Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The heterozygous R155C VCP mutation: Toxic in humans! Harmless in mice?

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ARTICLE INFO

Article history:
Received 30 July 2018
Accepted 4 August 2018
Available online xxx

Keywords:
VCP
p97
R155C VCP knock-in mice
IBMPFD
ALS
Multisystem proteinopathy

ABSTRACT

Heterozygous missense mutations in the human VCP gene cause inclusion body myopathy associated with Paget disease of bone and fronto-temporal dementia (IBMPFD) and amyotrophic lateral sclerosis (ALS). The exact molecular mechanisms by which VCP mutations cause disease manifestation in different tissues are incompletely understood. In the present study, we report the comprehensive analysis of a newly generated R155C VCP knock-in mouse model, which expresses the ortholog of the second most frequently occurring human pathogenic VCP mutation. Heterozygous R155C VCP knock-in mice showed decreased plasma lactate, serum albumin and total protein concentrations, platelet numbers, and liver to body weight ratios, and increased oxygen consumption and CD8+/Ly6C + T-cell fractions, but none of the typical human IBMPFD or ALS pathologies. Breeding of heterozygous mice did not yield in the generation of homozygous R155C VCP knock-in animals. Immunoblotting showed identical total VCP protein levels in human IBMPFD and murine R155C VCP knock-in tissues as compared to wild-type controls. However, while in human IBMPFD skeletal muscle tissue 70% of the total VCP mRNA was derived from the mutant allele, in R155C VCP knock-in mice only 5% and 7% mutant mRNA were detected in skeletal muscle and brain tissue, respectively. The lack of any obvious IBMPFD or ALS pathology could thus be a consequence of the very low expression of mutant VCP. We conclude that the increased and decreased fractions of the R155C mutant VCP mRNA in man and mice, respectively, are due to missense mutation-induced, divergent alterations in the biological half-life of the human and murine mutant mRNAs. Furthermore,

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https://doi.org/10.1016/j.bbrc.2018.08.038

our work suggests that therapy approaches lowering the expression of the mutant VCP mRNA below a critical threshold may ameliorate the intrinsic disease pathology.

1. Introduction

Missense mutations in the human VCP gene on chromosome 9p13-p12, which encodes an essential and evolutionarily highly conserved Triple-A ATPase (ATPase Associated with diverse cellular Activities) [1,2], cause various autosomal-dominantly inherited human diseases. Inclusion body myopathy (IBM) associated with Paget disease of the bone (PDB) and frontotemporal dementia (FTD), or IBMPFD (OMIM 605382), a late-onset multisystem disorder, was the first identified disease entity [3,4], followed by amyotrophic lateral sclerosis (ALS14) [5], Parkinson's disease (PD) [6,7], hereditary spastic paraplegia (HSP) [8,9], and Charcot-Marie-Tooth disease type 2 (HMSN2) [10]. Since the first description of pathogenic VCP mutations in 2004, over 40 different disease-causing missense mutations have been described [11].

The Mg^{2+}-dependent VCP protein exhibits a tripartite structure composed of an N-terminal CDC48 domain and the D1 and D2 domains that bind and hydrolyse ATP [12,13] (Fig. 1A). VCP monomers assemble into a ring-shaped hexamer with the D-domains forming a central cylinder surrounded by the CDC48 domains [14,15]. The multiple functions and the versatile cofactor binding of the VCP protein are essentially dependent on the energy derived from ATP hydrolysis [16]. VCP is centrally involved in multiple and diverse cellular processes such as membrane dynamics, protein quality control, cell cycle, apoptosis, and DNA damage response [17,18]. Moreover, recent work reported on mutation-specific effects on VCP interaction partners leading to alterations in the ratio of VCP monomers to hexamers [19] as well as functional consequences on endocytosis [20], endoplasmic reticulum associated degradation (ERAD) of proteins [21], ATPase activity [19], and 20S proteasome binding [22]. However, the exact molecular mechanisms leading from a VCP point mutation to specific disease manifestations affecting nervous, bone, and striated muscle tissues are currently unknown.

