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The Multiple Faces of Valosin-Containing Protein-Associated Diseases: Inclusion Body Myopathy with Paget's Disease of Bone, Frontotemporal Dementia, and Amyotrophic Lateral Sclerosis

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Abstract Inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD) is a progressive, fatal genetic disorder with variable penetrance, predominantly affecting three main tissue types: muscle (IBM), bone (PDB), and brain (FTD). IBMPFD is caused by mutations in the ubiquitously expressed valosin-containing protein (VCP) gene, a member of the AAA-ATPase superfamily. The majority of individuals who develop IBM have progressive proximal muscle weakness. Muscle biopsies reveal rimmed vacuoles and inclusions that are ubiquitin- and TAR DNA binding protein-43 (TDP-43)-positive using immunohistochemistry. PDB, seen in half the individuals, is caused by overactive osteoclasts and is associated clinically with pain, elevated serum alkaline phosphatase, and X-ray findings of coarse trabeculation and sclerotic lesions. FTD diagnosed at a mean age of 55 years in a third of individuals is characterized clinically by comprehension deficits, dysnomia, dyscalculia, and social unawareness. Ubiquitin- and TDP-43-positive neuronal inclusions are also found in the brain. Genotype–phenotype correlations are difficult with marked intra-familial and inter-familial variations being seen. Varied phenotypes within families include frontotemporal dementia, amyotrophic lateral sclerosis, Parkinsonism, myotonia, cataracts, and anal incompetence, among others. Cellular and animal models indicate pathogenetic disturbances in IBMPFD tissues including altered protein degradation, autophagy pathway alterations, apoptosis, and mitochondrial dysfunction. Currently, mouse and drosophila models carrying VCP mutations provide insights into the human IBMPFD pathology and are useful as tools for preclinical studies and testing of therapeutic strategies. In this review, we will explore the pathogenesis and clinical phenotype of IBMPFD caused by VCP mutations.

Keywords Valosin containing protein · Amyotrophic lateral sclerosis · Inclusion body myopathy · Paget's disease of bone · Frontotemporal dementia · Autophagy · NFKB · Ubiquitin · TAR DNA Binding Protein-43 · Endoplasmic reticulum associated degradation

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Introduction

Clinical Presentation: VCP and IBMPFD

Inclusion body myopathy associated with Paget's disease and frontotemporal dementia (IBMPFD; OMIM 167320) is a complex, autosomal dominant disorder identified by Kimonis et al. in 2000 (Kimonis et al. 2000; Watts et al. 2004), attributable to mutations in the valosin-containing protein (VCP) gene (Kimonis et al. 2000; Watts et al. 2004). This rare and progressive disease is characterized by three main clinical features found alone or in combination in affected individuals: myopathy, Paget's disease of bone, and frontotemporal dementia. Myopathy is the most common feature present in 80–90% of affected individuals with an age of onset in the 30s to 40s. Patients typically demonstrate progressive weakness and atrophy of the skeletal muscles of the pelvic and shoulder girdles (Fig. 1a, b). Muscle weakness progresses to involve other limb and, importantly, respiratory muscles and heart, with death from respiratory failure or cardiomyopathy and cardiac failure typically in their 40s to 60s.

Serum creatinine kinase concentration is usually normal to mildly elevated and electromyography shows myopathic changes and, in some individuals, neuropathic changes typical of amyotrophic lateral sclerosis (ALS). Histology of the affected muscle demonstrates rimmed vacuoles, and both myonuclear and sarcoplasmic inclusion bodies are immunoreactive for ubiquitin and TAR DNA binding protein-43 (TDP-43) (Kimonis et al. 2008a; Watts et al. 2004).

Paget's Disease of Bone

Paget's disease of bone (PDB) is observed in half the patients (Kimonis et al. 2000; Watts et al. 2003), typically with an early onset in the 30s to 40s. PDB is caused by excessive osteoclastic activity and increased bone turnover, resulting in susceptibility to long bone deformities (such as bowing) and fractures. PDB is often focal, and the bones most commonly involved are the skull, vertebrae, and pelvis (Fig. 1d). Pagetoid osteoclasts contain ubiquitinated nuclear and cytoplasmic inclusions. The diagnosis of PDB is based on elevated serum alkaline phosphatase, diagnostic changes of coarse trabeculation, cortical thickening, and spotty sclerosis in the skull, pelvis, hips, spine, and/or scapulae or typical findings of radionuclide scans. These findings may be present 10–15 years before the diagnosis of PDB can be made in pre-symptomatic individuals (Farpour et al. 2011; Kimonis et al. 2008b). Oral and intravenous bisphosphonates such as Zoledronic acid, known for their potent ability to suppress osteoclastic activity (Reid et al. 2005; Siris et al. 1996), are effective and potentially may be useful in preventing PDB in individuals at risk of VCP-associated PDB.

