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

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Abstract

In dorsal root ganglia (DRG), satellite glial cells (SGCs) tightly ensheath 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 β 3-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 $66 \pm 8\%$ at 3 days ($p < 0.01$, $n=4$ animals in each group) and $58 \pm 13\%$ 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.

Keywords

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

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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 nonmyelinating 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 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 than the calculated molecular mass of the HMGCS2 protein (about 56 kDa), likely due to the post-translational acetylation of 9 lysine residues in mitochondrial HMGCS2 (Shimazu et al., 2010), which increase molecular weight of the protein (Guan et al., 2010). Preincubation with excess HMGCS1 or HMGCS2 antigenic blocking peptides completely eliminated the HMGCS1 or HMGCS2 band, respectively (Fig. 1B, C). IHC on the DRG sections showed the distribution of ring-like immunopositivity of both HMGCS1 and HMGCS2 staining surrounding the Tubb3 or phosphorylated CaMKII positive primary sensory neuron somata, which are essentially negative for HMGCS1 (Fig. 2) or HMGCS2 (Fig. 3). The tissue labeling of HMGCS1 or HMGCS2 was completely eliminated by pre-incubation of the antibodies with excessive amount of each specific blocking peptide (Figs. 2, 3), but the HMGCS1 immunopositivity was not blocked by HMGCS2 peptide and *vice versa* (Data not shown). Scattered weak immunopositivity of HMGCS1 was found in S100-positive periaxonal glial cells in the attached root and spinal nerves (Fig. 2), but no HMGCS2-ir was visualized in MBP-labeled periaxonal glial cells in the DRG, root, and spinal nerves (Fig. 3). HMGCS1-ir signals are highly colocalized to HMGCS2 immunolabeling in DRG sections (Fig. 3). Moreover, neither HMGCS1 nor HMGCS2 was detected in the astrocytes, neurons, and myelin fibers in spinal cords (Figs. 2, 3) as well as in cutaneous innervating fibers in hindpaw (Data not shown) by the IHC technique used in this study. These results validate the high specificity of the antibodies in detection of their cognate antigens by immunoblot and IHC experiments, and also suggest that both HMGCS1 and HMGCS2 are abundant and preferentially expressed in the perineuronal or perisomatic sheath of nonmyelinating satellite glial cells (SGCs) in DRGs...

2.2. Colocalization of HMGCS1 and HMGCS2 with satellite glial cell markers

Having ascertained the specificity of the antibodies, we next determine the glial cell types that express HMGCS1 and HMGCS2 by double immunolabeling using HMGCS1 or HMGCS2 antibody with various authentic glial cell markers for SGCs including GFAP, GS, K_{ir}4.1, GLAST1, and GDNF, and SGCs/Schwann cell co-positive marker S100 (Pannese, 2010), as well as Iba1 for microglial cells. Results showed that both HMGCS1 (Fig. 4A) and HMGCS2 (Fig. 4B) were highly colabeled with each of the SGC markers but colocalization was not observed with Iba1. The colabeling of HMGCS1 with S100 was observed in the perisomatic SGCs (Fig. 4A), which, however, is not definitive evidence of HMGCS1 expression in Schwann cells since S100 is expressed in both SGCs and myelinating and

nonmyelinating glial cells (Pannese, 2010). As shown in Figs. 2 and 3, MBP, a major constituent of periaxonal myelin laminae (da Silva et al., 2014), was highly enriched in the periaxonal myelin sheath of Schwann cells whereas no MBP-ir was found in the perisomatic SGCs that were positive for HMGCS1 or HMGCS2, indicating that HMGCS1- and HMGCS2-positive perisomatic SGCs are non-myelinating. Overexpression of GDNF, which is expressed in both SGCs and neurons in DRGs, has been shown to downregulate HMGCS1 expression in neuronal progenitor cells (Pahnke et al., 2004). We also found that both HMGCS1 and HMGCS2 were highly colabeled with GDNF in SGCs (Fig. 4). Collectively, these results demonstrate that HMGCS1 and HMGCS2 were predominantly expressed in the SGCs and also weakly scattered in the periaxonal glial cells (likely nonmyelinating cells) in the initial segment of axon fibers of DRG neurons. HMGCS1 and HMGCS2 were not found in the resident microglial cells, and do not seem to be a major component of myelin membrane, and Schwann cells in peripheral nervous system, as well as astrocytes, oligodendrocytes, and neurons in spinal cords. Data characterized both HMGCS1 and HMGCS2 as the novel protein markers of SGCs.

