain homologs, divided into "classical" and "non-classical", was recently proposed [4,5]. In humans, 9 out of 15 calpain genes code for classical calpains, with alternative splicing variants also generated. On the basis of their expression profile, at least six genes are considered to be tissue-specific, and defects of the corresponding calpains are associated to tissue-specific diseases; among these, muscle Calpain-3 dysregulation gave rise in the past to the term "calpainopathy". Besides the large number of gene products and splice variants, the complexity of the calpain system also resides in the broad spectrum of substrates, demonstrated to be uniquely proteolyzed by calpains or shared with other proteolytic systems, mainly with caspases [6]. The structural basis of substrate recognition by calpains is not completely understood, but it is different from other intracellular proteolytic systems: for both proteasomal degradation [7] and chaperone-mediated autophagy [8] the substrate must be previously "labelled" with other molecules, i.e., ubiquitin and chaperones, respectively; caspases recognize short motifs of 4 aminoacids, where the caspase-specific residues are in position P1 and P4; differently, calpains recognize (not strictly but preferentially) PEST sequences (stretches of polypeptide chain rich in proline (P),[1] glutamate (E), serine (S) and threonine (T)) and/or higher order structures (general patterns of primary/secondary

* Corresponding author at: Department of Molecular and Developmental Medicine, University of Siena, via A. Moro, I-53100 Siena, Italy. Fax: +39 0577 234009.
E-mail addresses: dmonline@gmail.com (D. Moretti), barbara.delbello@unisi.it (B. Del Bello), giulia.allavena@unisi.it (G. Allavena), emilia.maellaro@unisi.it (E. Maellaro).

[1] *Abbreviations used:* P, proline; E, glutamate; S, serine; T, threonine; CAPN1, Calpain-1; CAPN2, Calpain-2; CBSW, type beta-sandwich; ERK, Extracellular signal-Related Kinase; EGF, Epidermal Growth Factor; PKCι, Protein Kinase C iota; PKA, Protein Kinase A; CAPN3, Calpain-3; AR, androgen receptors; HER2, Human Epithelial growth factor Receptor 2; XIAP, X-linked Inhibitor of Apoptosis; Gas2, Growth Arrest-Specific 2; CML, chronic myeloid leukemia; ER, endoplasmic reticulum; AIF, Apoptosis-Inducing Factor; PARP-1, poly(ADP-ribose) polymerase-1; Ambra1, Activating Molecule in Beclin1-Regulated Autophagy; HIF-1α, Hypoxia-Inducible Factor-1 α; VEGF, Vascular Endothelial Growth Factor; FAK, Focal Adhesion Kinase; MMP, Matrix Metallo-Protease; CAPN8, Calpain-8; CAPN9, Calpain-9; NSAIDs, non-steroidal anti-inflammatory drugs; G-Calpain, gastric calpain; CAPN6, Calpain-6; CAPN10, Calpain-10; T2DM, type 2 diabetes mellitus; ESCC, esophageal squamous cell carcinoma; LGMD2A, limb-girdle muscular dystrophy type 2A; NS, N-terminal sequence; IS1, insertion sequence 1; IS2, insertion sequence 2; NLS, nuclear localization sequence; BPV-2, bovine papillomavirus type 2; IFN-γ, interferon-γ; ROS, Reactive Oxygen Species.

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protein structures around the scissile bond) [9,10]. A further peculiarity is the functional fate of the processed targets. Differently from other proteases, calpains do not necessarily conduct a "degradative" type of proteolysis that destroys the substrate, but may conduct limited processing, after which the modified target protein may acquire an additional basic function or a novel function. Interestingly, it has been suggested [11] that calpains may generate protein fragments very similar to translated products of alternative transcripts, thus being a link between transcriptional and posttranslational regulation of cellular pathways [11–13].

The abundance of substrates cleaved by calpains, along with the "conservative" nature of substrate processing, explains the large number of cellular events, occurring under physiological and pathological conditions, in which calpains are involved. Among the pathological conditions, calpains have drawn a growing interest particularly in cancer research: (i) in tumor cell biology studies, demonstrating that calpains are involved in the natural history of cancer, from tumorigenesis to different cellular events typically associated to malignant progression; (ii) in *in vitro* pharmacological studies, where consolidated or novel compounds prove to exert an anticancer efficacy by interacting with (activating or inhibiting) calpains, which act as unique players of tumor cell response or together with other (proteolytic) systems. In addition to these experimental approaches, developed in cultured tumor cells and animal models, (iii) several clinical studies, performed on human tumor specimens, show significant correlations between calpains expression/activity and different tumor histopathological features and clinical parameters.

This review of current knowledge regarding the involvement of calpains in cancer first deals with "conventional", ubiquitous calpains (Calpain-1 and -2, and their endogenous inhibitor Calpastatin), which are the best characterized and whose involvement in tumor cell biology is well established. The review also emphasizes several "less famous" calpains (CAPN3, CAPN6, and CAPN8–10), mainly implicated in specific cancers, which are less characterized from a mechanistic point of view, but worth further study. Other human calpains (CAPN5, CAPN7, and CAPN11–16), which have no known role in cancer, are not discussed here.

