

Review Article

Killing Two Angry Birds with One Stone: Autophagy Activation by Inhibiting Calpains in Neurodegenerative Diseases and Beyond

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Proteolytic machineries execute vital cellular functions and their disturbances are implicated in diverse medical conditions, including neurodegenerative diseases. Interestingly, calpains, a class of Ca^{2+} -dependent regulatory proteases, can modulate the degradational system of autophagy by cleaving proteins involved in this pathway. Moreover, both machineries are common players in many molecular pathomechanisms and have been targeted individually or together, as a therapeutic strategy in experimental setups. In this review, we briefly introduce calpains and autophagy, with their roles in health and disease, and focus on their direct pathologically relevant interplay in neurodegeneration and beyond. The modulation of calpain activity may comprise a promising treatment approach to attenuate the deregulation of these two essential mechanisms.

1. Introduction

Proteolytic machineries of eukaryotic cells are key players in the regulation of protein function or the maintenance of cell homeostasis. Importantly, they act as modifiers of numerous neurodegenerative proteopathies, including classical medical conditions such as Alzheimer disease (AD), Parkinson disease (PD), and the group of polyglutamine (polyQ) disorders. This link is evident as the nature of these diseases, i.e., the occurrence of structurally abnormal toxic proteins, provokes an overload of these systems, leading to their disruption, loss of cellular integrity, and eventually neuronal demise [1]. Beyond neurodegeneration, proteostatic processes are implicated in further medical conditions like, for instance, cancer, cardiovascular disorders, and diabetes [2–4]. This multifarious involvement emphasizes the value of targeting these machineries therapeutically.

In this review, we focus on two major proteolytic machineries of the cell, the calpain protease system and autophagy, which both have been scrutinized in the context

of neurodegenerative disorders and other diseases for the last two decades. As often the case with complex cellular pathways, both proteolytic machineries are strongly interconnected and the deregulation of one of them inevitably leads to repercussion on the other. By shedding new light on the impact of calpains on autophagy and vice versa, we aim to work out points of vantage for therapeutic applications, which only target one but may hit both compromised proteolytic systems. Consequently, future disease-treating approaches may kill those rather angry birds, namely overactivated calpains and impaired autophagy, with only one stone.

2. Calpains and Autophagy in Neurodegeneration and Other Medical Conditions

2.1. Calpains

2.1.1. Calpain Basics. The regulation of protein structure, function, localization, or lifetime is mediated by a vast range

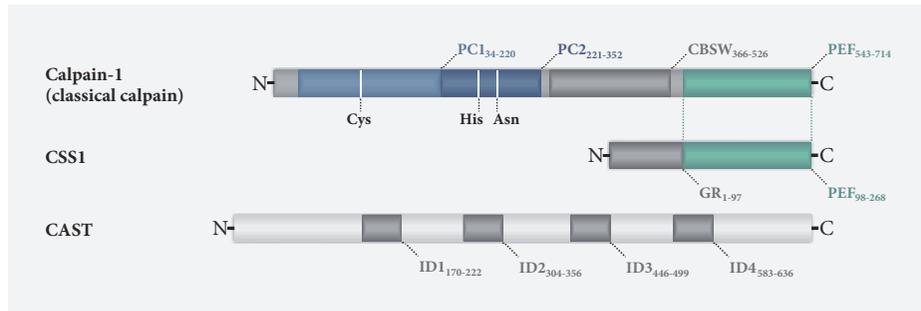


FIGURE 1: *Domain composition and structure of calpain-1, CSS1, and CAST.* Conventional classical calpains are present as a large protease unit, such as the here depicted calpain-1, and the calpain small subunit 1 (CSS1). Both share a C-terminal Ca^{2+} -binding penta-EF-hand (PEF) domain. Calpain-1 further contains an N-terminal proteolytic CysPc domain, consisting of core domains PC1 and PC2, which also bind Ca^{2+} ions. Amino acid positions of the catalytic triad of calpain-1 are indicated by vertical white lines. In addition, a calpain-like β -sandwich domain (CBSW) is located between the CysPc and the PEF domain. CSS1 features, moreover, an N-terminal glycine-rich (GR) hydrophobic domain. The endogenous inhibitor calpastatin (CAST) contains four structurally flexible inhibitory domains (ID1-4) of which each can inhibit one calpain molecule. Illustrations of calpain-1, CSS1, and CAST are based on data retrieved from the UniProt database (respective identifiers P07384-1, P04632-1, and P20810-1).

of posttranslational modifications (PTMs). Amongst those, proteolytic processing constitutes a profound mechanism, which spans from the removal of single amino acids to longer peptides or whole domains of the targeted protein. One class of enzymes responsible for this modification is calpains, firstly described as a Ca^{2+} -activated neutral proteinase in rat brain [5]. The later-promoted term ‘calpain’ is a portmanteau, which consists of the two syllables ‘cal’ in reference to Ca^{2+} or Ca^{2+} -binding proteins and ‘pain’ as an allusion to structurally related cysteine proteases like papain from plants or clostripain from *Clostridium* [6]. Calpain and their homologs can be found in unicellular and multicellular organisms, from animals, over plants, fungi, yeast, and down to bacteria [4].

Structurally, all calpains are characterized by their conserved proteolytic domain (CysPc), which is subdivided in the two protease core domains PC1 and PC2. Together with more than 40 different other protein domains or motifs, the CysPc domain forms multiple variants of calpains in a modular principle. The human genome encodes 15 different calpains, divided into two main groups: classical (calpains-1-14) and nonclassical calpains (calpain-5, calpain-6, calpain-7, calpain-10, calpain-15, and calpain-16). Classical calpains feature a C-terminal Ca^{2+} -binding penta-EF-hand (PEF) domain. Via this domain, members like calpain-1 and calpain-2, which are referred to as conventional classical calpains, exhibit a vital interaction with the regulatory calpain small subunit 1 (CSS1, formerly known as calpain-4) [7, 8]. Nonclassical calpains lack both the PEF domain and the interaction with a regulatory subunit [4, 8, 9]. The direct antagonist of these proteases is calpastatin (CAST), the only known endogenous, ubiquitously expressed, and highly specific proteinaceous inhibitor of classical calpains. Altogether, calpains, regulatory subunits, and CAST form the intracellular calpain system [10, 11]. A structural representation of calpain-1, CSS1, and CAST is shown in Figure 1. Calpain expression depends largely on the respective isoform: calpain-1, together with the regulatory subunit CSS1, is expressed ubiquitously, and isoforms such as calpain-2,

calpain-5, and calpain-10 are found in most cells. However, other calpains, like the skeletal muscle-specific calpain-3, show expression patterns restricted to distinct tissues [8].

The activation mechanism of calpains has been controversially discussed and led to the formulation of different explanatory scenarios [12]. However, X-ray crystallography of Ca^{2+} -bound calpain-2 together with CSS1 and CAST shed light on the precise mechanism: in a fully activated state, calpain-2-CSS1 heterodimer binds ten Ca^{2+} ions, of which eight are bound to the two PEF domains, and one Ca^{2+} is bound at each PC domain. The Ca^{2+} -binding induces structural rearrangements, which then allows the connection of the PC1 and PC2 core domains to a closed active state [13, 14]. *In vitro* studies demonstrated that Ca^{2+} concentrations necessary for activation of calpains were in a micro- to millimolar range, which is rather far beyond the nanomolar Ca^{2+} levels in cells under normal physiological conditions. Yet, this apparent contradiction is resolved, as the cellular microenvironment may provide the sufficient Ca^{2+} concentration [12].

Calpains feature a wide range of cellular functions and act in a regulatory way by performing limited proteolysis of substrates, such as enzymes and structural proteins [15, 16]. Their functional involvement ranges from remodeling cytoskeletal elements, regulating cell motility, cell cycle control, and proliferation, via controlling gene expression, inflammation, autophagy, and apoptosis, through to tuning signal transduction and synaptic plasticity in neurons [7, 17–19].

2.1.2. Calpains in Health and Disease. The important role of calpains in a healthy biological system becomes even clearer in the light of the wide-ranging implications of their malfunction in a multitude of human diseases. The deregulation of calpain function and mediation of molecular pathomechanisms by calpains were described in medical conditions such as myopathies, ophthalmic maladies, cardiovascular disorders, cancer, and neurodegeneration.

A whole group of diseases which are based on the direct dysfunctions of calpains was termed *calpainopathies*, comprising a wide spectrum of pathological manifestations [8]. Limb-girdle muscular dystrophy 2A (LGMD2A) was the first-described calpainopathy, which is caused by mutations in the gene encoding muscular calpain-3 (*CAPN3*) [20, 21]. Missense mutations in the calpain-5 gene (*CAPN5*) were associated with an autosomal-dominant form of neovascular inflammatory vitreoretinopathy (ADNIV) [22]. In cardiovascular injuries, mitochondrial calpain-1 was shown to mediate apoptotic effects [23–25]. Moreover, an intriguing association of calpains was made with diabetes, when calpain-10 was identified as a susceptibility gene for type 2 diabetes [26]. Mutations in the skin-specific calpain-12 were shown to worsen the clinical manifestation of autosomal recessive congenital ichthyosis [27].

Calpains also play a role in tumorigenesis by diversely acting on cancer cell migration, survival, and death, rendering these proteases a potential therapeutic target in oncology [28]. The proteases were shown to contribute to tumor progression and to exhibit deregulated expression patterns on one hand. On the other, calpains are acting as executioners of apoptotic cancer cell death, activated by anticancer drugs [29]. For instance, calpain-1 and calpain-2 demonstrated protumorigenic roles in HER2⁺ breast cancer models, as conditional deletion or knockout of *CSS1*, which is crucial for the activity of these conventional calpains, blocked or delayed tumorigenesis [30]. High calpain-2 expression was associated with the adverse clinical outcome of basal-like and triple-negative invasive breast cancer [31]. However, the proapoptotic or antineoplastic activity of capsaicin was found to be based on increased Ca²⁺ levels and, thereby, calpain-1 and calpain-2 activation, in models of human small cell lung cancer [32, 33].