2.3. Spinal nerve injury downregulates HMGCS1 but not HMGCS2 expression

There have been no reports of how peripheral nerve injury may modify HMGCSs expression in the SGCs of DRGs. We therefore next examined the influence of SNL, a well-established animal model of chronic neuropathic pain, on both HMGCS1 and HMGCS2 expression in DRGs at the acute stage (3 days) and late stage (28 days) after injury. As previously described (Wu et al., 2010), rats developed significant mechanical hypersensitivity following SNL. Reduced threshold for withdrawal from mild mechanical stimuli (von Frey testing) represents the development of mechanical allodynia, and exaggerated and sustained responses to fully noxious mechanical stimulation (Pin testing) represents the development of hyperalgesia. These changes lasted throughout the 4-weeks testing interval (Fig. 5A, B).

For the quantitative determination of HMGCS 1 and 2 protein levels, immunoblots were performed on DRG homogenates prepared from the rats 3 days and 28 days after SNL injury. The results showed that HMGCS1 protein level in the ipsilateral L5 DRGs was decreased to $66 \pm 8\%$ at 3 days and $58 \pm 13\%$ at 28 days of the basal levels in the contralateral L5 DRGs ($p < 0.01$, $n=4$ animals in each group, mean $\pm$ SD at 3 days; $p < 0.001$, $n=9$ animals in each group, mean $\pm$ SD at 28 days; Fig. 5C, D). These results are consistent with a previous report by proteomics analyses (Komori et al., 2007). GFAP protein levels were significantly increased in the axotomized DRGs at both stages compared to controls, reflecting SGC proliferation/activation. HMGCS2 protein levels in the L5 DRGs were comparable between two groups in both 3 days and 4 weeks after injury. IHC analyses were performed on the DRG sections harvested at 4 weeks following L5 SNL. In tissue from the control DRG sections, the expected ring-like immunopositivity of HMGCS1 was found surrounding the majority of the primary sensory neuron somata. In the contrary, sections from axotomized L5 DRGs after SNL showed HMGCS1-ir signals only in some SGCs surrounding large-sized neuron somata in the ipsilateral L5 DRGs while overall HMGCS1-ir intensity was clearly reduced, despite robust SGC proliferation/activation demonstrated by GFAP staining (Fig. 6A). HMGCS2 expression was retained in the ipsilateral L5 DRGs showing comparable immunopositivity and localization, as compared to controls (Fig. 6B).

3. Discussion

Here we have provided evidence for identification of HMGCS1 and HMGCS2 expression in the SGCs of DRGs. Their immunopositivity was colabeled with a variety of specific SGC proteins, indicating that HMGCSs may function as ‘housekeeping’ molecules in the diverse SGC populations (Nascimento et al., 2008). Although weakly detected in the periaxonal glial cells in the spinal nerve and root segments, HMGCSs were not found in spinal cord and peripheral nerve terminals, suggesting that the major function of HMGCSs may not be associated with myelin formation. HMGCS1 has been well characterized as one of the key enzymes of sterol biosynthesis (Goldstein and Brown, 1990). Thus, the specific localization in SGCs may indicate an important role for HMGCS1 in the lipid biosynthesis of SGCs. Moreover, our data also demonstrate that painful spinal nerve injury is correlated with down-regulation of HMGCS1 but not HMGCS2 expression in DRGs, raising the possibility that HMGCS1 may contribute to the pathophysiology of the nerve injury-induced neuropathic pain.