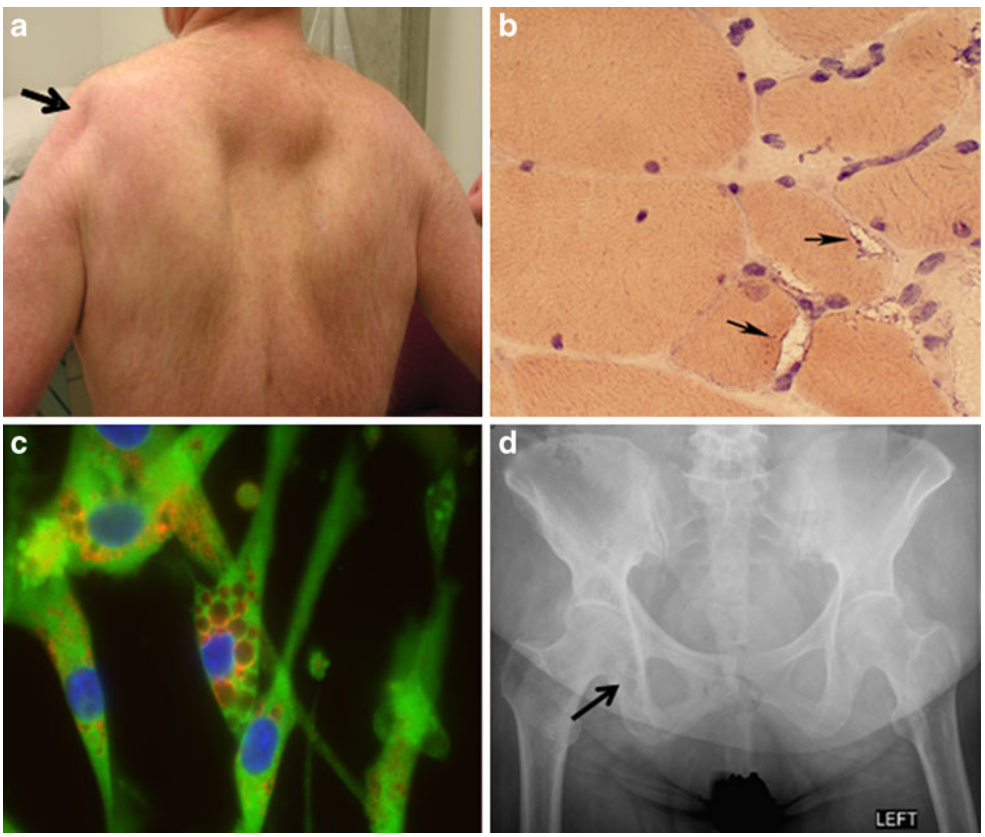

Fig. 1 Characteristics of IBMPFD. **a** Scapular winging and deltoid wasting (*arrow*) in a male with proximal limb girdle weakness. **b** Muscle biopsy from a patient with VCP myopathy showing two rimmed vacuoles (*arrows*). **c** Human patient mutant myoblasts were stained with VCP and LAMP-1 antibodies. Nuclei were stained with DAPI (magnification, ×63). IBMPFD patient myoblasts show marked vacuolization and LAMP staining, suggesting that the vacuoles are autophagosomic in origin. **d** Bone X-ray image of a 59-year-old female depicting coarse trabecular pattern and lytic changes of the right ileum (*arrow*), suggestive of Paget's disease of bone

Frontotemporal Dementia

Frontotemporal dementia (FTD) is a degenerative condition of the brain that primarily affects the frontal and anterior temporal lobes (Arnold et al. 2000; Turner et al. 1996; Zhukareva et al. 2001). FTD is associated with alterations in complex reasoning, mental flexibility and set shifting, working memory, judgment, expressive speech, and social awareness and regulation with relative preservation of memory. Common neuropathological findings in FTD are atrophy and neuronal loss in the frontal and temporal lobes. Approximately 55% of patients have ubiquitin-positive inclusions and 45% have tau-positive inclusions (Leyton and Hodges 2010). TDP-43 is a major component of inclusions characteristic of VCP-associated FTD and ALS pathology, placing VCP disease in a novel category of neurodegenerative diseases termed TDP-43 proteinopathies (Cairns et al. 2007; van der Zee et al. 2009).