Lastly, calpains are also implicated in neuronal injury, neurodegenerative disorders, and neuronal aging processes [1, 34]. For instance, these proteases execute Wallerian degeneration and mediate degenerative effects in traumatic brain injury [35–37]. A detrimental calpain overactivation has been detected in many neurodegenerative disorders such as AD, amyotrophic lateral sclerosis (ALS), PD, or the group of polyQ disorders [38–41]. Interestingly, calpains were associated with fragmentation of the respective disease proteins, leading to the generation of breakdown products with an increased toxicity compared to the full-length protein. This includes α -synuclein in PD or transactivation response element DNA-binding protein 43 (TDP-43) in ALS, as well as the polyQ disease proteins huntingtin in Huntington disease (HD) and ataxin-3 in Machado-Joseph disease (MJD). Resulting protein fragments were shown to be more harmful to cells or to readily form disease protein aggregates [42–46]. Consequently, inhibition of cleavage by genetically and pharmacologically targeting calpains or by rendering disease proteins cleavage-resistant ameliorated disease-related molecular and behavioral characteristics in respective models of those diseases [46–51]. Overexpression of CAST in animal models of AD and ALS showed beneficial effects by counteracting the intrinsic calpain overactivation [52–54]. Most recently, a neuronal calpainopathy was identified which is

caused by *CAPN1*-null mutations, leading to cerebellar ataxia and limb spasticity [55]. Furthermore, calpain-1 and calpain-2 seem to have opposing roles in neuronal function, mediating synaptic plasticity, and neuroprotection versus neurodegenerative effects [17]. Therefore, these circumstances have to be considered when targeting calpains for therapeutic purposes.

2.2. Autophagy

2.2.1. Autophagy Basics. Cellular homeostasis is the result of constantly ongoing self-renewing processes that assure elimination of malfunctioning or nonfunctional components, from proteins to organelles. These highly conserved processes feed into recycling mechanisms that provide the cell with nutrients and metabolites. PTMs, typically ubiquitination, can mark proteins for destruction, if they are nonfunctioning, aggregating, or long-lived, eventually handing them over to the cell's major protein-degradation pathways: the ubiquitin-proteasome system (UPS) and the autophagy lysosome pathway (ALP) [56, 57].

Classically, UPS targets are tagged with K48-linked ubiquitin chains, recognized by the 19S regulatory cap of the 26S proteasome, unfolded, and then cleaved in the 20S proteolytic core, generating small peptides [58, 59]. ALP, the other degradational system, allows specific as well as bulk degradation under energy- and nutrient-deficient conditions. This system can be subdivided into three different mechanisms: chaperone-mediated autophagy (CMA), microautophagy, and (macro-)autophagy. All of them have the shuttling of cargo to the lysosome in common, where hydrolases break the content down to single amino acids. Whereas CMA relies on a specific KFERQ pentapeptide recognition sequence for the chaperone-mediated transport to the lysosomal transporters [60, 61], microautophagy is a rather unspecific engulfment of cytoplasmic content at the lysosomal membrane [62].

Macroautophagy is characterized by the *de novo* formation of double-membrane structures that are formed in the vicinity of the endoplasmic reticulum (ER), at nucleation sites, even though the origin of the membranes is still not entirely resolved [63]. Membrane structures are created that form the phagophore (isolation membrane) by a steady growth into the vesicular structure, engulfing cellular material from proteins up to organelles. The mature autophagosomes finally fuse with lysosomes to autolysosomes, where the cargo is degraded [64]. The genes responsible for this process (autophagy-related genes, ATGs) have been found by reverse genetics in *Saccharomyces cerevisiae* and a multitude of homologues were shown to be conserved throughout many species and in humans [65]. The sensory components of this degradation mechanism are the mechanistic target of rapamycin complex 1 (mTORC1) and AMP-activated protein kinase (AMPK), which integrate signals about nutritional cues and growth factors or the energetic status of the cell, respectively. This leads to the rapid adaptation of anabolic processes and to the release of amino acids, through recycling of cellular material by their differential regulation of the

serine/threonine-protein kinase ULK1 [66, 67]. The formation of the autophagosome is classically initiated by the ULK1 (Atg1 in yeast) complex under nutrient deprivation. Beclin-1 is phosphorylated by ULK1 and VPS34 (Class III phosphatidylinositol 3-kinase (PI3K) in humans) is activated [68, 69]. In complex with VPS34, beclin-1 and ATG14 are involved in the nucleation of the phagophore and maturation of the autophagosome. The phagophore membranes are elongated via two ubiquitin-like systems (ATG12 and ATG8) by the reversible conjugation of several ATG gene products, which prime the growing ends for further protein interactions [70]. ATG5-conjugated ATG12 binds to ATG16L (E3-like protein) by E1-like (ATG7) and E2-like (ATG10) proteins. This complex at the extending phagophore allows the recruitment of the second ubiquitin-like system [71]. For this, ATG4-cleaved microtubule-associated protein 1 light chain 3 (LC3, Atg8 in yeast) is lipidated with phosphatidylethanolamine (PE). These modifications generate LC3-II [72], which is then incorporated into the double membrane. ATG7 and ATG3 function as E1-like and E2-like proteins, respectively, and LC3-II is conjugated to the ATG5/12/16L complex by ATG3, which drives the growth of the phagophore membrane [73, 74].

Autophagosomes can selectively engulf diverse forms of autophagic cargo, ranging from single proteins, over protein aggregates (aggrephagy), to whole organelles like mitochondria (mitophagy) and even proteasomes (proteaphagy) [75–78]. Cargo designated for degradation is detected by p62/SQSTM1, neighbor of BRCA1 gene 1 (NBR1), optineurin, Toll-interacting protein (TOLLIP), or other receptor proteins [78–80]. These receptors preferentially bind K63-polyubiquitin-tagged substrates and bring them in contact with the autophagosomes via a LC3-interacting region (LIR). The specific binding and the capacity of some proteins to act additionally as scaffolds for the recruitment of autophagic complexes ensure selective degradation [81].

Lastly, the mature autophagosomes fuse with lysosomes. This process requires several components, such as lysosome-associated membrane proteins (LAMPs) [82]. The degradation in the lysosome proceeds to the breakdown of proteinaceous cargo into single amino acids by cathepsins. Dysfunction of both degradative systems has been associated with neuronal aging and degeneration, bringing it into focus for therapeutic research [83].

2.2.2. Autophagy in Health and Disease. In line with its essential role in cellular homeostasis, autophagy is involved in major disease classes like cardiovascular, infectious, and metabolic disorders as well as cancer [84]. It has been generally challenging to delineate the exact roles of autophagy in cell survival and cell death [85]. Whilst this mechanism can have cell protective functions in regard to genomic integrity [86] and autophagy induction is a common therapeutic strategy in cancer, inhibition of this pathway has also shown its applicability in tumor treatment. This is mainly because autophagy can represent an escape mechanism for tumor cells and may be responsible for the development of resistances [87].

In the healthy nervous system, autophagy relieves neurons of protein and organelle damage. Moreover, it plays an important role in developmental organization processes [88], ensuring axonal homeostasis [89] and sustaining the pool of neuronal stem cells [90]. Autophagy is most vital during the neonatal starvation period and thus ubiquitous deletion of ATG core proteins results in neonatal or embryonic lethality [91]. In brain injury by hypoxia or trauma, autophagy is a critical and protective factor in cell survival, underlining its important role in the survival of neurons [92, 93]. Conditional knockout of core autophagy genes leads to decreased life span and phenotypes resembling neurodegeneration [94, 95]. Successful aging is especially relevant in postmitotic cells such as neurons [96]. The accumulation of long-lived organelles and proteins, as well as the reduced ability of cells to cope with stress imposed by those, is believed to be a major cause for late onset neurodegenerative diseases. As many different pathomechanisms may lead to neurodegeneration, various disease-specific deregulations of the autophagic pathway have been suggested [81, 97].

In several neurodegenerative disorders of the brain, an accumulation of autophagosomes and autophagic markers has been observed [84, 98, 99]. Importantly, the mere finding of an increased number of autophagosomes gives no information on whether the autophagic flux is increased or the elimination of autophagosomes is just inhibited [100]. Observations in HD have shown that patient brain and lymphoblasts feature increased numbers of autophagosomes [101, 102]. This upregulation of autophagosome formation is caused by the sequestration and inactivation of mTOR by mutant huntingtin [103] and is accompanied by a defect in cargo loading [104]. Further, critical autophagy regulating genes, such as beclin-1 and Ras homolog enriched in striatum (Rhes), show reduced function and protein levels in HD brain [105–107]. Despite an already increased autophagy and functionally disturbed autophagosomes, genetic or pharmacological induction of autophagy has, however, been proven effective in different HD models [108, 109] and comparable results were obtained for other polyQ disorders as well as for AD and PD [110–113]. On the other hand, lysosomal cathepsins, which are responsible for the degradation of cargo proteins in autophagy, were associated with cleavage of mutant huntingtin in HD or APP in AD and, thus, formation of toxic fragments. In this regard, inhibition of these proteases led to beneficial effects on the molecular disease phenotype [114, 115]. Interestingly, not only have the disease-causing proteins in polyQ disorders been found to be degraded by autophagy, but also accumulated evidence suggests a direct role of proteins like huntingtin or MJD's ataxin-3 in autophagy regulation. Huntingtin itself represents a very special case since its structure is related to several ATG proteins. Consequently, it has been implicated in the induction of autophagy [116, 117]. Huntingtin, in its physiological function, is proposed to act as a scaffold, which recruits autophagy-initiating factors and adapter proteins [117, 118]. More recent studies have also found ataxin-3 to be a regulator of autophagy initiation. Wild-type ataxin-3 is a deubiquitinase that is thought to cleave polyubiquitin chains from beclin-1, thereby saving it from

degradation and enabling autophagy [119]. By contrast, in MJD patient-derived fibroblasts, beclin-1 and autophagy levels were reduced, and beclin-1 overexpression rescued the deficit in autophagosome formation [120]. Interestingly, reduced beclin-1 levels are commonly detected in neurodegenerative disorders and aging brains, representing a limiting factor in autophagy induction and a driving factor in late onset proteinopathies [106, 121, 122].