Whereas glial cell-derived cholesterol has been recognized as an important source for neurons to maintain membrane-rich structures like axons and synapses (Nave and Trapp, 2008), there are few reports describing cholesterol biosynthesis in SGCs, and no reports on the significance of HMGCS1 regulation in SGCs as related to sensory function and pain mechanisms. In the peripheral nervous system, SGCs represent a class of specialized glial cells with unique properties different from Schwann cells. Although the functions of SGCs are largely unknown, they are generally described as performing comparable functions as astrocytes found in the CNS, with which they share some key proteins and similar features in response to injury (Ohara et al., 2009). In CNS, neuronal synaptogenesis requires local cholesterol production supplied by astrocytes through ApoE-containing lipoproteins (Goritz et al., 2002; Mauch et al., 2001). ApoE can also affect DRG cholesterol homeostasis and modulate the outgrowth of DRG neuronal processes (Cheng et al., 2007; Handelsmann et al., 1992). It is also evident that SGCs in sympathetic ganglia play a role in the regulating the synaptic environment, and nonmyelinating Schwann cells can enhance synaptogenesis at the neuromuscular junction (Feng and Ko, 2008; Ullian et al., 2004). Recent studies also demonstrate that DRG neurons communicate with each other and with glia through release of various mediators, suggesting that DRG neuron somata also possess ‘presynaptic-like’ or ‘sandwich synapse’ features in intraganglionic neuron-SGC-neuron communications (Gu et al., 2010; Ren and Dubner, 2010; Rozanski et al., 2013; Zhang et al., 2015). The majority of cholesterol in the peripheral nervous system resides in myelin, and the role of cholesterol metabolism in Schwann cells has been well documented, particularly with regards to degeneration and regeneration of axons (Fu et al., 1998; Goodrum, 1990, 1993; Singer and Bryant, 1969). However, it is unclear whether cholesterol regulation in SGCs plays a role in forming neuron-SGC-neuron structural and functional units. From this perspective, it is tempting to postulate that cholesterol regulation in SGCs of DRGs may also have a role in control of the perineuronal environment and facilitating non-synaptic neuron-SGC-neuron communication. Further investigation to elucidate the molecular details of this regulation under normal and injurious conditions may shed new lights on the mechanistic role of SGCs

in peripheral sensory function and help us identify new therapeutic targets to treat chronic pain.

It is noteworthy that the HMGCS1 molecule undergoes complex posttranslational modifications, including phosphorylation, lysine acetylation, S-nitrosylation, ubiquitination, and age-dependent sumoylation (Sapir et al., 2014). It is reported that HMGCS1 is significantly up-nitrosylated after spared nerve injury, and HMGCS1 is also a substrate of NAD-dependent protein deacetylase sirtuin-1 (SIRT1) (Hirschey et al., 2011) and decreased SIRT1 deacetylase activity in the spinal cord is a contributing factor for the development of neuropathic pain (Shao et al., 2014). However in the peripheral sensory system, the biological significance of these various modifications of HMGCS1 remains to be determined.

In conclusion, we report that HMGCSs is abundantly expressed in the SGCs of DRGs, and, that painful spinal nerve injury is associated with significantly downregulated HMGCS1 expression. These results may provide the basis for further investigation into the role of HMGCS1 expression in the SGCs and its relationship to sensory function and pain mechanisms.

4. Experimental procedures

4.1. Animals

Experiments were performed in adult male Sprague-Dawley (SD) rats (5–6 weeks old; 125–150 g body weight) purchased from Charles River Laboratories (Wilmington, MA). Rats were housed in standard 12 h cycle lighting and were allowed *ad libitum* access to food and water prior to and throughout the experimental protocols. All animal procedures were reviewed and approved by the Animal Care Committee of the Zablocki VA Medical Center Animal Studies Subcommittee and Medical College of Wisconsin IACUC.

4.2. Experimental spinal nerve injury and behavioral testing

Peripheral nerve injury by a L5 spinal nerve ligation model (L5 SNL) was induced in isoflurane-anesthetized animals by tight ligation and transection of the right L5 spinal nerve between the DRG and the beginning of the spinal nerve (Fischer et al., 2011). Behavioral evaluation by mechanical withdrawal threshold testing (von Frey) and noxious punctate mechanical stimulation (Pin test) was carried out as previously described (Fischer et al., 2011). Briefly, von Frey test, began with the 2.8 g (g) filament, applied with just enough force to bend the fiber and held for 1 s (s). If a response was observed, the next smaller filament was applied, and if no response was observed, the next larger was applied, until a reversal occurred, defined as a withdrawal after a previous lack of withdrawal, or *vice versa*. Following a reversal event, four more stimulations were performed following the same pattern. The forces of the filaments before the reversal, and the four filaments applied following the reversal, were used to calculate the 50% withdrawal threshold. Rats not responding to any filament were assigned a score of 25 g. Noxious punctate mechanical stimulation (Pin test) was performed using the point of a 22 g spinal anesthesia needle that was applied to the center of the hindpaw with enough force to indent the skin but not

puncture it. Five applications were separated by at least 10 s, which was repeated after 2 min, making a total of 10 touches. For each application, the induced behavior was either a very brisk, simple withdrawal with immediate return of the foot to the cage floor, or a sustained elevation with grooming that included licking and chewing, and possibly shaking, which lasted at least 1 s. This hyperalgesic behavior is specifically associated with place avoidance (Wu et al., 2010). Hyperalgesia was quantified by tabulating hyperalgesia responses as a percentage of total touches.