In the present study, we generated and characterized a R155C VCP knock-in mouse model expressing the ortholog of the human R155C VCP mutation, which has been shown to cause both IBMPFD [3,4] and ALS [5]. Though the failure to generate mice homozygous for the R155C VCP missense mutation clearly denotes a toxic effect not compatible with life, our comprehensive analyses of heterozygous mice resulted in multiple aberrant parameters, but did not reveal any VCP-typical pathology, as previously described in a R155H VCP knock-in mouse model [23]. The here presented findings are discussed in the context of the human and murine codon 155-related VCP pathologies.

2. Materials and methods

2.1. Animals

In the present study, we used heterozygous R155C (c.463_465delCGG > insTGT) VCP knock-in mice (B6J.B6-*Vcp*$^{tm2.1Ccrs}$) and their wild-type siblings. The mouse model was generated according to our specifications (CSC, RS) by genOway, Lyon, France. Routine genotyping was performed by PCR (primer pair 51546cre 5′-CAGTTCTCATGCCTCTCTGAAGGATAATGT-3′ and 51547cre 5′-TCTACAACTTTGAACTCCACAGCACGC-3′). In addition, individual mice were genotyped by Southern blotting, and the presence of the R155C VCP point mutation was verified by sequencing; for details see Fig. 1B–E. Mice were housed in isolated ventilated cages (IVC) under specific and opportunistic pathogen-free (SOPF) conditions at a standard environment with free access to water and food. Health monitoring was done as recommended by the Federation of European Laboratory Animal Science Associations (FELASA). Mice were handled in accordance with the German Animal Welfare Act (Tierschutzgesetz) as well as the German Regulation for the protection of animals used for experimental purposes or other scientific purposes (Tierschutz-Versuchstierverordnung). All investigations were approved by the governmental office for animal care (Landesamt für Natur, Umwelt und Verbraucherschutz North Rhine-Westphalia (LANUV NRW), Recklinghausen, Germany (reference numbers 8.87–50.10.47.09.014 and 84–02.05.40.14.057)).

2.2. Phenotypic analysis in the German Mouse Clinic

Heterozygous R155C VCP knock-in mice and wild-type control littermates were subjected to a systematic, comprehensive phenotyping screen at the German Mouse Clinic at the Helmholtz Zentrum München (http://www.mouseclinic.de) as described previously [24–27]. This screen covers a broad range of parameters in the areas of allergy, behavior, cardiovascular function, clinical chemistry, dysmorphology, energy metabolism, eye analysis and vision, hematology, immunology, neurology, and pathology. For further details see legend to Fig. 3.

2.3. Analysis of skeletal muscle tissue sections

Dissected murine skeletal muscle specimens were immediately frozen in liquid nitrogen-cooled isopentane. A human tissue sample was derived from a diagnostic skeletal muscle biopsy of a previously reported patient with a heterozygous R155C VCP mutation [28]. Cryostat sections of 6 μm thickness were placed on microscope slides, air-dried for 30 min, stained using routine histochemistry protocols [29], and images were captured using an Olympus CX41 light microscope (Olympus, Hamburg, Germany). Ultrathin sections for transmission electron microscopy were prepared as described in Ref. [30] and examined with a Zeiss LEO 900 electron microscope (Carl Zeiss GmbH, Oberkochen, Germany).

2.4. Analysis of skeletal muscle tissue lysates and antibodies

For SDS-PAGE snap frozen samples of dissected muscles were ground, solubilized, and homogenized essentially according to [31–33]. The trypsin-like proteasomal activity of soleus muscles was determined as described in detail in Ref. [34]. Primary antibodies for immunoblotting were: VCP, mouse monoclonal antibody, 1:2000 in PBS-T with 5% milk powder, #11433, Abcam; strumpellin, rabbit polyclonal antibody, 1:500 in PBS-T, #sc-87445, Santa Cruz; sequestosome-1/p62, rabbit polyclonal antibody, 1:3000 in PBS-T, #P0067, Sigma Aldrich; BAG-3, rabbit polyclonal antibody, 1:3000 in PBS-T, #10599-1-AP, Proteintech; TDP-43 rabbit polyclonal antibody, 1:1000 in PBS-T, #12892-1-AP, Proteintech; ULK1, rabbit polyclonal antibody, 1:1000 in PBS-T, #ab65056,

Please cite this article in press as: C.S. Clemen, et al., The heterozygous R155C VCP mutation: Toxic in humans! Harmless in mice?, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.08.038