Aside from polyQ disorders, different levels of autophagy deregulation have been reported for AD, PD, and ALS. An increased PI3K/AKT/mTOR signaling was shown in AD, as well as a defect in lysosomal clearance caused by A β [63, 121]. Autophagy induction by various means has been successfully tested in animal models of AD, and several substances have been evaluated in clinical trials [112, 123, 124]. All types of autophagy have been implicated in PD pathogenesis and macroautophagy, in particular, has been linked to mitochondrial dysfunction, due to ineffective mitophagy [125]. The PINK/parkin pathway, which is based on two proteins known to be causative for PD when mutated, regulates mitophagy and, therefore, controls mitochondrial number and quality. Moreover, the accumulation of α -synuclein has been found to interfere with mitochondrial turnover [126]. The genetic activation of autophagy by beclin-1 expression as well as pharmacological approaches were able to rescue disease phenotypes in PD animal models [113, 127, 128]. In ALS, the E478G mutation in the autophagy receptor optineurin leads to defective degradation of mitochondria [129]. Also, for the protein C9orf72, a regulatory function in autophagy induction has been proposed [130].

The general translation of findings on autophagy's role in cell and animal disease models to human patients and clinical applications poses a big challenge. Open questions remain about the exact dynamics of deregulation in autophagic processes in different neurodegenerative disorders, e.g., in terms of aging and tissue specificity. Furthermore, treatment approaches targeting mTOR and AMPK signaling pathways suffer from complications, such as pleiotropic effects or occurring toxicities. Thus, despite its compromised functionality in diseases, it is still unclear to which extent the autophagic clearance can be therapeutically exploited. In the pursuit of new targets for autophagy modulation, the calpain system could represent an approach to indirectly upregulate autophagy and thereby reestablish cell homeostasis.

2.3. Interplay between the Autophagy Pathway and the Calpain System

2.3.1. Impact of Calpains on Autophagy. Due to their limited proteolytic activity and intrinsic substrate specificity, calpains are considered as modulator proteases, allowing them to regulate protein functions and, thereby, various cellular pathways. It is, therefore, obvious that calpains exert respective modulatory effects on autophagy. In many disease conditions and models, calpains were shown to negatively regulate autophagy, making enhanced calpain activation a conceivable contributory factor in the impaired activation of this degradation pathway.

Diverse studies have shown that the impact of calpains on autophagy occurs on multiple levels, as summarized in Figure 2. For instance, the α -subunit of heterotrimeric G proteins ($G_{s\alpha}$) appears to be a substrate for calpains. Cleavage leads to its activation, which in turn activates adenylyl cyclase. This results in an accumulation of cAMP, which then culminates in the inhibition of phagophore formation through activation of phospholipase C and, consequently, increased generation of inositol triphosphate (IP₃) [131, 132]. Furthermore, ATG5 is cleaved and inactivated by calpains, leading to a disturbance of the ATG12-ATG5 complex formation and, as a consequence, of the expansion of the phagophore membrane [133, 134]. Interestingly, calpain-cleaved ATG5 was shown to translocate to mitochondria and induce apoptosis by blocking the antiapoptotic function of Bcl-xL. Thus, calpain cleavage of ATG5 constitutes a switch between autophagy and apoptosis [134]. Moreover, calpain overactivation as a result of anoxia-reoxygenation in cells or ischemia-reperfusion injuries *in vivo* demonstrated detrimental effects on autophagy via breakdown of beclin-1, ATG3, or ATG7, while calpain knockdown or overexpression of respective substrates counteracted the autophagic impairments [135–137]. Nearly all ATG proteins were shown to be cleaved by calpains *in vitro*, without characterizing, however, the biological relevance of their proteolysis [138]. Aside from proteins implicated in the proper formation of autophagosomes, calpains also target autophagy receptors, such as p62/SQSTM1 and optineurin, which may lead to a compromised cargo binding [138–140]. Autophagosome maturation might also be affected by calpains, as LAMP2 was shown to be cleaved by these proteases, leading to lysosomal permeabilization [141, 142]. Finally, due to their well-established role in microfilament dynamics, it was hypothesized that calpains may interfere with the dynamic changes of the cytoskeleton coupled to autophagosome formation [143]. Of note, the HD disease protein huntingtin, which is involved in autophagic processes, is a known calpain substrate, suggesting functional repercussions when proteolytically fragmented [43, 117, 118]. Interestingly, depletion of CSS1, whose knockout leads to early embryonic lethality in mice, induced lysosomal defects and blocked autophagy in cell-based experiments. The latter effect was attributed to the substantial calpain cleavage of Bif-1, which allows the scission of Golgi components and their targeting to nascent autophagosomes [144–146].

As neurodegenerative conditions, cancer, cardiovascular diseases, and diabetes have in common a reported deregulation of proteases and disturbances of the autophagic flux, further studies on the involvement of calpains in autophagy are of particular relevance. In the following sections, we will discuss the interplay between calpains and autophagy in a choice of those maladies.

2.3.2. Interplay of Calpains and Autophagy in Diabetes, Ischemia and Cancer. Both calpain activation and deregulated autophagy are implicated in the molecular pathomechanisms of many common health conditions with unsolved or complex etiologies. Myriads of them feature an impaired Ca²⁺ homeostasis as a primary trigger for these disturbances.

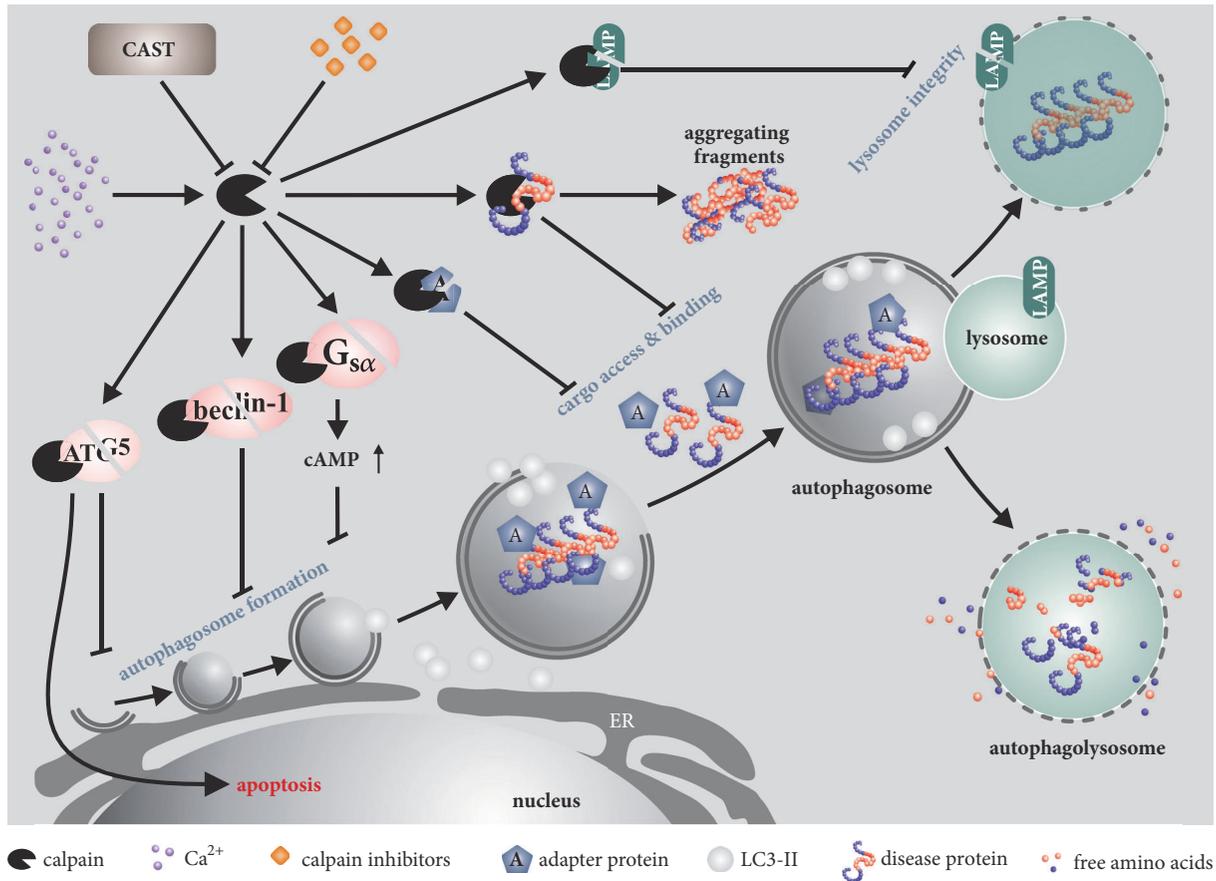


FIGURE 2: Calpain targets in the autophagic machinery. Calpains can impair protein clearance on different levels of the ALP. By cleaving signal transduction molecules, like G_{sα}, or autophagic proteins, like beclin-1 and ATG5, calpains lead to a reduction of autophagy initiation and can, in the case of ATG5 cleavage, act as a switch from autophagy to apoptosis. Moreover, the cleavage of adapter proteins (optineurin, p62/SQSTM1), cargo (e.g., disease proteins), or lysosome-associated proteins (LAMPs) can change the dynamics of cargo degradation, thereby causing a defect in protein homeostasis. The inhibitory function of CAST reduces calpain activity and thereby leads to increased autophagy levels. Additionally, it prevents the cleavage of disease proteins into toxic or strongly aggregating fragments, rendering more soluble, full-length forms of the protein more accessible to autophagy.

In type 2 diabetes (T2D), amylin (or islet amyloid polypeptide, IAPP), a peptide hormone which is cosecreted with insulin in a ratio 1:100, was shown to accumulate in affected pancreatic β cells, forming amyloid deposits and, eventually, leading to cell death [147]. Autophagy has been suggested as a defending mechanism in β cells against the proteotoxicity of amylin, and a known dysfunction of the ALP in T2D may further contribute to detrimental effects [148, 149]. Interestingly, toxic amylin oligomers were shown to lead to intracellular membrane disruption, increased cytoplasmic Ca²⁺ concentrations, and, consequently, overactivation of the calpain system, specifically calpain-2, in cell models, mice, and pancreatic tissues from humans with T2D [150, 151]. This overactivation ultimately leads to critical autophagic dysfunctions [150].