4.3. Immunohistochemistry (IHC)

Animals tested were terminally anesthetized. L5 DRGs, as well as the corresponding levels of the spinal cords, the sciatic nerves at middle thigh level, and the medial plantar skin of hindpaws were dissected, post-fixed in 10% buffered formalin, and processed for paraffin embedding and sectioning. IHC double staining was performed to characterize cellular specificity and distribution of target molecules in sections, as described in previous publication (Yu et al., 2011). In brief, 5 μ m-thick sections were deparaffinized, rehydrated through graded alcohol, and treated by heat-induced epitope retrieval in 10 mM citrate buffer, pH 6.0. Sections were first immunolabeled with the selected primary antibodies at appropriate dilutions (Table 1). All antibodies were diluted in 1 $\times$ phosphate buffered saline (PBS), containing 0.05% Triton X-100 and 3% bovine serum albumin (BSA). Non-immune immunoglobulin G (IgG) control (SCB) was replaced for the first antibody as the negative control. The appropriate fluorophore-conjugated (Alexa 488 or Alexa 594, 1:2000) secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used to reveal immune complexes. The sections were washed three times 5 min each with PBS containing 0.05% tween 20 between incubations. To confirm specificity of HMGCS1 and HMGCS2 immunolabeling, antibody solutions were preincubated with the specific immunogenic peptide (5 μ g/ml, SCB) for 2 h (hr) prior to IHC. The sections were examined and images acquired on a Nikon TE2000-S fluorescence microscope (El Segundo, CA), equipped with an Optronics QuantiFire digital camera and acquisition software (Ontario, NY), as well as filters suitable for selectively detecting the green, red and blue fluorescence. For each comparative experiment, all images were acquired with identical settings for detector gain and under a 20 $\times$ objective with 0.5 numerical aperture at 2048 $\times$ 2048 pixel resolution. For double-label colocalization, images from the same section but showing different antigen signals were overlaid.

4.4. Immunoblots

DRG protein homogenates were extracted using 1 $\times$ RIPA buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, with 0.1% Triton X100 and protease inhibitor cocktail) and protein concentration determined by using the BCA kit (Pierce, Rockford, IL). DRG homogenates (20 μ g protein per sample) were size separated using 10% SDS-PAGE gels, transferred to 0.22 μ m nitrocellulose membranes, and blocked for 1hr in 5% skim milk. The blots were subsequently incubated overnight at 4 $^{\circ}$ C with a polyclonal goat anti-HMGCS1 (1:400, SCB), a mouse monoclonal anti-HMGCS2 (1:400, SCB), and a polyclonal rabbit GFAP (1:2000, Dako). To confirm the band specificity of HMGCS1 and HMGCS2 detection, the antibody solutions were preincubated with the specific HMGCS1 or HMGCS2 blocking peptides (5 μ g/ml, SCB), respectively, for 2hr prior

to immunoblotting. Immunoreactive proteins were detected by enhanced chemiluminescence (Pierce, Rockford, IL) after incubation for 1hr with HRP-conjugated second antibodies (1:2000, SCB). The same membrane was stripped at 60°C for 30 min with stripping buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol and reprobed with anti GAPDH (1:2000, Sigma-Aldrich) or Tubb3 (1:1000, SCB). Densitometry of bands of interests was analyzed using ImageJ v.1.46. Ratios of the band density of the target proteins to the housekeeping gene were calculated and the percentage changes of target proteins in the experimental samples compared with those from the control samples (Liu et al., 2016).

4.5. Statistics

Statistical analysis was performed with GraphPad PRISM 6 (GraphPad Software, San Diego, CA). Behavioral changes over baseline and between groups for von Frey measurements were made using repeated measures two-way ANOVA and *post-hoc* analysis with Bonferroni test. Pin test results in discrete numerical data without normal distribution so conservative nonparametric analysis was performed by Friedman's test for analysis of variance and Dunn's test for *post hoc* analysis. The differences of targeted gene expression by immunoblots were compared with a two-tailed unpaired *t*-test. Results are reported as mean and standard deviation (SD). $P < 0.05$ were considered statistically significant.

