Neurobiology of Disease 45 (2012) 711–722

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

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

Synaptic dysfunction in progranulin-deficient mice

Terri L. Petkau [a], Scott J. Neal [a], Austen Milnerwood [c,d], Ada Mew [a], Austin M. Hill [a], Paul Orban [a], Jenny Gregg [a], Ge Lu [a], Howard H. Feldman [b,f], Ian R.A. Mackenzie [e], Lynn A. Raymond [b,c,d], Blair R. Leavitt [a,b,d,*]

[a] Centre for Molecular Medicine & Therapeutics, Department of Medical Genetics, University of British Columbia, and Children's and Women's Hospital, 980 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4
[b] Division of Neurology, Department of Medicine, University of British Columbia Hospital, S 192-2211 Wesbrook Mall, Vancouver, BC, Canada V6T 2B5
[c] Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada V6T 1Z3
[d] Brain Research Centre, University of British Columbia, Vancouver, BC, Canada V6T 1Z3
[e] Department of Pathology and Laboratory Medicine, University of British Columbia and Vancouver General Hospital, 855 West 12th Avenue, Vancouver, BC, Canada
[f] Bristol-Myers Squibb, Neuroscience Global Clinical Research, 5 Research Parkway, Wallingford CT, 06492, USA

ARTICLE INFO

Article history:
Received 4 May 2011
Revised 7 October 2011
Accepted 16 October 2011
Available online 25 October 2011

Keywords:
Progranulin
Frontotemporal dementia
Synaptic dysfunction
Long-term potentiation
Neuronal morphology
Mouse model

ABSTRACT

Progranulin haploinsufficiency is a common cause of familial frontotemporal dementia (FTD), but the role of progranulin in the brain is poorly understood. To investigate the role of murine progranulin (Grn) in the CNS *in vivo*, we generated mice targeted at the progranulin locus (*Grn*) using a gene-trap vector. Constitutive progranulin knockout mice (GrnKO) show moderate abnormalities in anxiety-related behaviors, social interactions, motor coordination, and novel object recognition at 8 months of age, many of which differ between males and females. Analysis of synaptic transmission in 10–12 month old GrnKO male mice indicates altered synaptic connectivity and impaired synaptic plasticity. Additionally, apical dendrites in pyramidal cells in the CA1 region of the hippocampus in GrnKO males display an altered morphology and have significantly decreased spine density compared to wild-type (WT) mice. The observed changes in behavior, synaptic transmission, and neuronal morphology in GrnKO mice occur prior to neuropathological abnormalities, most of which are apparent at 18 but not at 8 months of age. We conclude that progranulin deficiency leads to reduced synaptic connectivity and impaired plasticity, which may contribute to FTD pathology in human patients.

Introduction

Progranulin (GRN), encoded by a single gene (*GRN*) on human chromosome 17, is a 593 amino acid secreted glycoprotein consisting of 7.5 repeats of a highly conserved 12-cysteinyl granulin motif (Bhandari et al., 1992). Cleavage of the mature protein yields short (~6–25 kDa) biologically active granulin peptides that may have distinct roles from the full-length protein (Bateman and Bennett, 1998; Plowman et al., 1992). The orthologous murine gene (*Grn*) is located on chromosome 11 and encodes a 589 amino acid protein sharing a similar predicted structure and high sequence identity at both the DNA (76%) and protein (79%) levels with human *GRN* and GRN respectively. In the periphery, GRN is a pleiotropic protein involved in wound healing, inflammation, tumorigenesis, and development (Eriksen and Mackenzie, 2008; He and Bateman, 2003; Ong and Bateman, 2003).

Autosomal dominant loss-of-function mutations in *GRN* are a major cause of familial frontotemporal dementia (FTD) (Baker et al., 2006; Cruts et al., 2006). FTD is a neurodegenerative disorder characterized by prominent atrophy of the frontal and temporal lobes of the brain. It occurs in mid to later life and, despite a high degree of clinical heterogeneity, typically causes profound behavioral abnormalities, semantic impairment, and progressive aphasia (McKhann et al., 2001).