Calpain overactivation is a general response during ischemia-reperfusion in many different tissues, when anaerobic metabolism decreases the active Ca²⁺ efflux and limits its reuptake by the ER, thereby producing Ca²⁺ overload in

the cell. In the eye, heart, or liver, for instance, this overload leads to the deleterious overactivation of calpains, which then excessively cleave structural and functional proteins [152–154]. Following retinal ischemic injury *in vivo*, calpains were shown to fragment and inactivate beclin-1, resulting in the deregulation of autophagy [136]. A direct impact of calpains on autophagy remains unproven in the heart muscle; however, in fatty livers, calpain-2 inhibited autophagy by cleaving ATG3 and ATG7, thereby contributing to ischemia-reperfusion injuries [137]. In livers of obese mice, a baseline impairment of autophagy, due to ATG7 depletion, was associated with a dramatic increase in calpain-2 protein expression [155].

In cancer cells, calpains are often mediating the switch between protective autophagy and desired apoptosis (e.g., via ATG5). Inhibiting their activity may be disadvantageous in anticancer treatments, whereas activating them may be beneficial [156, 157]. Investigations on human metastatic melanoma cells treated with cisplatin revealed that this

chemotherapeutic induces calpain activation and inhibits basal autophagy, while autophagy activation by calpain inhibition acts as a prosurvival response [158, 159]. A nonclassical calpain demonstrated a different effect regarding the control of the autophagy system in sarcoma cells. Knockdown of calpain-6, which is strongly upregulated in cells with tumor-initiating and metastatic capacities, suppressed autophagy as well as hypoxia-dependent prevention of senescence entry [160, 161]. The Kaposi sarcoma-associated herpesvirus inhibits autophagy and impairs monocyte differentiation into dendritic cells as an immune evasion strategy, by reducing CAST expression and consequently leading to decreased ATG5 levels [162].

2.3.3. Linking Calpain Activation and Autophagy in Neurodegeneration. The neurodegenerative disorders AD, ALS, HD, MJD, and PD exhibit an overactivation of calpains and disturbances of the autophagic pathway [1, 8, 81]. Considering the known implications of calpains in autophagy, a link between both pathways is also apparent in neurodegeneration.

In HD, calpains have been early identified as a disease modifier, being overactivated in the disease context and leading to cleavage of polyQ-expanded huntingtin [43, 49]. Likewise, a deregulation of autophagy was shown for HD, which is further emphasized by wild-type huntingtin's physiological involvement in this pathway [117, 118, 163]. A direct connection between calpain overactivation and autophagy deregulation has yet not been made for HD; however, the knockdown of a calpain homologue in an HD *Drosophila* model and CAST overexpression in HD mice reduced polyQ toxicity of an N-terminal huntingtin fragment and improved behavioral signs, by activating autophagy [164]. This upregulation of the autophagic pathway was attributed to cleavage inhibition of the calpain substrate G_{sc} , as shown earlier in cell and zebrafish models of HD [131]. Respective effects also cannot be ruled out as a contributing factor in two of our preclinical studies, where we treated two HD animal models with the experimental drug olesoxime, thereby not only reducing calpain overactivation, huntingtin fragmentation, and aggregate formation, but also ameliorating the behavioral phenotype [47, 165]. In an MJD zebrafish model, calpain inhibition reduced polyQ-expanded ataxin-3 levels in an autophagy-dependent manner [166]. In a conditional α -synuclein-expressing mouse model of PD, the environmental neurotoxin paraquat was shown to activate calpains, leading to inhibition of autolysosomal clearance and, thereby, accumulation of both calpain-cleaved and insoluble α -synuclein species [167].

Calpains were, furthermore, suggested to act as a switch between two modes of cell death in hippocampal neural stem cells, as low calpain activity triggered by insulin deprivation resulted in a preference for autophagic cell death over apoptosis [168]. In cortical neurons, autophagy was shown to fail preventing glucose deprivation/reintroduction-induced neuronal death due to lysosomal permeabilization, which resulted from a calpain-mediated LAMP2 cleavage [141].

2.4. Activating Autophagy via Calpain Inhibition as a Therapeutic Approach

2.4.1. Genetic Approaches for Calpain Inhibition to Stimulate Autophagy. In the previous paragraphs we have highlighted the relevance of calpains and autophagy in various human medical conditions, as well as the interplay of both proteolytic machineries. The fact that calpains have a direct regulatory impact on the autophagic system suggests the assumption that exclusively targeting these proteases may target both their deregulation and the autophagic dysfunction. Not having reached clinical applicability yet, multiple cell-based or *in vivo* disease models have delivered general proofs of concept using genetic or pharmacological approaches.

Typical genetic strategies comprise the overexpression of the endogenous calpain inhibitor CAST, or the knockdown and knockout of calpain isoforms as well as of CSS1. In a human IAPP transgenic mouse model of T2D, overexpression of CAST was shown to be protective against the loss and dysfunction of pancreatic β cells and preventing diabetes onset by restoring the vital ALP [150]. Moreover, the intravitreal injection of siRNA directed against CSS1 reduced calpain activation and beclin-1 cleavage in an *in vivo* model for retinal ischemic injuries [136]. In a mouse model for bone sarcoma, knockdown of the nonclassical calpain-6 blocked tumor development, and overexpression of this protease in an osteosarcoma cell line increased autophagic flux, which could rather favor tumorigenesis [161].

In models of neurodegenerative disorders, CAST overexpression and calpain knockdown were protective against toxicity of the respective disease proteins. RNAi-mediated knockdown of the calpain homologue *CalpA* in *Drosophila* models of HD and tauopathy ameliorated the disease-related phenotypes in an autophagy-dependent manner [164]. HD mice transgenic for CAST showed an activation of autophagy, leading to reduced mutant huntingtin protein and aggregate levels, attenuating disease symptoms, such as tremor and motor phenotype [164]. In line with these findings, our group showed that HD mice with ablated CAST expression presented, in addition to calpain overactivation and the consequent increase of huntingtin cleavage and aggregation, disturbances in autophagy [169]. Analogously, positive effects of CAST overexpression as well as negative consequences of an CAST knockout on disease protein toxicity were shown in further cell and mouse models of neurodegenerative disorders, such as ALS, PD, and SCA3, without, however, linking it directly to autophagy [44, 45, 48, 50, 52–54, 170].

Excessively calpain inhibition can be, nevertheless, detrimental, as the inhibition of calpain activity by CAST overexpression in mouse hearts resulted in a progressive cardiomyopathy characterized by accumulation of protein aggregates, formation of autophagosomes, and disruption of sarcomere integrity [171]. Moreover, in CAST transgenic mice, postinfarct scar healing was impaired, leading to an increased mortality [172].

2.4.2. Pharmacological Approaches for Calpain Inhibition to Stimulate Autophagy. Pharmacological inhibition of calpains for stimulating autophagy can be achieved in two modes: first,

by targeting calpains directly with specific inhibitors and, second, by aiming at calpain-activating mechanisms (i.e., primary elements of the cellular Ca^{2+} homeostasis). Accordingly, studies in different fields of clinical investigations have tested these respective approaches.

In a model for retinal ischemic injuries, administration of calpain inhibitors MDL 28170 and SJA6017 prevented calpain overactivation and cleavage of beclin-1 [136]. Pharmacological inhibition of calpain-2, not calpain-1, suppressed anoxia-reoxygenation-induced loss of autophagy proteins beclin-1 and ATG7, avoiding the onset of mitochondrial permeability transition and decreasing cell death after reoxygenation in rat hepatocytes [135]. Similar results were achieved by preventing a Ca^{2+} overload in mouse livers after ischemia-reperfusion, using the anticonvulsant/antiepileptic drug carbamazepine, which suppressed the calpain-mediated autophagic flux impairment and likewise prevented the loss of beclin-1 and ATG7 [173]. Furthermore, inhibition of calpain-2 in steatotic livers restored autophagic flux both in livers from obese rats, after ischemia-reperfusion, and in free fatty acid-treated hepatocytes [174]. In obese mice, administration of calpain inhibitors MDL 28170 and PD150606 resulted in rescued ATG7 level, eventuating in lower ER stress, enhanced hepatic insulin action, and systemic glucose tolerance [155].

In the field of polyQ diseases, diverse approved compounds have been rising as a promising reliever, through calpain inhibition, for those disorders. In PC12 and neuroblastoma cells expressing an exon 1 fragment of mutant huntingtin, inhibition of L-type Ca^{2+} ion channels (e.g., with verapamil) enhanced the autophagic clearance of the soluble and aggregated disease protein in a calpain-inhibiting manner. These effects could also be observed by direct inhibition of calpains using calpeptin [131]. In line with this, treatment of an MJD zebrafish model with calpeptin resulted in lowered mutant ataxin-3 expression by increased autophagy, which furthermore ameliorated the model's motor phenotype [166]. With regard to its significant effects on calpain activation and mutant huntingtin aggregation in two animal models of HD, the voltage-dependent anion channel-targeting experimental drug olesoxime may most likely exert positive effects on autophagy. However, respective evidence still needs to be provided [47, 165].

3. Conclusions

As presented in this review, many *in vivo* and *in vitro* studies have furnished evidence for the crosstalk between calpains and autophagy, two important proteolytic cell machineries. The interaction is grounded on calpains' functional role as *modulator proteases*, which cleave and modify the activity of multiple substrate proteins, in this context, involved in the autophagy pathway. Thereby, calpains regulate autophagy by influencing autophagosome formation, substrate recognition, and cargo degradation.

This direct mechanistic impact highlights calpains as a point of vantage for therapeutically targeting autophagy. Especially in neurodegenerative diseases, but also other medical conditions such as diabetes or ischemia, where calpains

are known to be overactivated, genetic or pharmacological strategies to inhibit these proteases can attenuate concomitant autophagic disturbances. Still, a further and broader dissection of the interplay is necessary, which may cover its potential involvement in other diseases and additional underinvestigated processes along the autophagy pathway. Notwithstanding the known detrimental effects of an exhaustive calpain inhibition or an excessive autophagy activation, the general role of calpains in autophagy regulation may present downstream advantages, either by increasing cell viability such as in diabetes and neurodegeneration, or by facilitating apoptotic cell death upon chemotherapy in cancer.

For this reason, a broad discernment on both proteolytic machineries and their intersections may be useful for understanding deregulation of calpains and autophagy in neurodegenerative diseases and beyond, thereby contributing to the development of additional treatment strategies for a multitude of medical conditions.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

All authors contributed equally to this study.