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References

- Aledo R, Mir C, Dalton RN, Turner C, Pie J, Hegardt FG, Casals N, Champion MP. Refining the diagnosis of mitochondrial HMG-CoA synthase deficiency. *J Inher Metab Dis*. 2006; 29:207–211. [PubMed: 16601895]
- Ayte J, Gil-Gomez G, Haro D, Marrero PF, Hegardt FG. Rat mitochondrial and cytosolic 3-hydroxy-3-methylglutaryl-CoA synthases are encoded by two different genes. *Proc Natl Acad Sci USA*. 1990; 87:3874–3878. [PubMed: 1971108]
- Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell*. 2009; 139:267–284. [PubMed: 19837031]
- Chen G, Fang X, Yu M. Regulation of gene expression in rats with spinal cord injury based on microarray data. *Mol Med Rep*. 2015; 12:2465–2472. [PubMed: 25936407]
- Cheng H, Jiang X, Han X. Alterations in lipid homeostasis of mouse dorsal root ganglia induced by apolipoprotein E deficiency: a shotgun lipidomics study. *J Neurochem*. 2007; 101:57–76. [PubMed: 17241120]
- Costa FA, Moreira Neto FL. Satellite glial cells in sensory ganglia: its role in pain. *Rev Bras Anesthesiol*. 2015; 65:73–81. [PubMed: 25497752]
- Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci*. 2009; 32:1–32. [PubMed: 19400724]
- Cotter DG, Ercal B, Huang X, Leid JM, d'Avignon DA, Graham MJ, Dietzen DJ, Brunt EM, Patti GJ, Crawford PA. Ketogenesis prevents diet-induced fatty liver injury and hyperglycemia. *J Clin Invest*. 2014; 124:5175–5190. [PubMed: 25347470]
- D'Antonio M, Michalovich D, Paterson M, Droggiti A, Woodhoo A, Mirsky R, Jessen KR. Gene profiling and bioinformatic analysis of Schwann cell embryonic development and myelination. *Glia*. 2006; 53:501–515. [PubMed: 16369933]