In VCP disease, clinical FTD is observed in a third of patients with an average age of onset in the mid-50s and is characterized by dysnomia, dyscalculia, comprehension deficit, paraphasic errors, and relative preservation of memory. In later stages, auditory comprehension deficits for simpler one-step commands, alexia, and agraphia may be seen (Kimonis et al. 2000; Watts et al. 2003). The diagnosis of FTD requires a thorough understanding of family history of dementia, with assessment of behavioral and personality changes, in addition to neuropsychological testing. The frontal assessment battery is useful at the bedside to supplement the neurologic examination and the Frontal Behavioral Inventory checklist given to the caregiver to cover common behavioral symptoms. Currently, there are no curative treatments for any variant of FTD. However, selective serotonin reuptake inhibitors are sometimes used in patients to control obsessive symptoms and mood (Roberson 2011). Thus, there is an urgent need to develop therapeutic strategies and novel agents for the disease.

Less common phenotypic features that have been reported in VCP disease include cardiomyopathy, hepatic steatosis, cataracts, sensory motor axonal neuropathy, pyramidal tract dysfunction, sphincter disturbance, sensorineural hearing loss, and amyotrophic lateral sclerosis-like features (Djamshidian et al. 2009; Guyant-Marechal et al. 2006; Haubenberger et al. 2005; Hubbers et al. 2007; Kumar et al. 2010; Miller et al. 2009).

VCP and ALS

ALS is an adult-onset progressive neurodegenerative disease involving both upper and lower motor neuron degeneration (Leigh and Wijesekera 2010). Patients typically present asymmetric focal weakness of the extremities or bulbar findings (dysarthria, dysphagia). Muscle atrophy and weakness progress to affect other muscles, ultimately leading to death from respiratory failure 3–5 years after symptom onset. ALS affects all populations, having a lifetime risk of 1 in 350 to 1 in 600 for males and 1 in 450 to 1 in 800 for females, with a reported population incidence of 1.5–2.5 per 100,000 per year (Alonso et al. 2009; Logroscino et al. 2008; McGuire et al. 1996; Traynor et al. 1999).

About 5% of individuals with ALS meet the clinical criteria of Neary et al. (1998) for a diagnosis of FTD. Impairment in executive functioning is seen in 30% to 50% of individuals with ALS, but do not meet these criteria (Lomen-Hoerth et al. 2003; Strong et al. 2003). Approximately 5–10% of ALS patients have familial amyotrophic lateral sclerosis (fALS). The remainder of patients has simplex or sporadic disease, the etiology of which remains unknown. In familial fALS, 15% of cases are attributable to mutations in the superoxide dismutase (SOD1) gene, and 3% of cases are due to pathogenic variants in the TDP-43 or FUS/TLS genes (Lagier-Tourenne et al. 2010). TDP-43 has been identified as a major disease protein in ubiquitinated inclusions, and recently, mutations have been found in ALS (Ling et al. 2010). Pathological TDP-43 is hyperphosphorylated and ubiquitinated from the affected central nervous system regions in sporadic and familial ALS (Sreedharan et al. 2008) and neurotoxic in vivo. FUS is a functional homolog of TDP-43 and plays an important role in RNA processing and micro-RNA biogenesis. Patients with ALS with FUS mutations develop large cytoplasmic aggregates within the spinal motor neurons (Kwiatkowski et al. 2009; Vance et al. 2009).

Until recently, work in fALS has focused on mutations in the FUS, TDP-43, and SOD1 genes in providing insights into motor neuron pathogenesis. Johnson et al. (2010) performed exomic sequencing in an Italian family with ALS and identified the R191Q mutation. Screening of VCP in 78 familial ALS and 210 isolated cases identified VCP mutations including p.R159G, p.R159C, p.R191Q, p.D592N, and p.R155H, suggesting that VCP mutations account for 2% of fALS. The frequency in different populations needs to be established by screening larger fALS and sALS cohorts.

The molecular mechanisms and by which VCP mutations cause motor neuron degeneration needs further investigation. However, the involvement of VCP in ALS lends support to the importance of disrupted autophagy and ubiquitinated proteasomal degradation in its pathogenesis. By further deepening our understanding of the underlying causes and signaling mechanisms of the ALS phenotype, we hope to find genetic therapeutic strategies aimed to ameliorate the disease (Shaw 2010).