The function of Grn in the brain is not well understood. Grn is expressed in neurons throughout the brain and in microglia (Petkau et al., 2010). Recent evidence indicates that Grn may also be expressed at much lower levels in astrocytes and ependymal cells (Ahmed et al., 2010). In different *in vitro* paradigms, Grn deficiency enhances neuron vulnerability to excitotoxic and oxidative stress (Guo et al., 2010; Yin et al., 2010a), alters TAR DNA binding protein 43 (TDP-43) localization and solubility (Guo et al., 2010; Kleinberger et al., 2010), enhances neurite outgrowth (Gao et al., 2010; Ryan et al., 2009; Van Damme et al., 2008), and alters the stress response (Piscopo et al.,

* Corresponding author at: Centre for Molecular Medicine & Therapeutics, Department of Medical Genetics, University of British Columbia, and Children's and Women's Hospital, 980 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4. Fax: +1 604 875 3819.
E-mail address: bleavitt@cmmt.ubc.ca (B.R. Leavitt).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2011.10.016

2010). Finally, Grn-deficient macrophages and microglia are cytotoxic to neurons in culture (Yin et al., 2010a).

Grn is an androgen-inducible gene involved in the sexual differentiation of the brain, and mice with a targeted disruption of the gene show alterations in sexual behaviors (Kayasuga et al., 2007) and increased anxiety (Chiba et al., 2009). Others have reported increased depression- and disinhibition-like behavior, as well as deficits in social recognition and impaired spatial learning in aged Grn-deficient mice (Yin et al., 2010b). Grn-deficient mice display progressive neuropathological changes including the accumulation of ubiquitin immunoreactivity and lipofuscinosis, as well as increased microgliosis and astrocytosis (Ahmed et al., 2010; Yin et al., 2010a,b).

Despite clear evidence that Grn deficiency in mice causes behavioral abnormalities and progressive neuropathological changes, the mechanism(s) through which Grn exerts its effects *in vivo* is not known. We report the first evidence of reduced basal synaptic efficacy, impaired synaptic plasticity, and abnormal neuronal morphology in 10–12 month old GrnKO male mice, and suggest that strategies aimed at increasing or maintaining synaptic connectivity may prove beneficial in the treatment of FTD.

Materials and methods

Construction of the targeting vector

Using the mouse genomic sequence available from the Mouse Genome Assembly (March 2006) polymerase chain reaction (PCR) primers were designed to amplify the *Grn* locus in three pieces from mEMS128 embryonic stem (ES) cell (strain of origin: 129S1/SvImJ) DNA [5′ long arm of homology (7080 bp from −4.4 kb to intron 2), an internal fragment to be flanked by loxP sites ('floxed'; 538 bp including exons 3 and 4) and a 3′ short arm of homology (2613 bp from intron 4 to intron 12)]. The PCR products were inserted into the pFlrt1 vector (Lefebvre et al., 1997). The conditional targeting vector was then modified by sub-cloning a lacZ/neomycin fusion protein (βgeo) coding sequence from the gene-trap vector pIFS into the pFlrt1 construct where it replaces the neomycin resistance cassette (referred to as 'pFleo' allele). The complete βgeo cassette comprises a consensus splice acceptor, and an internal ribosome entry site (IRES) preceding the βgeo coding sequence and a polyadenylation signal following the stop codon, such that it is spliced downstream of *Grn* exons 1–4 and expressed as would be native *Grn*. The IRES serves to uncouple the translation of the reporter from that of the *Grn* sequence. The βgeo cassette is situated between FRT sites, allowing its removal via the introduction of Flp recombinase. Subsequent introduction of Cre recombinase excises exons 3 and 4 of *Grn*, creating a constitutive null allele. All analyses reported here were performed in mice carrying the full gene-trap construct and expressing *lacZ*.