- da Silva TF, Eira J, Lopes AT, Malheiro AR, Sousa V, Luoma A, Avila RL, Wanders RJ, Just WW, Kirschner DA, Sousa MM, Brites P. Peripheral nervous system plasmalogens regulate Schwann cell differentiation and myelination. *J Clin Invest*. 2014; 124:2560–2570. [PubMed: 24762439]
- Di Narzo AF, Kozlenkov A, Ge Y, Zhang B, Sanelli L, May Z, Li Y, Fouad K, Cardozo C, Koonin EV, Bennett DJ, Dracheva S. Decrease of mRNA editing after spinal cord injury is caused by down-regulation of ADAR2 that is triggered by inflammatory response. *Sci Rep*. 2015; 5:12615. [PubMed: 26223940]
- Feng Z, Ko CP. Schwann cells promote synaptogenesis at the neuromuscular junction via transforming growth factor-beta1. *J Neurosci: Off J Soc Neurosci*. 2008; 28:9599–9609.
- Ferrari LF, Lotufo CM, Araldi D, Rodrigues MA, Macedo LP, Ferreira SH, Parada CA. Inflammatory sensitization of nociceptors depends on activation of NMDA receptors in DRG satellite cells. *Proc Natl Acad Sci USA*. 2014; 111:18363–18368. [PubMed: 25489099]
- Fischer G, Kostic S, Nakai H, Park F, Sapunar D, Yu H, Hogan Q. Direct injection into the dorsal root ganglion: technical, behavioral, and histological observations. *J Neurosci Methods*. 2011; 199:43–55. [PubMed: 21540055]
- Fu Q, Goodrum JF, Hayes C, Hostettler JD, Toews AD, Morell P. Control of cholesterol biosynthesis in Schwann cells. *J Neurochem*. 1998; 71:549–555. [PubMed: 9681444]
- Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature*. 1990; 343:425–430. [PubMed: 1967820]
- Goodrum JF. Cholesterol synthesis is down-regulated during regeneration of peripheral nerve. *J Neurochem*. 1990; 54:1709–1715. [PubMed: 2324745]
- Goodrum JF. Cholesterol synthesis in regenerating peripheral nerve is not influenced by serum cholesterol levels. *J Neurochem*. 1993; 60:1564–1566. [PubMed: 8455042]
- Goritz C, Mauch DH, Nagler K, Pfrieger FW. Role of glia-derived cholesterol in synaptogenesis: new revelations in the synapse-glia affair. *J Physiol*. 2002; 96:257–263.
- Griffin JW, Thompson WJ. Biology and pathology of nonmyelinating Schwann cells. *Glia*. 2008; 56:1518–1531. [PubMed: 18803315]
- Gu Y, Chen Y, Zhang X, Li GW, Wang C, Huang LY. Neuronal soma-satellite glial cell interactions in sensory ganglia and the participation of purinergic receptors. *Neuron glia Biol*. 2010; 6:53–62. [PubMed: 20604979]
- Guan KL, Yu W, Lin Y, Xiong Y, Zhao S. Generation of acetyllysine antibodies and affinity enrichment of acetylated peptides. *Nat Protoc*. 2010; 5:1583–1595. [PubMed: 21085124]
- Hanani M. Satellite glial cells in sensory ganglia: from form to function. *Brain Res Brain Res Rev*. 2005; 48:457–476. [PubMed: 15914252]
- Handelmann GE, Boyles JK, Weisgraber KH, Mahley RW, Pitas RE. Effects of apolipoprotein E, beta-very low density lipoproteins, and cholesterol on the extension of neurites by rabbit dorsal root ganglion neurons in vitro. *J Lipid Res*. 1992; 33:1677–1688. [PubMed: 1464751]
- Hirschey MD, Shimazu T, Capra JA, Pollard KS, Verdin E. SIRT1 and SIRT3 deacetylate homologous substrates: *AceCS1,2* and *HMGCS1,2*. *Aging*. 2011; 3:635–642. [PubMed: 21701047]
- Huang LY, Gu Y, Chen Y. Communication between neuronal somata and satellite glial cells in sensory ganglia. *Glia*. 2013; 61:1571–1581. [PubMed: 23918214]
- Komori N, Takemori N, Kim HK, Singh A, Hwang SH, Foreman RD, Chung K, Chung JM, Matsumoto H. Proteomics study of neuropathic and nonneuropathic dorsal root ganglia: altered protein regulation following segmental spinal nerve ligation injury. *Physiol Genom*. 2007; 29:215–230.
- Liu Z, Wang F, Fischer G, Hogan QH, Yu H. Peripheral nerve injury induces loss of nociceptive neuron-specific Galphai-interacting protein in neuropathic pain rat. *Mol Pain*. 2016; 12
- Mathews ES, Mawdsley DJ, Walker M, Hines JH, Pozzoli M, Appel B. Mutation of 3-hydroxy-3-methylglutaryl CoA synthase I reveals requirements for isoprenoid and cholesterol synthesis in oligodendrocyte migration arrest, axon wrapping, and myelin gene expression. *J Neurosci: Off J Soc Neurosci*. 2014; 34:3402–3412.
- Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A, Pfrieger FW. CNS synaptogenesis promoted by glia-derived cholesterol. *Science*. 2001; 294:1354–1357. [PubMed: 11701931]