VCP Disease Has a Worldwide Distribution

To date, there are approximately 20 missense mutations discovered in the VCP gene (Ju and Weihl 2010), and IBMPFD has been reported in more than 39 families worldwide (Table 1). IBMPFD cases have been reported in German (Djamshidian et al. 2009; Kimonis et al. 2008b; Schroder et al. 2005), Italian (Bersano et al. 2009; Gidaro et al. 2008), French, Spanish (Stojkovic et al. 2009), Austrian (Haubenberger et al. 2005), Belgian (van der Zee et al. 2009), Brazilian (Fanganiello et al. 2011), British (Miller et al. 2009; Rohrer et al. 2011), Australian (Kumar et al. 2010), and Korean (Kim et al. 2011) patients. Recently, Rohrer et al. (2011) identified a novel exon 2 I27V VCP variant in a man with frontotemporal dementia, elevated alkaline phosphatase, and a family history of progressive muscle weakness and personality changes in his father, and also in a woman with isolated progressive dysarthria; this mutation was not identified in healthy control populations (Rohrer et al. 2011). Interestingly, Kaleem et al. (2007) sequenced 188 individuals with Alzheimer's disease and from the set of sibling pairs that suggested linkage to chromosome 9 and found the R95H in one individual with pathologically confirmed AD (Kaleem et al. 2007). Although these clinical cases provide the pathogenic and phenotypic spectrum of VCP-associated IBMPFD, further analyses are needed to define the genotype–phenotype relationships.

Characterization of VCP

Linkage studies have localized the IBMPFD gene mutation to VCP on chromosome 9p21.1-p12 (Kovach et al. 2001).

Table 1 List of VCP missense mutations

	Amino acid mutation	c.DNA Base change (ORF)	Exon location	Domain location	No. of affected families	References
1	I27V	79A>G	2	N terminus	1	Rohrer et al. (2011)
2	R95H	284G>A	3	N terminus	1	Kaleem et al. (2007)
3	R93C	277C>T	3	N terminus	4	Hubbers et al. (2007); Guyant-Marechal et al. (2006); Krause et al. (2007)
4	R95G	283C>G	3	N terminus	2	Watts et al. (2004)
5	R95C	283C>T	3	N terminus	1	Kimonis et al. (2008a)
6	P137L	410C>T	4	N terminus	1	Gidaro et al. (2008)
7	R155C	463C>T	5	N terminus	5	Hubbers et al. (2007); Watts et al. (2004); Schroder et al. (2005); Guyant-Marechal et al. (2006); Gidaro et al. (2008)
8	R155H	464G>A	5	N terminus	8	Hubbers et al. (2007); Watts et al. (2004)
9	R155P	464G>C	5	N terminus	1	Watts et al. (2004)
10	R155S	463C>A	5	N terminus	1	Vesa et al. (2009)
11	R155L	N/A	5	N terminus	1	Gidaro et al. (2008)
12	G157R	469G>C	5	N terminus	1	Djamshidian et al. (2009)
13	R159H	476G>A	5	N terminus	2	Haubenberger et al. (2005)
14	R159C	476G>A	5	N terminus	2	Bersano et al. (2009); Spina et al. (2008)
15	R191Q	572G>A	5	Linker 1	1	Watts et al. (2004); Spina et al., (2008)
16	L198W	593T>G	6	Linker 1	1	Watts et al. (2007)
17	A232E	695C>A	6	Junction (L1-D1)	1	Watts et al. (2004)
18	T262A	N/A	7	AAA D1	1	Spina et al. (2008)
19	N387H	1159A>C	10	AAA D1	1	Watts et al. (2007)
20	A439S	N/A	11	Linker 2	1	Gidaro et al. (2008)

Later, disease mutations were identified within the VCP gene (Watts et al. 2004). VCP and its homologs TER94, CDC48, and VCP-like ATPase are highly conserved in evolution. VCP belongs to the type II AAA-ATPase gene superfamily characterized by two ATPase domains (D1 and D2; Confalonieri and Duguet 1995; Wang et al. 2004), two linker domains (L1 and L2), as well as the amino terminal and carboxyl terminal domains. Mutations are mainly located in three different domains—R95H, R93C, R95C/G, P137L, R155H/P/C/S/L, G157R, and R159H/C within the N domain; R191Q and L198W within the linker domains; and A232E, T262A, N387H, and A439S within the D1 ATPase domain (Kumar et al. 2010; Stojkovic et al. 2009; Weihl et al. 2009; Table 1).