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References

- [1] M. P. Mattson and T. Magnus, "Ageing and neuronal vulnerability," *Nature Reviews Neuroscience*, vol. 7, no. 4, pp. 278–294, 2006.
- [2] E.-L. Eskelinen and P. Saftig, "Autophagy: a lysosomal degradation pathway with a central role in health and disease," *Biochimica et Biophysica Acta*, vol. 1793, no. 4, pp. 664–673, 2009.
- [3] E. Jankowska, J. Stoj, P. Karpowicz, P. A. Osmulski, and M. Gaczynska, "The proteasome in health and disease," *Current Pharmaceutical Design*, vol. 19, no. 6, pp. 1010–1028, 2013.
- [4] H. Sorimachi, S. Hata, and Y. Ono, "Calpain chronicle—an enzyme family under multidisciplinary characterization," *Proceedings of the Japan Academy Series B: Physical and Biological Sciences*, vol. 87, no. 6, pp. 287–327, 2011.
- [5] G. Guroff, "A Neutral, calcium-activated proteinase from the soluble fraction of rat brain," *Journal of Biological Chemistry*, vol. 239, pp. 149–55, 1964.
- [6] T. Murachi, K. Tanaka, M. Hatanaka, and T. Murakami, "Intracellular Ca^{2+} -dependent protease (CALPAIN) and its high-molecular-weight endogenous inhibitor (CALPASTATIN)," *Advances in Enzyme Regulation*, vol. 19, pp. 407–424, 1980.
- [7] D. E. Goll, V. F. Thompson, H. Q. Li, W. Wei, and J. Y. Cong, "The calpain system," *Physiological Reviews*, vol. 83, no. 3, pp. 731–801, 2003.

- [8] Y. Ono, T. C. Saido, and H. Sorimachi, "Calpain research for drug discovery: Challenges and potential," *Nature Reviews Drug Discovery*, vol. 15, no. 12, pp. 854–876, 2016.
- [9] W. R. Dayton, D. E. Goll, M. G. Zeece, R. M. Robson, and W. J. Reville, "A Ca²⁺-activated protease possibly involved in myofibrillar protein turnover. purification from porcine muscle," *Biochemistry*, vol. 15, no. 10, pp. 2150–2158, 1976.
- [10] I. Nishiura, K. Tanaka, S. Yamato, and T. Murachi, "The occurrence of an inhibitor of Ca²⁺-dependent neutral protease in rat liver," *The Journal of Biochemistry*, vol. 84, no. 6, pp. 1657–1659, 1978.
- [11] A. Wendt, V. F. Thompson, and D. E. Goll, "Interaction of calpastatin with calpain: A review," *Biological Chemistry*, vol. 385, no. 6, pp. 465–472, 2004.
- [12] R. L. Campbell and P. L. Davies, "Structure-function relationships in calpains," *Biochemical Journal*, vol. 447, no. 3, pp. 335–351, 2012.
- [13] R. A. Hanna, R. L. Campbell, and P. L. Davies, "Calcium-bound structure of calpain and its mechanism of inhibition by calpastatin," *Nature*, vol. 456, no. 7220, pp. 409–412, 2008.
- [14] T. Moldoveanu, K. Gehring, and D. R. Green, "Concerted multi-pronged attack by calpastatin to occlude the catalytic cleft of heterodimeric calpains," *Nature*, vol. 456, no. 7220, pp. 404–408, 2008.
- [15] D. A. duVerle, Y. Ono, H. Sorimachi, and H. Mamitsuka, "Calpain cleavage prediction using multiple kernel learning," *PLoS ONE*, vol. 6, no. 5, p. e19035, 2011.
- [16] P. Tompa, P. Buzder-Lantos, A. Tantos et al., "On the sequential determinants of calpain cleavage," *The Journal of Biological Chemistry*, vol. 279, no. 20, pp. 20775–20785, 2004.
- [17] M. Baudry and X. Bi, "Calpain-1 and calpain-2: the yin and yang of synaptic plasticity and neurodegeneration," *Trends in Neurosciences*, vol. 39, no. 4, pp. 235–245, 2016.
- [18] J. Ji, L. Su, and Z. Liu, "Critical role of calpain in inflammation," *Biomedical Reports*, vol. 5, no. 6, pp. 647–652, 2016.
- [19] B. Ravikumar, S. Sarkar, J. E. Davies et al., "Regulation of mammalian autophagy in physiology and pathophysiology," *Physiological Reviews*, vol. 90, no. 4, pp. 1383–1435, 2010.
- [20] E. Gallardo, A. Saenz, and I. Illa, "Limb-girdle muscular dystrophy 2A," *Handbook of Clinical Neurology*, vol. 101, pp. 97–110, 2011.
- [21] I. Richard, O. Broux, V. Allamand et al., "Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A," *Cell*, vol. 81, no. 1, pp. 27–40, 1995.
- [22] V. B. Mahajan, J. M. Skeie, A. G. Bassuk et al., "Calpain-5 mutations cause autoimmune uveitis, retinal neovascularization, and photoreceptor degeneration," *PLoS Genetics*, vol. 8, no. 10, p. e1003001, 2012.
- [23] Q. Chen, M. Paillard, L. Gomez et al., "Activation of mitochondrial μ -calpain increases AIF cleavage in cardiac mitochondria during ischemia-reperfusion," *Biochemical and Biophysical Research Communications*, vol. 415, no. 4, pp. 533–538, 2011.
- [24] M. A. Smith and R. G. Schnellmann, "Calpains, mitochondria, and apoptosis," *Cardiovascular Research*, vol. 96, no. 1, pp. 32–37, 2012.
- [25] J. Thompson, Y. Hu, E. J. Lesnfsky, and Q. Chen, "Activation of mitochondrial calpain and increased cardiac injury: beyond AIF release," *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 310, no. 3, pp. H376–H384, 2016.
- [26] Y. Horikawa, N. Oda, N. J. Cox et al., "Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus," *Nature Genetics*, vol. 26, no. 2, pp. 163–175, 2000.
- [27] R. Bochner, L. Samuelov, O. Sarig et al., "Calpain 12 function revealed through the study of an atypical case of autosomal recessive congenital ichthyosis," *Journal of Investigative Dermatology*, vol. 137, no. 2, pp. 385–393, 2017.
- [28] S. J. Storr, N. O. Carragher, M. C. Frame, T. Parr, and S. G. Martin, "The calpain system and cancer," *Nature Reviews Cancer*, vol. 11, no. 5, pp. 364–374, 2011.
- [29] D. Moretti, B. Del Bello, G. Allavena, and E. Maellaro, "Calpains and cancer: Friends or enemies?" *Archives of Biochemistry and Biophysics*, vol. 564, pp. 26–36, 2014.
- [30] J. A. MacLeod, Y. Gao, C. Hall, W. J. Muller, T. S. Gujral, and P. A. Greer, "Genetic disruption of calpain-1 and calpain-2 attenuates tumorigenesis in mouse models of HER2+ breast cancer and sensitizes cancer cells to doxorubicin and lapatinib," *Oncotarget*, vol. 9, no. 70, pp. 33382–33395, 2018.
- [31] S. J. Storr, K. W. Lee, C. M. Woolston et al., "Calpain system protein expression in basal-like and triple-negative invasive breast cancer," *Annals of Oncology*, vol. 23, no. 9, pp. 2289–2296, 2012.
- [32] J. R. Friedman, H. E. Perry, K. C. Brown et al., "Capsaicin synergizes with camptothecin to induce increased apoptosis in human small cell lung cancers via the calpain pathway," *Biochemical Pharmacology*, vol. 129, pp. 54–66, 2017.
- [33] J. K. Lau, K. C. Brown, A. M. Dom et al., "Capsaicin induces apoptosis in human small cell lung cancer via the TRPV6 receptor and the calpain pathway," *Apoptosis*, vol. 19, no. 8, pp. 1190–1201, 2014.
- [34] R. A. Nixon, "The calpains in aging and aging-related diseases," *Ageing Research Reviews*, vol. 2, no. 4, pp. 407–418, 2003.
- [35] M. Ma, T. A. Ferguson, K. M. Schoch et al., "Calpains mediate axonal cytoskeleton disintegration during Wallerian degeneration," *Neurobiology of Disease*, vol. 56, pp. 34–46, 2013.
- [36] K. E. Saatman, J. Creed, and R. Raghupathi, "Calpain as a therapeutic target in traumatic brain injury," *Neurotherapeutics*, vol. 7, no. 1, pp. 31–42, 2010.
- [37] J. T. Wang, Z. A. Medress, and B. A. Barres, "Axon degeneration: molecular mechanisms of a self-destruction pathway," *The Journal of Cell Biology*, vol. 196, no. 1, pp. 7–18, 2012.
- [38] A. Ferreira, "Calpain dysregulation in alzheimer's disease," *ISRN Biochemistry*, vol. 2012, Article ID 728571, 12 pages, 2012.
- [39] C. A. Matos, L. P. de Almeida, and C. Nóbrega, "Proteolytic cleavage of polyglutamine disease-causing proteins: Revisiting the toxic fragment hypothesis," *Current Pharmaceutical Design*, vol. 23, no. 5, pp. 753–775, 2017.
- [40] S. Samantaray, S. Ray, and N. Banik, "Calpain as a potential therapeutic target in parkinsons disease," *CNS & Neurological Disorders - Drug Targets*, vol. 7, no. 3, pp. 305–312, 2008.
- [41] A. L. Wright and B. Vissel, "CAST your vote: Is calpain inhibition the answer to ALS?" *Journal of Neurochemistry*, vol. 137, no. 2, pp. 140–141, 2016.
- [42] B. M. Dufty, L. R. Warner, S. T. Hou et al., "Calpain-cleavage of α -synuclein: Connecting proteolytic processing to disease-linked aggregation," *The American Journal of Pathology*, vol. 170, no. 5, pp. 1725–1738, 2007.
- [43] J. Gafni and L. M. Ellerby, "Calpain activation in huntington's disease," *The Journal of Neuroscience*, vol. 22, no. 12, pp. 4842–4849, 2002.
- [44] A. Haacke, F. U. Hartl, and P. Breuer, "Calpain inhibition is sufficient to suppress aggregation of polyglutamine-expanded ataxin-3," *The Journal of Biological Chemistry*, vol. 282, no. 26, pp. 18851–18856, 2007.

