Brain Research 1652 (2016) 62–70

Contents lists available at ScienceDirect

Brain Research

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

Research report

HMG-CoA synthase isoenzymes 1 and 2 localize to satellite glial cells in dorsal root ganglia and are differentially regulated by peripheral nerve injury

Fei Wang[a,b], Hongfei Xiang[a,c], Gregory Fischer[a], Zhen Liu[a], Matthew J. Dupont[a], Quinn H. Hogan[a,d], Hongwei Yu[a,d,*]

[a] Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA
[b] Medical Experiment Center, Shaanxi University of Chinese Medicine, Xianyang, Shaanxi 712046, PR China
[c] Department of Orthopedic Surgery, Affiliated Hospitals of Qingdao University, Qingdao 266000, PR China
[d] Zablocki Veterans Affairs Medical Center, Milwaukee, WI 53295, USA

ARTICLE INFO

Keywords:
HMG-CoA synthase
Satellite glial cells
Cholesterol
Neuropathic pain
Dorsal root ganglion, peripheral nerve injury

ABSTRACT

In dorsal root ganglia (DRG), satellite glial cells (SGCs) tightly ensheathe the somata of primary sensory neurons to form functional sensory units. SGCs are identified by their flattened and irregular morphology and expression of a variety of specific marker proteins. In this report, we present evidence that the 3-hydroxy-3-methylglutaryl coenzyme A synthase isoenzymes 1 and 2 (HMGCS1 and HMGCS2) are abundantly expressed in SGCs. Immunolabeling with the validated antibodies revealed that both HMGCS1 and HMGCS2 are highly colabeled with a selection of SGC markers, including GS, GFAP, K_{ir}4.1, GLAST1, GDNF, and S100 but not with microglial cell marker Iba1, myelin sheath marker MBP, and neuronal marker β3-tubulin or phosphorylated CaMKII. HMGCS1 but not HMGCS2 immunoreactivity in SGCs is reduced in the fifth lumbar (L5) DRGs that contain axotomized neurons following L5 spinal nerve ligation (SNL) in rats. Western blot showed that HMGCS1 protein level in axotomized L5 DRGs is reduced after SNL to 66 ± 8% at 3 days ($p < 0.01$, n=4 animals in each group) and 58 ± 13% at 28 days ($p < 0.001$, n=9 animals in each group) of its level in control samples, whereas HMGCS2 protein was comparable between injured and control DRGs. These results identify HMGCSs as the alternative markers for SGCs in DRGs. Downregulated HMGCS1 expression in DRGs after spinal nerve injury may reflect a potential role of abnormal sterol metabolism of SGCs in the nerve injured-induced neuropathic pain.

1. Introduction

The dorsal root ganglion (DRG) is a critical site in the peripheral somatosensory pathway since it harbors the somata of the primary sensory neurons, which transmit sensory information from the peripheral to the central nervous system (CNS) (Basbaum et al., 2009; Costigan et al., 2009). A unique feature of the DRG is that each neuronal soma with its initial axon segment is ensheathed by non-myelinating satellite glial cells (SGCs) to form discrete anatomical and functional sensory units (Griffin and Thompson, 2008; Hanani, 2005; Huang et al., 2013). SGCs can be identified by their laminar morphology and by the presence of specific proteins. A number of proteins have been identified in SGCs, including glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), S100 protein, ATP-sensitive inward rectifier potassium channel 10 (K_{ir}4.1), excitatory amino acid transporter 1 (GLAST1), connexin-43 (Cx43) subunit of gap junctions, P2Y purinoceptor 4 (P2Y4), soluble guanylate cyclase (sGC), and various N-methyl-D-aspartate receptor (NMDAr) subunits, neurotransmitter receptors, neurotrophins, as well as cell adhesion molecules (Ferrari et al., 2014; Gu et al., 2010; Pannese, 2010). Multiple protein expression indicates that SGCs may participate in diverse functions that are a current focus of pain research (Costa and Moreira Neto, 2015).