It has been reported that VCP is involved in a plethora of distinct cellular processes including homotypic membrane fusion, transcription activation, nuclear envelope reconstruction, DNA repair, post-mitotic organelle reassembly, cell cycle control, apoptosis (Jarosch et al. 2002; Rabinovich et al. 2002; Rabouille et al. 1998), and endoplasmic reticulum-associated degradation of proteins (ERAD) capable of destroying both integral membrane and luminal proteins. This latter activity functions as a quality control for newly synthesized polypeptides, which selectively eliminates aberrant proteins in the secretory pathway (Jarosch et al. 2002).

VCP forms homohexamers and binds to several different adapter proteins, enabling VCP to target specific substrates for degradation (Kondo et al. 1997; Meyer et al. 2000). Disease mutations of VCP cluster in the CDC48 domain located in the N terminus of the protein, which is involved in ubiquitin binding and protein–protein interactions (Dai and Li 2001; Rape et al. 2001). CDC48 interacts with a multitude of different cofactors transporting proteins from the ER into the cytosol and primarily ubiquitinated substrates of proteasomal degradation and ubiquitin system whose cellular functions remain unknown (Ye et al. 2001). The passage of ubiquitinated substrates to the 26S proteasome is in flux with the alternative route of degradation by autophagy (Korolchuk et al. 2009). VCP is important for the retro-translocation of misfolded ER proteins, and failure in this activity results in defective ERAD and ER stress responses (DeLaBarre et al. 2006). Weihl et al. (2006) have previously shown that mutant VCP fails to degrade prototypical ERAD substrates. Degradation of specific substrates including IkB, Unc-45b, and hypoxia-inducible factor-1α is mediated by VCP (Alexandru et al. 2008; Dai et al. 1998; Janiesch et al. 2007).

The most common R155H mutation has been shown to have a normal hexameric structure (Weihl et al. 2006), and elevated ATPase activity was identified in cells transfected with IBMPFD mutants (Kakizuka 2008). Fernandez-Saiz and Buchberger (2010) have demonstrated that mutant VCP induces conformational changes in the p97 N domain, the main binding site for regulatory cofactors, and exhibits strongly altered cofactor interactions, e.g. for binding of the ubiquitin ligase E4B was reduced whereas binding of ataxin 3 was enhanced. These results suggest that imbalanced cofactor binding to p97 is a key pathological feature of IBMPFD and potentially of other proteinopathies involving VCP/p97 (Kaiser et al., personal communication).

VCP and Autophagy

Recent studies have implicated VCP at the intersection of the autophagy and ubiquitin–proteasome signaling pathways, facilitating intracellular protein degradation (Balch et al. 2008). Autophagy has been postulated as a pathogenetic event occurring in Alzheimer's disease, Huntington's disease, and ALS, among other neurodegenerative and inflammatory disorders (Wong and Cuervo 2010). Autophagy is responsible for the degradation of defective organelles and the bulk degradation of the cytoplasm during starvation. On induction of autophagy, cytoplasmic constituents are first sequestered by a phagophore and subsequently form a double-membrane structure known as the autophagosome. Autophagosome-associated proteins including Light Chain 3 (LC3), γ-aminobutyric acid type A receptor-associated protein, and Golgi-associated ATPase enhancer of 16 kDa (GATE-16) are speculated in mediating the autophagosome–lysosome fusion. Autophagosomes fuse with lysosomal vesicles and form digestive autolysosomes (Rusten et al. 2008) in the cascade (Fig. 2).

Evidence suggests that the autophagic process is related to cell cycle regulation, starvation adaptation, aging, and cancer development. P62 plays a key role in the autophagic process and can interact with effector protein LC3 and mediate the uptake of aggregated proteins. One of the neuropathological hallmarks of ALS is the misfolding of proteins that may perturb a number of intracellular signaling and degradation pathways. The role of autophagy in sIBM and ALS pathogenesis has led to clinical trials such as lithium treatments (Crippa et al. 2010a, b; Pasquali et al. 2010; Siciliano et al. 2010).