- [45] J. Hübener, J. J. Weber, C. Richter et al., "Calpain-mediated ataxin-3 cleavage in the molecular pathogenesis of spinocerebellar ataxia type 3 (SCA3)," *Human Molecular Genetics*, vol. 22, no. 3, pp. 508–518, 2013.
- [46] T. Yamashita, T. Hideyama, K. Hachiga et al., "A role for calpain-dependent cleavage of TDP-43 in amyotrophic lateral sclerosis pathology," *Nature Communications*, vol. 3, p. 1307, 2012.
- [47] L. E. Clemens, J. J. Weber, T. T. Wlodkowski et al., "Olesoxime suppresses calpain activation and mutant huntingtin fragmentation in the BACHD rat," *Brain*, vol. 138, no. 12, pp. 3632–3653, 2015.
- [48] M. Diepenbroek, N. Casadei, H. Esmer et al., "Over expression of the calpain-specific inhibitor calpastatin reduces human alpha-Synuclein processing, aggregation and synaptic impairment in [A30P]αSyn transgenic mice," *Human Molecular Genetics*, vol. 23, no. 15, pp. 3975–3989, 2014.
- [49] J. Gafni, E. Hermel, J. E. Young, C. L. Wellington, M. R. Hayden, and L. M. Ellerby, "Inhibition of calpain cleavage of Huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus," *The Journal of Biological Chemistry*, vol. 279, no. 19, pp. 20211–20220, 2004.
- [50] A. T. Simões, N. Gonçalves, A. Koeppen et al., "Calpastatin-mediated inhibition of calpains in the mouse brain prevents mutant ataxin 3 proteolysis, nuclear localization and aggregation, relieving Machado-Joseph disease," *Brain*, vol. 135, no. 8, pp. 2428–2439, 2012.
- [51] J. J. Weber, M. Golla, G. Guaitoli et al., "A combinatorial approach to identify calpain cleavage sites in the Machado-Joseph disease protein ataxin-3," *Brain*, vol. 140, no. 5, pp. 1280–1299, 2017.
- [52] M. V. Rao, P. S. Mohan, C. M. Peterhoff et al., "Marked calpastatin (CAST) depletion in Alzheimer's disease accelerates cytoskeleton disruption and neurodegeneration: Neuroprotection by CAST overexpression," *The Journal of Neuroscience*, vol. 28, no. 47, pp. 12241–12254, 2008.
- [53] M. V. Rao, J. Campbell, A. Palaniappan, A. Kumar, and R. A. Nixon, "Calpastatin inhibits motor neuron death and increases survival of hSOD1G93A mice," *Journal of Neurochemistry*, vol. 137, no. 2, pp. 253–265, 2016.
- [54] M. V. Rao, M. K. McBrayer, J. Campbell et al., "Specific calpain inhibition by calpastatin prevents tauopathy and neurodegeneration and restores normal lifespan in tau P301L Mice," *The Journal of Neuroscience*, vol. 34, no. 28, pp. 9222–9234, 2014.
- [55] Y. Wang, J. Hersheson, D. Lopez et al., "Defects in the CAPN1 gene result in alterations in cerebellar development and cerebellar ataxia in mice and humans," *Cell Reports*, vol. 16, no. 1, pp. 79–91, 2016.
- [56] C. H. Ji and Y. T. Kwon, "Crosstalk and interplay between the ubiquitin-proteasome system and autophagy," *Molecules and Cells*, vol. 40, no. 7, pp. 441–449, 2017.
- [57] A. Lilienbaum, "Relationship between the proteasomal system and autophagy," *International Journal of Biochemistry and Molecular Biology*, vol. 4, no. 1, pp. 1–26, 2013.
- [58] G. L. Grice and J. A. Nathan, "The recognition of ubiquitinated proteins by the proteasome," *Cellular and Molecular Life Sciences*, vol. 73, no. 18, pp. 3497–3506, 2016.
- [59] A. Ciechanover, "Proteolysis: from the lysosome to ubiquitin and the proteasome," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 1, pp. 79–87, 2005.
- [60] S. Kaushik, U. Bandyopadhyay, S. Sridhar et al., "Chaperone-mediated autophagy at a glance," *Journal of Cell Science*, vol. 124, no. 4, pp. 495–499, 2011.
- [61] J. F. Dice, "Chaperone-mediated autophagy," *Autophagy*, vol. 3, no. 4, pp. 295–299, 2007.
- [62] W. W. Li, J. Li, and J. K. Bao, "Microautophagy: lesser-known self-eating," *Cellular and Molecular Life Sciences*, vol. 69, no. 7, pp. 1125–1136, 2012.
- [63] B. Boland, A. Kumar, S. Lee et al., "Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease," *The Journal of Neuroscience*, vol. 28, no. 27, pp. 6926–6937, 2008.
- [64] C. He and D. J. Klionsky, "Regulation mechanisms and signaling pathways of autophagy," *Annual Review of Genetics*, vol. 43, pp. 67–93, 2009.
- [65] Z. Xie and D. J. Klionsky, "Autophagosome formation: core machinery and adaptations," *Nature Cell Biology*, vol. 9, no. 10, pp. 1102–1109, 2007.
- [66] D. Egan, J. Kim, R. J. Shaw, and K.-L. Guan, "The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR," *Autophagy*, vol. 7, no. 6, pp. 643–644, 2011.
- [67] J. Kim, M. Kundu, B. Viollet, and K. Guan, "AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1," *Nature Cell Biology*, vol. 13, no. 2, pp. 132–141, 2011.
- [68] J.-M. Park, C. H. Jung, M. Seo et al., "The ULK1 complex mediates MTORC1 signaling to the autophagy initiation machinery via binding and phosphorylating ATG14," *Autophagy*, vol. 12, no. 3, pp. 547–564, 2016.
- [69] R. C. Russell, Y. Tian, H. Yuan et al., "ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase," *Nature Cell Biology*, vol. 15, no. 7, pp. 741–750, 2013.
- [70] Y. Ohsumi, "Molecular dissection of autophagy: Two ubiquitin-like systems," *Nature Reviews Molecular Cell Biology*, vol. 2, no. 3, pp. 211–216, 2001.
- [71] N. Fujita, T. Itoh, H. Omori, M. Fukuda, T. Noda, and T. Yoshimori, "The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy," *Molecular Biology of the Cell (MBoC)*, vol. 19, no. 5, pp. 2092–2100, 2008.
- [72] J. Hemelaar, V. S. Lelyveld, B. M. Kessler, and H. L. Ploegh, "A single protease, Apg4B, is specific for the autophagy-related ubiquitin-like proteins GATE-16, MAPI-LC3, GABARAP, and Apg8L," *The Journal of Biological Chemistry*, vol. 278, no. 51, pp. 51841–51850, 2003.
- [73] N. Mizushima, A. Kuma, Y. Kobayashi et al., "Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate," *Journal of Cell Science*, vol. 116, no. 9, pp. 1679–1688, 2003.
- [74] N. Mizushima, A. Yamamoto, M. Hatano et al., "Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells," *The Journal of Cell Biology*, vol. 152, no. 4, pp. 657–667, 2001.
- [75] V. Cohen-Kaplan, I. Livneh, N. Avni et al., "p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 47, pp. E7490–E7499, 2016.
- [76] L. Galluzzi, E. H. Baehrecke, A. Ballabio et al., "Molecular definitions of autophagy and related processes," *EMBO Journal*, vol. 36, no. 13, pp. 1811–1836, 2017.
- [77] I. Kim, S. Rodriguez-Enriquez, and J. J. Lemasters, "Selective degradation of mitochondria by mitophagy," *Archives of Biochemistry and Biophysics*, vol. 462, no. 2, pp. 245–253, 2007.