Conventional calpains

Calpain structure

CAPN1 and *CAPN2* code for ubiquitous CAPN1 (Calpain-1) and CAPN2 (Calpain-2) large subunits (80 kDa) (sharing almost 60% sequence), also termed "conventional" calpains. Both active enzymes are heterodimers formed by each large subunit and a shared regulatory subunit, CAPNS1[30K] (also known as Calpain-4), coded by *CAPNS1*. The large, catalytic subunit comprises four domains (Fig. 1): the N-terminal anchor helix region, which is a short prodomain; the conserved CysPc catalytic domain, composed of two protease core domains (PC1 and PC2); the CBSW (calpain-type beta-sandwich) domain, involved in the conformational changes during calcium binding; and the PEF(L) (penta-EF-hand) domain [14]. The small regulatory subunit contains two domains: GR, a glycine-rich hydrophobic domain at N-terminus, and the PEF(S) (penta-EF-hand) domain [15] (Fig. 1). The first four EF-hands of both large and small subunits are involved in binding calcium, while the fifth EF-hand elicits the homophilic association for active heterodimer formation [16,17]. Calpain-1 and -2 are also referred to as "classical" calpains, where the classical structure comprises both the CBSW and PEF domains.

Calpain activity regulation

Calpain-1 and Calpain-2 were originally termed μ-calpain and m-calpain, respectively, on the basis of the calcium concentration (μM or mM range) required for their optimal activity *in vitro*: in the presence of calcium ions, in fact, the Cys, His, and Asn residues get closer and form the catalytic site [18,19]. High calcium concentrations in the cytosol can be achieved through diverse mechanisms in damaged, dying cells. In fact, several established drugs, including genistein [20,21], cisplatin and oxaliplatin [22–25], and resveratrol [26], induce calpain activation and cell death, following alterations of calcium homeostasis that raise the cytosolic calcium levels. The list of novel compounds (mainly plant-derived) and their derivatives which induce tumor cell death by this mechanism

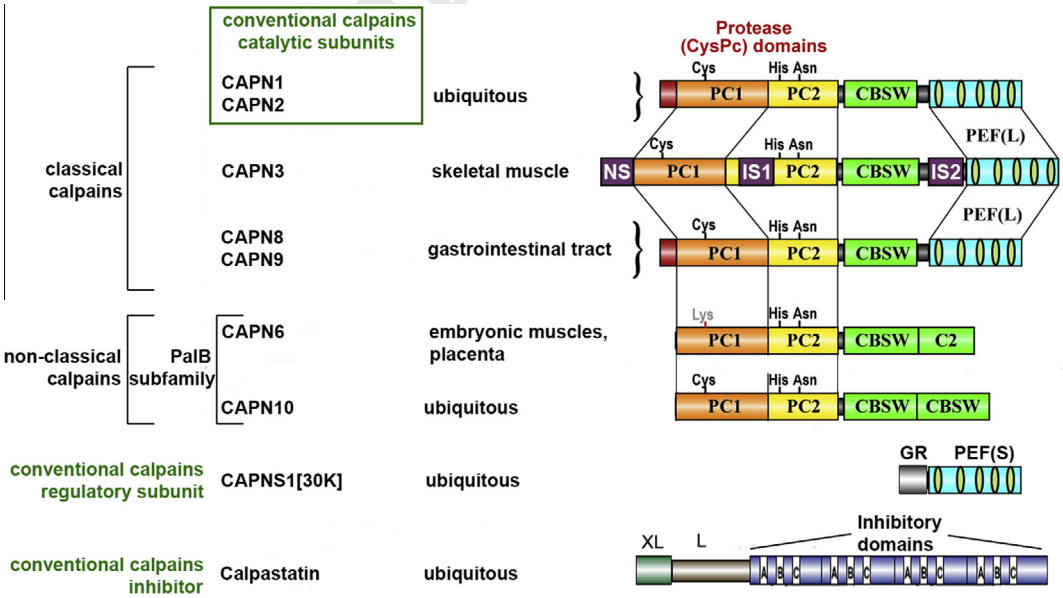

Fig. 1. Schematic structure of the calpains examined in this review. These include conventional calpains (the large, catalytic subunits CAPN1 and CAPN2 and the small, regulatory subunit CAPNS1[30K]), their endogenous inhibitor Calpastatin (the longest isoform is reported), CAPN3, CAPN8 and CAPN9, CAPN6 and CAPN10. Domain structures are defined into the text. At 2013 FASEB Summer Research Conference (SRC) on calpains it was proposed and approved to rename domain III (formerly called C2-domain-like (C2L) domain) to CBSW (calpain-type beta-sandwich) domain. The figure was modified from http://calpain.net/structure/human.html.

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