Involvement of the NFκB Pathway in VCP Disease

In Paget's disease of bone, NFκappaB (NFκB) is known to be upregulated and stimulates osteoclastic activity (Daroszewska and Ralston 2006). NFκB is a major nuclear transcription factor known to regulate the expression of many genes involved in inflammation, cellular proliferation, anti-apoptosis, and immune system development (Aggarwal 2004; Hayden and Ghosh 2004). The NFκB family consists of five members retained in the cytoplasm, including p50, p52, p65 (RelA), c-Rel, and RelB, forming various homo- and heterodimers. These dimers bind to specific inhibitors of

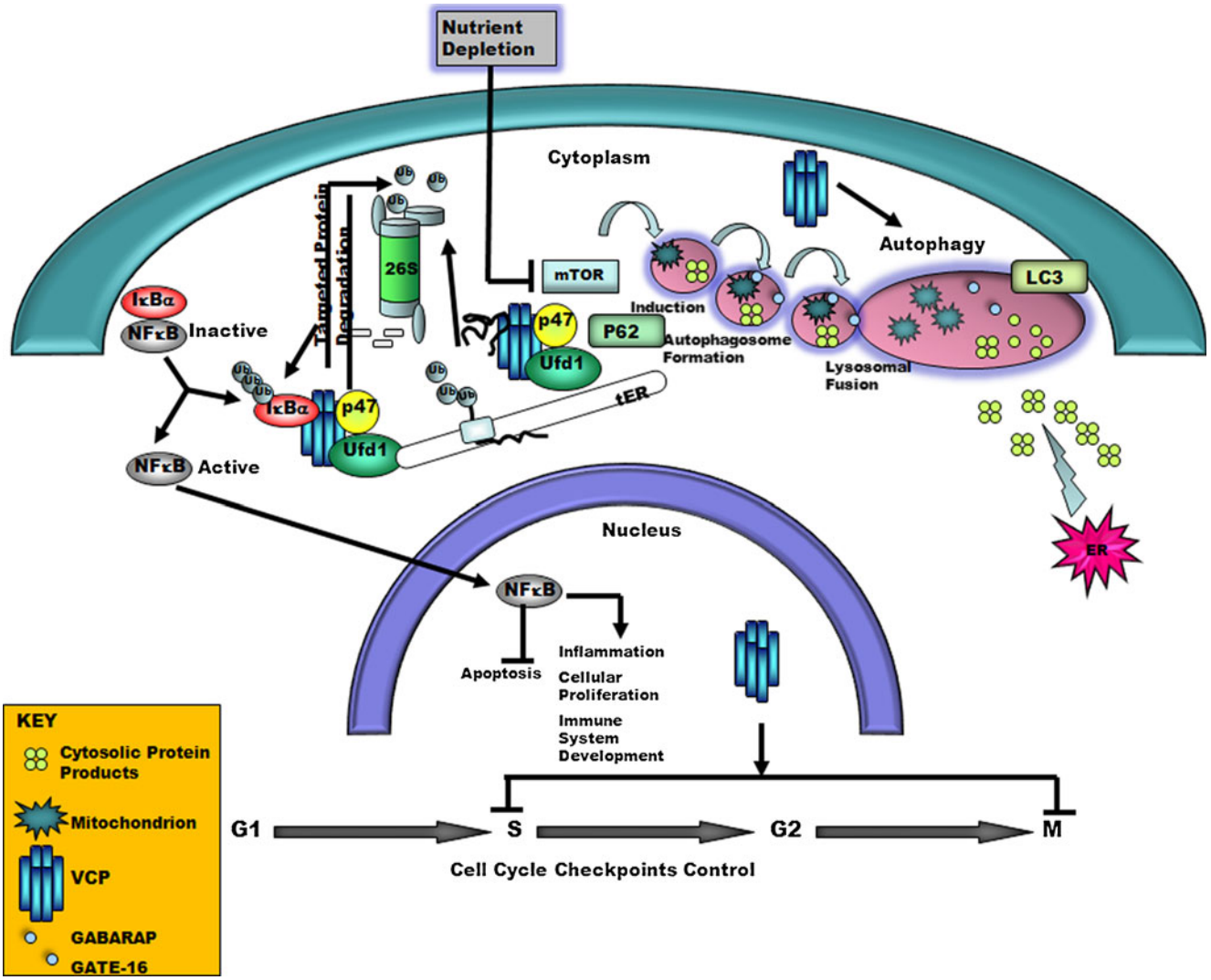

Fig. 2 Proposed functions and mechanisms of VCP. VCP is involved in a plethora of cellular functions including targeted protein degradation by ubiquitin/proteasome system, surveillance of misfolded proteins, nuclear membrane condensation, membrane fusion, apoptosis, and cell cycle control. Suggested mechanisms of VCP include signaling transduction cascades such as the ubiquitin/proteasome, NFκB, lysosomal, and mTOR/autophagy. Nutrient depletion, endoplasmic stress, and normal VCP lead to mTOR inhibition, subsequently leading to induction, autophagosome formation, and lysosomal fusion, ultimately concluding in the autophagy signaling cascade. The activation of the NFκB pathway by lack of inhibition by IkB leads to the translocation of NFκB into the nucleus, subsequently leading to inflammation, cellular proliferation, development of the immune system, and inhibition of apoptosis

NFκB (IκBs), masking the nuclear localization sequence in the complex and thereby maintaining it in an inactive state (Hayden and Ghosh 2004).