- Nascimento RS, Santiago MF, Marques SA, Allodi S, Martinez AM. Diversity among satellite glial cells in dorsal root ganglia of the rat. *Braz J Med Biol Res.* 2008; 41:1011–1017. [PubMed: 19030716]
- Nave KA, Trapp BD. Axon-glial signaling and the glial support of axon function. *Annu Rev Neurosci.* 2008; 31:535–561. [PubMed: 18558866]
- Ohara PT, Vit JP, Bhargava A, Romero M, Sundberg C, Charles AC, Jasmin L. Gliopathic pain: when satellite glial cells go bad. *Neurosci: Rev J Bringing Neurobiol Neurol Psychiatry.* 2009; 15:450–463.
- Pahnke J, Mix E, Knoblich R, Muller J, Zschiesche M, Schubert B, Koczan D, Bauer P, Bottcher T, Thiesen HJ, Lazarov L, Wree A, Rolfs A. Overexpression of glial cell line-derived neurotrophic factor induces genes regulating migration and differentiation of neuronal progenitor cells. *Exp Cell Res.* 2004; 297:484–494. [PubMed: 15212950]
- Pannese E. The structure of the perineuronal sheath of satellite glial cells (SGCs) in sensory ganglia. *Neuron glia Biol.* 2010; 6:3–10. [PubMed: 20604977]
- Ren K, Dubner R. Interactions between the immune and nervous systems in pain. *Nat Med.* 2010; 16:1267–1276. [PubMed: 20948535]
- Rozanski GM, Nath AR, Adams ME, Stanley EF. Low voltage-activated calcium channels gate transmitter release at the dorsal root ganglion sandwich synapse. *J Physiol.* 2013; 591:5575–5583. [PubMed: 24000176]
- Sapir A, Tsur A, Koorman T, Ching K, Mishra P, Bardenheier A, Podolsky L, Bening-Abu-Shach U, Boxem M, Chou TF, Broday L, Sternberg PW. Controlled sumoylation of the mevalonate pathway enzyme HMGs-1 regulates metabolism during aging. *Proc Natl Acad Sci USA.* 2014; 111:E3880–E3889. [PubMed: 25187565]
- Shao H, Xue Q, Zhang F, Luo Y, Zhu H, Zhang X, Zhang H, Ding W, Yu B. Spinal SIRT1 activation attenuates neuropathic pain in mice. *PloS One.* 2014; 9:e100938. [PubMed: 24959710]
- Shimazu T, Hirschey MD, Hua L, Dittenhafer-Reed KE, Schwer B, Lombard DB, Li Y, Bunkenborg J, Alt FW, Denu JM, Jacobson MP, Verdin E. SIRT3 deacetylates mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 and regulates ketone body production. *Cell Metab.* 2010; 12:654–661. [PubMed: 21109197]
- Singer M, Bryant SV. Movements in the myelin Schwann sheath of the vertebrate axon. *Nature.* 1969; 221:1148–1150. [PubMed: 4975278]
- Tshala-Katumbay D, Monterroso V, Kayton R, Lasarev M, Sabri M, Spencer P. Probing mechanisms of axonopathy. Part I: protein targets of 1,2-diacetylbenzene, the neurotoxic metabolite of aromatic solvent 1,2-diethylbenzene. *Toxicol Sci: Off J Soc Toxicol.* 2008; 105:134–141.
- Ullian EM, Christopherson KS, Barres BA. Role for glia in synaptogenesis. *Glia.* 2004; 47:209–216. [PubMed: 15252809]
- Wu HE, Gemes G, Zoga V, Kawano T, Hogan QH. Learned avoidance from noxious mechanical simulation but not threshold semmes weinstein filament stimulation after nerve injury in rats. *J Pain: Off J Am Pain Soc.* 2010; 11:280–286.
- Yu H, Fischer G, Jia G, Reiser J, Park F, Hogan QH. Lentiviral gene transfer into the dorsal root ganglion of adult rats. *Mol Pain.* 2011; 7:63. [PubMed: 21861915]
- Zhang Y, Zhao S, Rodriguez E, Takatoh J, Han BX, Zhou X, Wang F. Identifying local and descending inputs for primary sensory neurons. *J Clin Investig.* 2015; 125:3782–3794. [PubMed: 26426077]

A Alignment of HMGCS1 and HMGCS2 C-termini

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.

Fig. 2.

HMGCS1 immunofluorescent histochemistry (IHC). IHC in DRG sections shows ring-like immunopositivity of HMGCS1 staining (A, green) surrounding the majority of Tubb3-positive primary sensory neurons (red) that are negative for HMGCS1 (B). HMGCS1 immunolabeling was eliminated (C) to negative control level (D) by incubation of the antibody with an excess of the antigenic peptide (BP). HMGCS1 was not found in MBP-positive (+) myelin sheaths ensheathing the axons within DRG (E), although weakly scattered immunopositivity was visible in the S-100 positive periaxonal glial cells in attached root (F) and spinal nerve (G). No HMGCS1 was found in GFAP+ astrocytes (H), MBP+ myelin fibers (I), and pCaMKII+ neurons (J) in spinal cord sections. Scale bar: 100 μ m for all.

Fig. 3.

IHC for HMGCs2. IHC in DRG sections shows ring-like immunopositivity of HMGCs2 staining (A, green) surrounding the majority of pCaMKII-positive primary sensory neurons (red) that are negative for HMGCs2 (B, inset is high-power view). HMGCs2 immunolabeling was eliminated (C) by incubation of the antibody with an excess of the antigenic peptide (BP). HMGCs2 was not found in MBP-positive (+) myelin sheaths ensheathing the axons within DRG (D), attached root (E), and spinal nerve (F). No HMGCs1 was found in GFAP+ astrocytes (G), MBP+ myelin fibers (H), and pCaMKII+

neurons (Not shown) in spinal cord sections. HMGCS2 is highly colabeled with HMGCS2 (I). Scale bar: 100 μ m for all.