- [78] T. Johansen and T. Lamark, "Selective autophagy mediated by autophagic adapter proteins," *Autophagy*, vol. 7, no. 3, pp. 279–296, 2011.
- [79] A. Stolz, A. Ernst, and I. Dikic, "Cargo recognition and trafficking in selective autophagy," *Nature Cell Biology*, vol. 16, no. 6, pp. 495–501, 2014.
- [80] K. Lu, I. Psakhye, and S. Jentsch, "A new class of ubiquitin-Atg8 receptors involved in selective autophagy and polyQ protein clearance," *Autophagy*, vol. 10, no. 12, pp. 2381–2382, 2014.
- [81] F. M. Menzies, A. Fleming, A. Caricasole et al., "Autophagy and Neurodegeneration: Pathogenic Mechanisms and Therapeutic Opportunities," *Neuron*, vol. 93, no. 5, pp. 1015–1034, 2017.
- [82] Y. Tanaka, G. Guhde, A. Suter et al., "Accumulation of autophagic vacuoles and cardiomyopathy LAMP-2-deficient mice," *Nature*, vol. 406, no. 6798, pp. 902–906, 2000.
- [83] A. Ciechanover and Y. T. Kwon, "Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies," *Experimental & Molecular Medicine*, vol. 47, no. 3, p. e147, 2015.
- [84] N. Mizushima, B. Levine, A. M. Cuervo, and D. J. Klionsky, "Autophagy fights disease through cellular self-digestion," *Nature*, vol. 451, no. 7182, pp. 1069–1075, 2008.
- [85] G. Kroemer and B. Levine, "Autophagic cell death: the story of a misnomer," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 12, pp. 1004–1010, 2008.
- [86] A. T. Vessoni, E. C. Filippi-Chiela, C. F. M. Menck, and G. Lenz, "Autophagy and genomic integrity," *Cell Death & Differentiation*, vol. 20, no. 11, pp. 1444–1454, 2013.
- [87] J. M. M. Levy, C. G. Towers, and A. Thorburn, "Targeting autophagy in cancer," *Nature Reviews Cancer*, vol. 17, no. 9, pp. 528–542, 2017.
- [88] G. Maria Fimia, A. Stoykova, A. Romagnoli et al., "Ambral regulates autophagy and development of the nervous system," *Nature*, vol. 447, no. 7148, pp. 1121–1125, 2007.
- [89] M. Komatsu, J. W. Qing, G. R. Holstein et al., "Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 36, pp. 14489–14494, 2007.
- [90] C. Wang, C.-C. Liang, Z. C. Bian, Y. Zhu, and J.-L. Guan, "FIP200 is required for maintenance and differentiation of postnatal neural stem cells," *Nature Neuroscience*, vol. 16, no. 5, pp. 532–542, 2013.
- [91] A. Kuma, M. Hatano, M. Matsui et al., "The role of autophagy during the early neonatal starvation period," *Nature*, vol. 432, no. 7020, pp. 1032–1036, 2004.
- [92] S. Carloni, G. Buonocore, and W. Balduini, "Protective role of autophagy in neonatal hypoxia-ischemia induced brain injury," *Neurobiology of Disease*, vol. 32, no. 3, pp. 329–339, 2008.
- [93] S. Erlich, A. Alexandrovich, E. Shohami, and R. Pinkas-Kramarski, "Rapamycin is a neuroprotective treatment for traumatic brain injury," *Neurobiology of Disease*, vol. 26, no. 1, pp. 86–93, 2007.
- [94] T. Hara, K. Nakamura, M. Matsui et al., "Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice," *Nature*, vol. 441, no. 7095, pp. 885–889, 2006.
- [95] M. Komatsu, S. Waguri, T. Chiba et al., "Loss of autophagy in the central nervous system causes neurodegeneration in mice," *Nature*, vol. 441, no. 7095, pp. 880–884, 2006.
- [96] D. C. Rubinsztein, G. Mariño, and G. Kroemer, "Autophagy and aging," *Cell*, vol. 146, no. 5, pp. 682–695, 2011.
- [97] E. Wong and A. M. Cuervo, "Autophagy gone awry in neurodegenerative diseases," *Nature Neuroscience*, vol. 13, no. 7, pp. 805–811, 2010.
- [98] D. C. Rubinsztein, M. DiFiglia, N. Heintz et al., "Autophagy and its possible roles in nervous system diseases, damage and repair," *Autophagy*, vol. 1, no. 1, pp. 11–22, 2005.
- [99] A. Sittler, M.-P. Muriel, M. Marinello, A. Brice, W. den Dunnen, and S. Alves, "Deregulation of autophagy in postmortem brains of Machado-Joseph disease patients," *Neuropathology*, vol. 38, no. 2, pp. 113–124, 2018.
- [100] X.-J. Zhang, S. Chen, K.-X. Huang, and W.-D. Le, "Why should autophagic flux be assessed?" *Acta Pharmacologica Sinica*, vol. 34, no. 5, pp. 595–599, 2013.
- [101] E. Sapp, C. Schwarz, K. Chase et al., "Huntingtin localization in brains of normal and Huntington's disease patients," *Annals of Neurology*, vol. 42, no. 4, pp. 604–612, 1997.
- [102] E. Nagata, A. Sawa, C. A. Ross, and S. H. Snyder, "Autophagosome-like vacuole formation in Huntington's disease lymphoblasts," *NeuroReport*, vol. 15, no. 8, pp. 1325–1328, 2004.
- [103] S. Sarkar and D. C. Rubinsztein, "Huntington's disease: Degradation of mutant huntingtin by autophagy," *FEBS Journal*, vol. 275, no. 17, pp. 4263–4270, 2008.
- [104] M. Martinez-Vicente, Z. Tallozy, E. Wong et al., "Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease," *Nature Neuroscience*, vol. 13, no. 5, pp. 567–576, 2010.
- [105] A. Hodges, A. D. Strand, A. K. Aragaki et al., "Regional and cellular gene expression changes in human Huntington's disease brain," *Human Molecular Genetics*, vol. 15, no. 6, pp. 965–977, 2006.
- [106] M. Shibata, T. Lu, T. Furuya et al., "Regulation of intracellular accumulation of mutant huntingtin by beclin 1," *The Journal of Biological Chemistry*, vol. 281, no. 20, pp. 14474–14485, 2006.
- [107] S. Subramaniam and S. H. Snyder, "Huntington's Disease is a disorder of the corpus striatum: Focus on Rhes (Ras homologue enriched in the striatum)," *Neuropharmacology*, vol. 60, no. 7–8, pp. 1187–1192, 2011.
- [108] S. Sarkar, B. Ravikumar, R. A. Floto, and D. C. Rubinsztein, "Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies," *Cell Death & Differentiation*, vol. 16, no. 1, pp. 46–56, 2009.
- [109] B. Ravikumar, C. Vacher, Z. Berger et al., "Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease," *Nature Genetics*, vol. 36, no. 6, pp. 585–595, 2004.
- [110] A. Fleming, T. Noda, T. Yoshimori, and D. C. Rubinsztein, "Chemical modulators of autophagy as biological probes and potential therapeutics," *Nature Chemical Biology*, vol. 7, no. 1, pp. 9–17, 2011.
- [111] F. M. Menzies, J. Huebener, M. Renna, M. Bonin, O. Riess, and D. C. Rubinsztein, "Autophagy induction reduces mutant ataxin-3 levels and toxicity in a mouse model of spinocerebellar ataxia type 3," *Brain*, vol. 133, no. 1, pp. 93–104, 2010.
- [112] P. Spilman, N. Podlutskaya, M. J. Hart et al., "Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease," *PLoS ONE*, vol. 5, no. 4, p. e9979, 2010.
- [113] B. Spencer, R. Potkar, M. Trejo et al., "Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in α -synuclein models of Parkinson's and Lewy body

- diseases," *The Journal of Neuroscience*, vol. 29, no. 43, pp. 13578–13588, 2009.
- [114] M. S. Kindy, J. Yu, H. Zhu, S. S. El-Amouri, V. Hook, and G. R. Hook, "Deletion of the cathepsin B gene improves memory deficits in a transgenic alzheimer's disease mouse model expressing A β PP containing the wild-type β -secretase site sequence," *Journal of Alzheimer's Disease*, vol. 29, no. 4, pp. 827–840, 2012.
- [115] T. Ratovitski, E. Chighladze, E. Waldron, R. R. Hirschhorn, and C. A. Ross, "Cysteine proteases bleomycin hydrolase and cathepsin Z mediate N-terminal proteolysis and toxicity of mutant huntingtin," *The Journal of Biological Chemistry*, vol. 286, no. 14, pp. 12578–12589, 2011.
- [116] J. S. Steffan, "Does Huntingtin play a role in selective macroautophagy?" *Cell Cycle*, vol. 9, no. 17, pp. 3401–3413, 2010.
- [117] J. Ochaba, T. Lukacsovich, G. Csikos et al., "Potential function for the Huntingtin protein as a scaffold for selective autophagy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 47, pp. 16889–16894, 2014.
- [118] Y.-N. Rui, Z. Xu, B. Patel et al., "Huntingtin functions as a scaffold for selective macroautophagy," *Nature Cell Biology*, vol. 17, no. 3, pp. 262–275, 2015.
- [119] A. Ashkenazi, C. F. Bento, T. Ricketts et al., "Polyglutamine tracts regulate beclin 1-dependent autophagy," *Nature*, vol. 545, no. 7652, pp. 108–111, 2017.
- [120] I. Onofre, N. Mendonça, S. Lopes et al., "Fibroblasts of Machado Joseph Disease patients reveal autophagy impairment," *Scientific Reports*, vol. 6, no. 1, p. 28220, 2016.
- [121] M. M. Lipinski, B. Zheng, T. Lu et al., "Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 32, pp. 14164–14169, 2010.
- [122] F. Pickford, E. Masliah, M. Britschgi et al., "The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid β accumulation in mice," *The Journal of Clinical Investigation*, vol. 118, no. 6, pp. 2190–2199, 2008.
- [123] M. S. Uddin, A. Stachowiak, A. Al Mamun et al., "Autophagy and Alzheimer's disease: From molecular mechanisms to therapeutic implications," *Frontiers in Aging Neuroscience*, vol. 10, p. 04, 2018.
- [124] D.-S. Yang, P. Stavrides, P. S. Mohan et al., "Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits," *Brain*, vol. 134, no. 1, pp. 258–277, 2011.
- [125] F. Gao, J. Yang, D. Wang et al., "Mitophagy in parkinson's disease: pathogenic and therapeutic implications," *Frontiers in Neurology*, vol. 8, p. 527, 2017.
- [126] B. Wang, N. Abraham, G. Gao, and Q. Yang, "Dysregulation of autophagy and mitochondrial function in Parkinson's disease," *Translational Neurodegeneration*, vol. 5, no. 1, p. 19, 2016.
- [127] C. Malagelada, Z. H. Jin, V. Jackson-Lewis, S. Przedborski, and L. A. Greene, "Rapamycin protects against neuron death in vitro and in vivo models of Parkinson's disease," *The Journal of Neuroscience*, vol. 30, no. 3, pp. 1166–1175, 2010.
- [128] E. Santini, M. Heiman, P. Greengard, E. Valjent, and G. Fisone, "Inhibition of mTOR signaling in parkinson's disease prevents L-DOPA-induced dyskinesia," *Science Signaling*, vol. 2, no. 80, p. ra36, 2009.
- [129] Y. C. Wong and E. L. F. Holzbaur, "Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 42, pp. E4439–E4448, 2014.
- [130] P. M. Sullivan, X. Zhou, A. M. Robins et al., "The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway," *Acta Neuropathologica Communications*, vol. 4, no. 1, p. 51, 2016.
- [131] A. Williams, S. Sarkar, P. Cuddon et al., "Novel targets for Huntington's disease in an mTOR-independent autophagy pathway," *Nature Chemical Biology*, vol. 4, no. 5, pp. 295–305, 2008.
- [132] P. Rivero-Ríos, J. Madero-Pérez, B. Fernández, and S. Hilfiker, "Targeting the autophagy/lysosomal degradation pathway in Parkinson's disease," *Current Neuropharmacology*, vol. 14, no. 3, pp. 238–249, 2016.
- [133] H. G. Xia, L. Zhang, G. Chen et al., "Control of basal autophagy by calpain1 mediated cleavage of ATG5," *Autophagy*, vol. 6, no. 1, pp. 61–66, 2010.
- [134] S. Yousefi, R. Perozzo, I. Schmid et al., "Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis," *Nature Cell Biology*, vol. 8, no. 10, pp. 1124–1132, 2006.
- [135] J.-S. Kim, T. Nitta, D. Mohuczy et al., "Impaired autophagy: a mechanism of mitochondrial dysfunction in anoxic rat hepatocytes," *Hepatology*, vol. 47, no. 5, pp. 1725–1736, 2008.
- [136] R. Russo, L. Berliocchi, A. Adornetto et al., "Calpain-mediated cleavage of Beclin-1 and autophagy deregulation following retinal ischemic injury in vivo," *Cell Death & Disease*, vol. 2, no. 4, p. e144, 2011.
- [137] Q. Zhao, Z. Guo, W. Deng et al., "Calpain 2-mediated autophagy defect increases susceptibility of fatty livers to ischemia-reperfusion injury," *Cell Death & Disease*, vol. 7, no. 4, p. e2186, 2016.
- [138] J. M. Norman, G. M. Cohen, and E. T. W. Bampton, "The in vitro cleavage of the hAtg proteins by cell death proteases," *Autophagy*, vol. 6, no. 8, pp. 1042–1056, 2010.
- [139] A. Colunga, D. Bollino, A. Schech, and L. Aurelian, "Calpain-dependent clearance of the autophagy protein p62/SQSTM1 is a contributor to Δ pK oncolytic activity in melanoma," *Gene Therapy*, vol. 21, no. 4, pp. 371–378, 2014.
- [140] C. Kim, N. Yun, Y. M. Lee et al., "Gel-Based protease proteomics for identifying the novel calpain substrates in dopaminergic neuronal cell," *The Journal of Biological Chemistry*, vol. 288, no. 51, pp. 36717–36732, 2013.
- [141] C. Gerónimo-Olvera, T. Montiel, R. Rincon-Heredia, S. Castro-Obregón, and L. Massieu, "Autophagy fails to prevent glucose deprivation/glucose reintroduction-induced neuronal death due to calpain-mediated lysosomal dysfunction in cortical neurons," *Cell death & disease*, vol. 8, no. 6, p. e2911, 2017.
- [142] G. E. Villalpando Rodriguez and A. Torriglia, "Calpain 1 induce lysosomal permeabilization by cleavage of lysosomal associated membrane protein 2," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1833, no. 10, pp. 2244–2253, 2013.
- [143] F. Demarchi, C. Bertoli, T. Copetti, E.-L. Eskelinen, and C. Schneider, "Calpain as a novel regulator of autophagosome formation," *Autophagy*, vol. 3, no. 3, pp. 235–237, 2007.
- [144] F. Demarchi, C. Bertoli, T. Copetti et al., "Calpain is required for macroautophagy in mammalian cells," *The Journal of Cell Biology*, vol. 175, no. 4, pp. 595–605, 2006.
- [145] E. Marcassa, M. Raimondi, T. Anwar et al., "Calpain mobilizes Atg9/Bif-1 vesicles from golgi stacks upon autophagy induction by thapsigargin," *Biology Open*, vol. 6, no. 5, pp. 551–562, 2017.