There are now data to implicate increased NFκB activity in the pathogenesis of IBMPFD (Custer et al. 2010). Mechanistic considerations regarding how VCP mutations might contribute to NFκB activity begin with understanding how VCP affects NFκB's repressor, IκBα. In 1998, Dai et al. (Dai et al. 1998) reported evidence suggesting that VCP co-precipitates with IκBα and that this interaction is important for IκBα's degradation. The IBMPFD/myogenic transcriptome consisting of differentially expressed genes broadly matches the NFκB transcriptosome known to be involved in myogenesis, prompting investigation of the terminal stages of myogenesis. Specifically, intracellular adhesion molecule 1, myogenin, and m-cadherin are all direct or indirect targets of NFκB, and most are involved in myogenesis and myotube formation. VCP interacts with IκB alpha and by implication is a regulator of NFκB activity. Upregulation of the NFκB pathway is also seen in Duchenne muscular dystrophy and limb girdle muscular dystrophy type 2A (Acharyya et al. 2007; Graham et al. 2010; Haslbeck et al. 2005). The NFκappaB pathway impacts skeletal muscle atrophy in one of three ways, either by upregulation of proteins involved in the ubiquitin/proteasome system, inflammatory cytokines, or via the

inhibition of myogenesis. Studies are currently dissecting the molecular mediators in skeletal muscle causing hypertrophy and atrophy in hopes of finding therapeutic targets (Bhatnagar and Kumar 2010). Therapies using pharmacological and genetic approaches that normalize NFκB activity via restoration of native levels of its inhibitor may allow for more physiologic myotube formation and potentially ameliorate the weakness in both skeletal and respiratory muscles in VCP disease.

IBMPFD: VCP *In Vitro* and Animal Models

IBMPFD Pathogenesis in Myoblasts

Cellular and animal models have been helpful in clarifying the role of ERAD, autophagy, apoptosis, and mitochondrial dysfunction in the pathogenesis of IBMPFD. Myoblasts from patients identified enlarged ubiquitin-containing vacuoles that accumulate membrane-bound Light Chain 3 (LC3-II), a marker of autophagy (Vesa et al. 2009). Upon activation of autophagy, cytosolic LC3-I undergoes proteolytic cleavage and is converted to the membrane-bound form, LC3II, which is localized to the autophagosomal membranes (Mizushima et al. 2004). Lysosomal-associated membrane protein 1 (LAMP-1) and LAMP-2 proteins demonstrated differential *N*-glycosylation in mutant myoblasts by Western blotting (Vesa et al. 2009) and immunohistochemical analyses (Fig. 1c). Patients' myoblasts thus serve as a useful *in vitro* model to recapitulate human disease and may further be used for dissecting molecular signaling cascades and for the development of therapeutic strategies and advances.

Mouse Models

Mouse models have been created in order to investigate the in vivo effects of VCP mutations as well as to further our understanding of the pathogenesis and mechanisms of IBMPFD. Both human and mouse VCP proteins consist of 806 amino acids, and the mouse protein differs by only one amino acid residue at position 684 when compared with the human protein.

Muller et al. (2007) reported that the deletion of p97 (VCP/CDC48) results in early embryonic lethality in the mouse, demonstrating the importance of p97 as an essential gene in mouse development. Homozygous $p97^{-/-}$ mice died at pre-implantation stages, thus indicating a significant role for p97 in the coordination of cellular events in embryonic development. Heterozygous mice lacking one p97 allele and having one wild-type allele were indistinguishable from their wild-type littermates. Weihl et al. (2007) reported VCP transgenic mice with p97/VCP-WT or p97/VCP mutations under a muscle-specific promoter. These mice became weaker at 6 months of age and depicted abnormal muscle pathology including disorganized membrane morphology and vacuolation with reduced caveolin-3 expression as compared with wild-type mice. Custer et al. (2010) has reported R155H mutant transgenic mice under the control of the CMV-enhanced chicken beta actin promoter, recapitulating pathology in muscle, brain, and bone typical of human IBMPFD. These mice developed muscle weakness, including rimmed vacuoles and TDP-43/ubiquitin-positive myopathy.