Hydroxymethylglutaryl-CoA synthase (HMGCS) is a key enzyme converting acetyl-CoA and acetoacetyl-CoA into HMG-CoA, an intermediate in both sterol synthesis and ketogenesis (Cotter et al., 2014; Goldstein and Brown, 1990). There are at least two isoenzymes of HMGCS (EC 2.3.3.10) encoded by two different genes (Ayte et al., 1990). HMGCS1 is located in cytoplasm and participates in sterol biosynthesis. HMGCS2 functions as a key enzyme for ketogenesis and

* Corresponding author at: Department of Anesthesiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA.
E-mail address: hyu@mcw.edu (H. Yu).

http://dx.doi.org/10.1016/j.brainres.2016.09.032
Received 4 March 2016; Received in revised form 19 September 2016; Accepted 22 September 2016
Available online 23 September 2016
0006-8993/

is located in the mitochondrial matrix. Inherited HMGCS2 deficiency in human causes metabolic disorder presenting with hypoketotic hypoglycemia, encephalopathy, and hepatomegaly (Aledo et al., 2006). Mutation of HMGCS1 in zebrafish leads to abnormality in oligodendrocyte development and myelin gene expression (Mathews et al., 2014). Based on microarray and proteomics data, HMGCS1 was reported to be significantly downregulated in the ipsilateral lumbar (L) 5 DRG of L5 spinal nerve ligation (L5 SNL) rats and in the spinal cords of rats with spinal cord injury or chemical-induced spinal cord axonopathy, and sciatic nerve injury (Chen et al., 2015; D'Antonio et al., 2006; Di Narzo et al., 2015; Komori et al., 2007; Tshala-Katumbay et al., 2008). Nevertheless, little is known regarding the cellular localization of HMGCS 1 and 2 in the peripheral sensory nervous system.

Here we report that, in the peripheral nerve system, both HMGCS1 and HMGCS2 are abundantly expressed in the SGCs of DRGs. Both HMGCS isoforms are highly colabeled with a selection of SGCs markers but colocalization was not found with microglial cells, myelinating Schwann cells, and neuronal markers. Interestingly, HMGCS1 but not HMGCS2 protein level and immunoreactivity (ir) are reduced in DRGs that have been axotomized by spinal nerve injury. These data indicate that HMGCSs can be used as alternative markers for SGCs in DRGs, and that painful neuropathy induced by spinal nerve injury was associated with significant suppression of HMGCS1 expression in DRGs, suggesting a possible role of abnormal sterol metabolism of SGCs in neuropathic pain following nerve injury.

2. Results

2.1. Specificity determination of HMGCS1 and HMGCS2 antibodies and HMGCSs expression in the DRGs

The specificity of HMGCS1 and HMGCS2 antibodies was validated by immunoblot and IHC with antigen adsorption. By immunoblotting, the HMGCS1 antibody that was raised against a unique peptide mapping the HMGCS1 protein C-terminus (which is absent in the HMGCS2 protein) revealed a clean, strong band at the estimated molecular weight of the target protein (about 57 kDa) in the homogenates of DRGs obtained from naïve adult rats (Fig. 1A). HMGCS2 antibody was raised against a HMGCS2 proximal C-terminal peptide that has about 48% homologous to the corresponding sequence of HMGCS1 and produced a single strong band at ~64 kDa. This is larger