Generation of constitutive Grn knockout mice

Two positive ES cell clones were injected into C56BL/6J blastocysts by the Transgenic Core Facility at the Center for Molecular Medicine and Therapeutics/Child and Family Research Institute (Vancouver, Canada). Chimeras were identified and crossed to C57BL/6J mice to establish germline transmission of the pFleo gene-trap allele (Allele accession ID: MGI: 4867226). Heterozygous pFleo mice ($Grn^{+/fleo}$) were then backcrossed on to the C57BL/6J background. Cohorts of mice were generated by mating two heterozygous $Grn^{+/fleo}$ animals and using WT littermates as controls. Analysis of behavior and neuronal morphology in $Grn^{fleo/fleo}$ mice (GrnKO) at 8–10 months of age were done after two backcrosses (N = 2); electrophysiology and neuropathology were assessed in a mixed cohort of early generation (N = 2) and incipient congenic (N = 5) mice.

All animal work was approved by the UBC Animal Care Committee and the Canadian Council on Animal Care.

Real-time quantitative PCR

Cortical tissue from 2 $Grn^{+/+}$, 7 $Grn^{+/fleo}$, and 3 $Grn^{fleo/fleo}$ mice at 3 months of age was collected and immediately frozen at −80 °C. Homogenization of each sample was performed using a Fastprep Homogenizer (ThermoScientific). Total RNA from homogenized tissues was isolated using the PureLink RNA mini kit (Invitrogen). Reverse transcription was carried out using the Superscript VILO kit (Invitrogen) according to the manufacturer's instructions. Quantitative analyses of mRNA expression were performed using PowerSYBR®green mix according to the manufacturer's instructions (Applied Biosystems). Amplification of cDNA was performed using the StepOne Plus Real-Time PCR System (Applied Biosystems). The WT Grn allele was amplified using primers that spanned the junction between exons 4 and 5 (forward primer, 5-CTGTAGTGCAGATGGGAAATCCTGCT-3′; reverse primer, 5′-GTGGCAGAGTCAGGACATTCAAACT-3′); the targeted allele was amplified using the same forward primer and a reverse primer within the splice acceptor site of the targeting construct (reverse primer, 5′-AAAGACCGCGAAGAGTTTGTCCTC-3′). Quantification of mRNA levels was calculated using the standard curve method, with amplification of target mRNA and control genes in separate wells. Standard curves were created using 10-fold serial dilutions of whole brain cDNA from heterozygous-targeted animals. Each sample was run in duplicate. The relative amount of mRNA in each well was calculated as the ratio between the target mRNA and the endogenous level of the *HPRT* transcripts.

Immunohistochemistry

Immunohistochemistry was performed as previously described (Mackenzie et al., 2006, Petkau et al., 2010 #585) on 5 μm paraffin-embedded brain sections from 3 WT and 3 GrnKO mice at 8 and 18 months of age.

Behavioral tests

Prior to initiating any behavior tests, mice were allowed to acclimatize to the testing room for 10 min. The equipment for each test was cleaned with ethanol in between animals, and females were tested before males. All video analysis was done using EthoVision XT 7.0 software (Noldus, VA). A modified SHIRPA examination specific for behavior and cognition phenotyping was performed according to the protocol from the EMPReSS database (http://empress.har.mrc.ac.uk). Briefly, mice were evaluated in a viewing jar for body position, tremor, palpebral closure, coat appearance, lacrimation, and defecation; in an open field arena for transfer arousal, locomotor activity, gait, tail elevation, startle response, and touch escape; and under manual restraint for positional passivity, trunk curl, limb grasping, pinna and corneal reflexes, limb grasping, contact righting, biting, and vocalization. Immediately following the SHIRPA evaluation, grip strength was tested using an automated digital grip strength meter (Columbus Instruments, OH). Mice were placed on the wire grid and allowed to grab on with all four feet for approximately 5 s before being gently pulled off by the tail. The maximum force produced was recorded. Each mouse was given five consecutive trials, and the highest and lowest scores were disregarded so that the score for each mouse is the average of the three middle scores. The open field test consisted of one 10-minute trial per day over three consecutive days in a 50 cm × 50 cm × 20 cm arena. The center zone was defined as a square covering 16% of the total arena area (20 cm × 20 cm central square). Spontaneous motor activity was measured using an automated open-field system (San Diego Instruments, San Diego, CA). The activity of mice in the open field was assessed in the dark during the light cycle and was measured automatically as the number of photobeam breaks during each trial. Activity was recorded for the duration of a 30 min period. For the resident–intruder test