Additionally, Badadani et al. (2010) have generated a VCP knock-in mouse model with the common $R155H^{+/-}$ mutation, typical of human IBMPFD pathogenesis. The mouse model demonstrated progressive muscle weakness and vacuolization of myofibrils and centrally located nuclei beginning at approximately 6 months of age. MicroCT images showed radiolucency of distal femurs and proximal tibiae in knock-in mice, and CT morphometrics showed decreased trabecular pattern and increased cortical wall thickness. Bone histology and bone marrow-derived macrophage cultures in these mice revealed increased osteoclastogenesis observed by tartrate-resistant acid phosphatase staining suggestive of PDB. Immunohistochemical analyses exhibited progressive cytoplasmic accumulation of TDP-43 and ubiquitin-positive inclusion bodies in the quadriceps myofibrils and brain of these mice. Increased LC3-II staining of brain sections representing increased number of autophagosomes suggested impaired autophagy in the brain pathogenesis. Our hypothesis is that immature autophagosomes accumulate in tissues secondary to impaired lysosome–autophagosome fusion, further contributing to tissue pathology. Increased apoptosis was demonstrated by elevated caspase-3 activity and increased TUNEL-positive nuclei. Therefore, the VCP $R155H^{+/-}$ knock-in mice exhibit tissue pathology typical of human IBMPFD disease and represent a useful model for preclinical and future therapeutic studies (Badadani et al. 2010). We can use these approaches to dissect and understand the mechanisms and signaling cascades underlying the developmental, physiological, and pathological processes in VCP disease (Fig. 2).

Drosophila Models

Drosophila contains a single VCP homolog, TER94, which interestingly shares approximately over 80% identity of protein sequences with human VCP. *Drosophila* models have recapitulated a variety of diseases including IBMPFD. In *Drosophila* VCP (TER94 in *Drosophila melanogaster*), loss of function has been identified as a dominant repressor of expanded poly-glutamine-induced neuronal degeneration. Ritson et al. (2010) developed and characterized the first *Drosophila* model to elucidate the pathogenic mecha-

nisms of IBMPFD. They demonstrated that mutations in VCP lead to TDP-43 redistribution to the cytoplasm, thereby inducing degeneration in vivo.

Chang et al. (2011) developed *Drosophila* IBMPFD models by introducing amino acid substitutions at three residues in TER94 (R152H, R188Q, and A229E). These mutants exhibit learning deficits, tissue degeneration in muscle, and nervous systems typical of VCP disease. Chang et al. (2011) also demonstrated that an increase in cellular ATP could suppress the TER94 mutant phenotypes and that, conversely, a decrease in ATP would enhance the phenotype, thus linking ATP levels with the pathogenesis of IBMPFD. The *Drosophila* model of IBMPFD may thus aid in the development of novel therapeutic strategies/agents for this disease.

Therapeutic Approaches

There are no disease-modifying treatments to prevent the progression of the VCP-associated myopathy or FTD. PDB can be treated with bisphosphonates. We are improving our understanding of how disease mutations in VCP affect its function, but the pathogenic mechanisms for the progressive proximal muscle weakness, as well as for the inclusion and vacuole formation in IBMPFD muscle fibers, remain unknown. To be able to develop treatments, it is necessary to understand and elucidate the pathological molecular cascades resulting in the clinical manifestation of the disease. Mouse and *Drosophila* models recapitulating the IBMPFD phenotype in addition to *in vitro* systems will be useful for preclinical studies and in developing therapeutic strategies and novel drugs for the treatment of this disease as well as for several muscular and neurodegenerative diseases.

Future Directions

IBMPFD is a multisystem disorder caused by disease mutations in VCP, and pathology includes the degeneration of tissue and accumulation of ubiquitin and TDP-43-positive protein aggregates in the affected tissue. IBMPFD is an under-diagnosed disease, and VCP mutation testing should be considered in an individual with two or more of the associated characteristics. Increased cofactor binding of VCP with the multiple adaptor proteins appears to be a key pathogenic mechanism in VCP-associated disease. The specific mechanism(s) whereby VCP mutations result in IBMPFD remain unknown. Various signaling networks are currently being connected to mutations in the multifunctional chaperone VCP, including the NFκB, autophagy, and apoptotic pathways (Fig. 2). Elucidating the basic molecular signaling cascades involved in IBMPFD disease will ultimately lead to a better understanding of IBMPFD. The animal models recapitulate the IBMPFD pathology and offer the prospect of novel therapeutic interventions.

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Conflict of Interest Statement None declared.

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