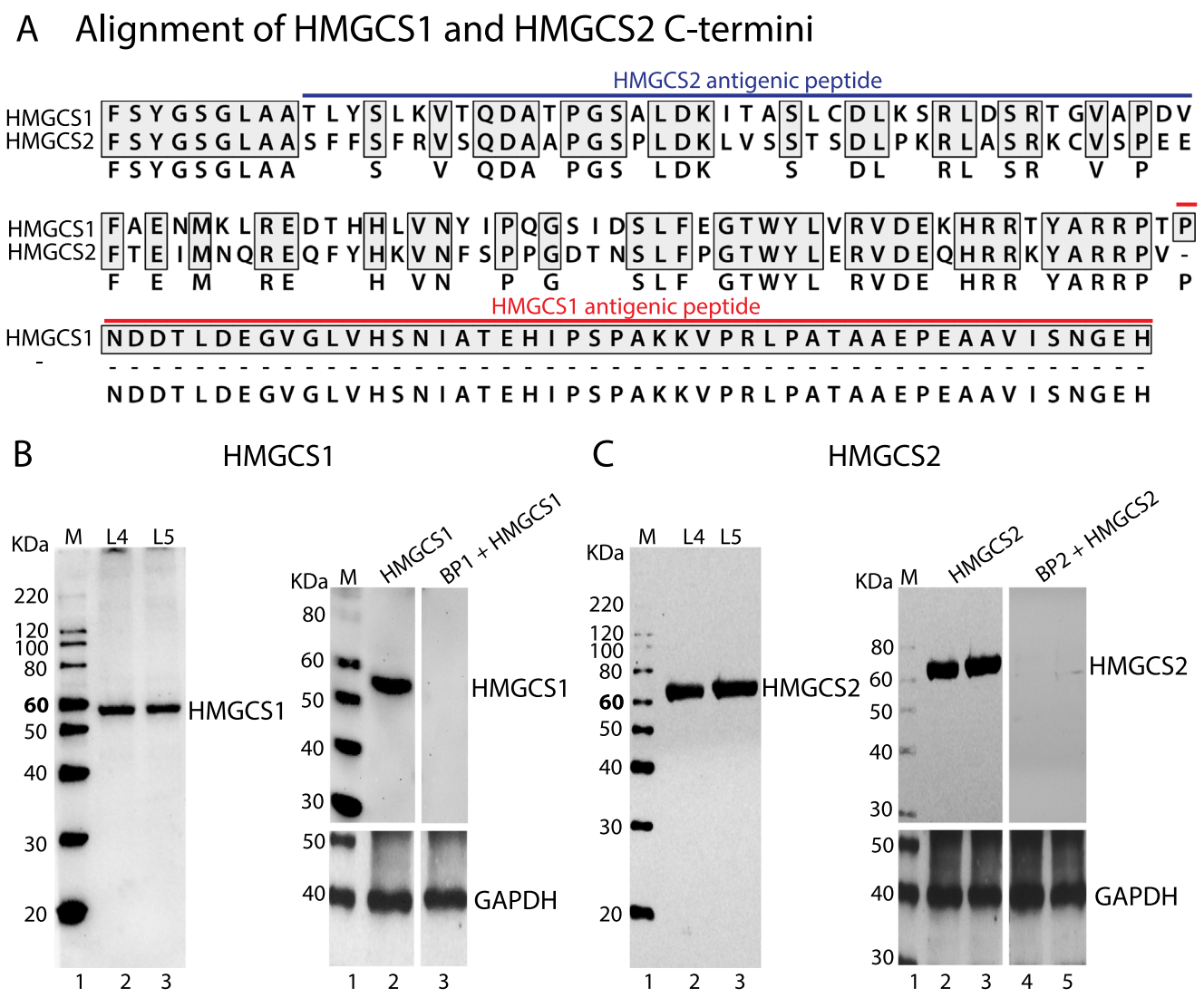

Fig. 1. Determination of the specificity of HMGCS1 and HMGCS2 antibodies. (A) Alignment of C-terminal amino acid sequences of HMGCS1 and HMGCS2. Identical residues are highlighted in gray. Unique antigenic peptide of HMGCS1 is highlighted in red line and HMGCS2 antigenic peptide highlighted in blue line. (B) HMGCS1 and (C) HMGCS2 western blots revealed a clean band of HMGCS1 protein (about 57 kDa) and HMGCS2 protein (about 64 kDa), respectively, in the homogenates of lumbar (L) 4 (lane 2) and L5 (lane 3) DRGs from naïve adult rats. Lane 1: protein molecular weight ladders (MagicMark XP Protein Standard, life Technologies, Carlsbad, CA). Right panels show that pre-incubation with excess antigenic blocking peptide (BP) for HMGCS1 (B) and HMGCS2 (C) eliminated the band, suggesting specificity of detection.