Fig. 4. Colocalization of HMGCS1 and HMGCS2 with glial cell markers. (A, B) Representative results of double immunolabeling of HMGCS1 (A) and HMGCS2 (B) with multiple authentic glial cell markers (GFAP, GS, Iba1, Kir4.1, GLAST1, GDNF or S100), labeled at the top with staining indicated at the left of the panels. Magnified images were counterstained with Hoechst (blue). Note that sensory neuron nuclei are weakly stained by Hoechst, as is typically seen. Scale bar: 100 μ m for all panels.

Fig. 5.

Immunoblotting of HMGCs in spinal nerve injury. (A, B) Rats with L5 SNL developed mechanical allodynia [von Frey, A. ### and *** $p < 0.001$ for comparison to baseline (BL) and between groups, respectively] and hyperalgesia (Pin, B. # $p < 0.05$, ## $p < 0.01$ for comparison to BL and * $p < 0.05$, ** $p < 0.01$ for comparison between groups). (C, D) Representative immunoblots of HMGC1, HMGC2, GFAP, and Tubb3 on L5 DRG lysates at three days (C) and 28 days (D) after L5 SNL and control samples as indicated. Bar chart in right panels of C and D are the results of densitometry analysis (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$). No significant change of HMGC2 protein level in the ipsilateral (ipsi.) L5 DRGs was observed compared to the contralateral (contra.) L5 DRGs.

Fig. 6. IHC comparison of HMGCSs expression in L5 DRGs of spinal nerve injury. (A) Four weeks after L5 SNL, HMGCS1-ir intensity is apparently reduced in the ipsilateral (ipsi.) L5 DRGs. (B) HMGCS2 expression was retained in the ipsilateral L5 DRG showing comparable immunopositivity and localization, as compared to the contralateral (contra.) L5 DRG. GFAP staining revealed marked glial cell activation in the ipsilateral L5 DRGs while GFAP is weakly detectable in SGCs in the contralateral L5 DRGs. Scale bar: 100 μ m for all panels.

Table 1

Primary antibodies used in this study.

Antibody ^a	Host	Supplier/Catalog# ^b	Dilution
HMGCS1	Goat polyclonal	SCB/sc-32422	IHC, 1:200; Wb, 1:400
HMGCS2	Mouse monoclonal	SCB/sc-393256	IHC, 1:200; Wb, 1:400
Tubb3	Mouse monoclonal	SCB/sc-80016	IHC, 1:400; Wb, 1:1000
pCaMKII	Rabbit polyclonal	SCB/sc-12886 R	1:2000
GFAP	Rabbit polyclonal	Dako/Z0334	IHC, 1:1000; Wb, 1:2000
GS	Rabbit polyclonal	SCB/sc-6640 R	1:600
Iba1	Rabbit polyclonal	Wako/019-19741	1:1000
K _{ir} 4.1	Guinea pig polyclonal	Alomone Labs/AGP-012	1:100
GLAST1	Rabbit polyclonal	Alomone Labs/AGC-021	1:100
GDNF	Rabbit polyclonal	SCB/sc-328	1:500
S100	Mouse monoclonal	NeoMarkers/MS296P1	1:200
MBP	Goat polyclonal	SCB/sc-13912	1:1000

^aAntibody abbreviations: HMGCS1, *HMG CoA synthase 1*; HMGCS2, *HMG CoA synthase 2*; Tubb3, *β3-Tubulin*; pCaMKII, phosphorylated calcium/calmodulin-depen-dent kinase II; GFAP, *Glial fibrillary acidic protein*; GS, *Glutamine synthetase*; Iba1, *Ionized calcium binding adaptor molecule 1*; K_{ir}4.1, *ATP-sensitive inward rectifier potassium channel 10*; GLAST1, *Excitatory amino acid transporter 1*; GDNF, *Glial cell line-derived neurotrophic factor*; S100, *S-100 calcium-binding protein*; MBP, *Myelin basic protein*.

^bSCB, Santa Cruz Biotechnology, Santa Cruz, CA; Dako, Carpinteria, CA; Wako, Richmond, VA; Alomone Labs, Jerusalem, Israel; NeoMarkers, Fremont, CA.