- [146] U.-J. P. Zimmerman, L. Boring, U. H. Pak, N. Mukerjee, and K. K. W. Wang, "The calpain small subunit gene is essential: Its inactivation results in embryonic lethality," *IUBMB Life*, vol. 50, no. 1, pp. 63–68, 2000.
- [147] K. Pillay and P. Govender, "Amylin uncovered: A review on the polypeptide responsible for type II diabetes," *BioMed Research International*, vol. 2013, Article ID 826706, 17 pages, 2013.
- [148] M. Masini, M. Bugliani, R. Lupi et al., "Autophagy in human type 2 diabetes pancreatic beta cells," *Diabetologia*, vol. 52, no. 6, pp. 1083–1086, 2009.
- [149] J. F. Rivera, S. Costes, T. Gurlo, C. G. Glabe, and P. C. Butler, "Autophagy defends pancreatic β cells from human islet amyloid polypeptide-induced toxicity," *The Journal of Clinical Investigation*, vol. 124, no. 8, pp. 3489–3500, 2014.
- [150] T. Gurlo, S. Costes, J. D. Hoang, J. F. Rivera, A. E. Butler, and P. C. Butler, " β Cell-specific increased expression of calpastatin prevents diabetes induced by islet amyloid polypeptide toxicity," *JCI Insight*, vol. 1, no. 18, p. e89590, 2016.
- [151] C. J. Huang, T. Gurlo, L. Haataja et al., "Calcium-activated calpain-2 is a mediator of beta cell dysfunction and apoptosis in type 2 diabetes," *The Journal of Biological Chemistry*, vol. 285, no. 1, pp. 339–348, 2010.
- [152] M. V. Hoang, L. E. Smith, and D. R. Senger, "Calpain inhibitors reduce retinal hypoxia in ischemic retinopathy by improving neovascular architecture and functional perfusion," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1812, no. 4, pp. 549–557, 2011.
- [153] V. Kohli, W. Gao, C. A. Camargo, and P. A. Clavien, "Calpain is a mediator of preservation-reperfusion injury in rat liver transplantation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 17, pp. 9354–9359, 1997.
- [154] C. Neuhof and H. Neuhof, "Calpain system and its involvement in myocardial ischemia and reperfusion injury," *World Journal of Cardiology*, vol. 6, no. 7, pp. 638–652, 2014.
- [155] L. Yang, P. Li, S. Fu, E. S. Calay, and G. S. Hotamisligil, "Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance," *Cell Metabolism*, vol. 11, no. 6, pp. 467–478, 2010.
- [156] S. K. Bhutia, R. Dash, S. K. Das et al., "Mechanism of autophagy to apoptosis switch triggered in prostate cancer cells by antitumor cytokine melanoma differentiation-associated gene 7/interleukin-24," *Cancer Research*, vol. 70, no. 9, pp. 3667–3676, 2010.
- [157] S. Lépine, J. C. Allegood, Y. Edmonds, S. Milstien, and S. Spiegel, "Autophagy induced by deficiency of sphingosine-1-phosphate phosphohydrolase 1 is switched to apoptosis by calpain-mediated autophagy-related gene 5 (Atg5) cleavage," *The Journal of Biological Chemistry*, vol. 286, no. 52, pp. 44380–44390, 2011.
- [158] B. Del Bello, D. Moretti, A. Gamberucci, and E. Maellaro, "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*, vol. 26, no. 19, pp. 2717–2726, 2007.
- [159] B. Del Bello, M. Toscano, D. Moretti, and E. Maellaro, "Cisplatin-induced apoptosis inhibits autophagy, which acts as a pro-survival mechanism in human melanoma cells," *PLoS ONE*, vol. 8, no. 2, p. e57236, 2013.
- [160] A. Marion, F.-X. Dieudonné, A. Patiño-García, F. Lecanda, P. J. Marie, and D. Modrowski, "Calpain-6 is an endothelin-1 signaling dependent protective factor in chemoresistant osteosarcoma," *International Journal of Cancer*, vol. 130, no. 11, pp. 2514–2525, 2012.
- [161] C. Andrique, L. Morardet, L. K. Linares et al., "Calpain-6 controls the fate of sarcoma stem cells by promoting autophagy and preventing senescence," *JCI Insight*, vol. 3, no. 17, 2018.
- [162] R. Santarelli, M. Granato, G. Pentassuglia et al., "KSHV reduces autophagy in THP-1 cells and in differentiating monocytes by decreasing CAST/calpastatin and ATG5 expression," *Autophagy*, vol. 12, no. 12, pp. 2311–2325, 2016.
- [163] D. D. O. Martin, R. J. Heit, M. C. Yap, M. W. Davidson, M. R. Hayden, and L. G. Berthiaume, "Identification of a post-translationally myristoylated autophagy-inducing domain released by caspase cleavage of huntingtin," *Human Molecular Genetics*, vol. 23, no. 12, pp. 3166–3179, 2014.
- [164] F. M. Menzies, M. Garcia-Arencibia, S. Imarisio et al., "Calpain inhibition mediates autophagy-dependent protection against polyglutamine toxicity," *Cell Death & Differentiation*, vol. 22, no. 3, pp. 433–444, 2015.
- [165] J. J. Weber, M. M. Ortiz Rios, O. Riess, L. E. Clemens, and H. P. Nguyen, "The calpain-suppressing effects of olesoxime in Huntington's disease," *Rare Diseases*, vol. 4, no. 1, p. e1153778, 2016.
- [166] M. Watchon, K. C. Yuan, N. Mackovski et al., "Calpain inhibition is protective in machado-joseph disease zebrafish due to induction of autophagy," *The Journal of Neuroscience*, vol. 37, no. 32, pp. 7782–7794, 2017.
- [167] S. Nuber, D. Tadros, J. Fields et al., "Environmental neurotoxic challenge of conditional alpha-synuclein transgenic mice predicts a dopaminergic olfactory-striatal interplay in early PD," *Acta Neuropathologica*, vol. 127, no. 4, pp. 477–494, 2014.
- [168] K. M. Chung, H. Park, S. Jung et al., "Calpain determines the propensity of adult hippocampal neural stem cells to autophagic cell death following insulin withdrawal," *Stem Cells*, vol. 33, no. 10, pp. 3052–3064, 2015.
- [169] J. J. Weber, S. J. Kloock, M. Nagel et al., "Calpastatin ablation aggravates the molecular phenotype in cell and animal models of Huntington disease," *Neuropharmacology*, vol. 133, pp. 94–106, 2018.
- [170] M. L. Tradewell and H. D. Durham, "Calpastatin reduces toxicity of SODIG93A in a culture model of amyotrophic lateral sclerosis," *NeuroReport*, vol. 21, no. 15, pp. 976–979, 2010.
- [171] A. S. Galvez, A. Diwan, A. M. Odley et al., "Cardiomyocyte degeneration with calpain deficiency reveals a critical role in protein homeostasis," *Circulation Research*, vol. 100, no. 7, pp. 1071–1078, 2007.
- [172] F. Wan, E. Letavernier, C. J. Le Saux et al., "Calpastatin overexpression impairs postinfarct scar healing in mice by compromising reparative immune cell recruitment and activation," *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 309, no. 11, pp. H1883–H1893, 2015.
- [173] J.-S. Kim, J.-H. Wang, T. G. Biel et al., "Carbamazepine suppresses calpain-mediated autophagy impairment after ischemia/reperfusion in mouse livers," *Toxicology and Applied Pharmacology*, vol. 273, no. 3, pp. 600–610, 2013.
- [174] A. Liu, E. Guo, J. Yang et al., "Ischemic preconditioning attenuates ischemia/reperfusion injury in rat steatotic liver: Role of heme oxygenase-1-mediated autophagy," *Oncotarget*, vol. 7, no. 48, pp. 78372–78386, 2016.