The Journal of Veterinary Medical Science

NOTE

Anatomy

Expression of prosaposin and its G protein-coupled receptor (GPR) 37 in mouse cochlear and vestibular nuclei

Aimi FUYUKI[1)], Saki YAMAMOTO[1)], Md Shahriar Hasan SOHEL[2)], Takeshi HOMMA[2)], Kai KITAMURA[1)], Sawa ONOUCHI[1,2)], Shouichiro SAITO[1,2)]*

[1)]Laboratory of Veterinary Anatomy, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan
[2)]Laboratory of Veterinary Anatomy, The Joint Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan

J. Vet. Med. Sci.
85(3): 266–270, 2023
doi: 10.1292/jvms.22-0552

Received: 14 December 2022
Accepted: 14 January 2023
Advanced Epub:
24 January 2023

ABSTRACT. Prosaposin is a precursor of lysosomal hydrolases activator proteins, saposins, and also acts as a secretory protein that is not processed into saposins. Prosaposin elicits neurotrophic function via G protein-coupled receptor (GPR) 37, and prosaposin deficiency causes abnormal vestibuloauditory end-organ development. In this study, immunohistochemistry was used to examine prosaposin and GPR37 expression patterns in the mouse cochlear and vestibular nuclei. Prosaposin immunoreactivity was observed in neurons and glial cells in both nuclei. GPR37 immunoreactivity was observed in only some neurons, and its immunoreactivity in the vestibular nucleus was weaker than that in the cochlear nucleus. This study suggests a possibility that prosaposin deficiency affects not only the end-organs but also the first center of the vestibuloauditory system.

KEYWORDS: auditory organ, autophagy, exocytosis, immunofluorescence, vestibular organ

Prosaposin is a glycoprotein that is widely conserved in vertebrates. It is a precursor of lysosomal hydrolases activator proteins, saposins. The lysosomal enzymes require activator proteins for their hydrolysis function [21], and in the lysosome, prosaposin is processed into 4 kinds of sphingolipid hydrolase activators, saposin A [10, 11, 18, 28, 34], B [18, 28, 34], C [10, 11, 18, 28, 34], and D [18, 19, 28, 34]. Not only inside the cell, prosaposin could be found in cerebrospinal fluid [13, 14], and is known to act as a neurotrophic factor [29, 30]. Prosaposin can activate mitogen-activated protein kinase (MAPK) via G protein-coupled receptor (GPR), and GPR37 is one of the candidate receptors for prosaposin [23, 24]. In mammals, prosaposin deficiency induces hypertrophy of Deiters' cells and inner sulcus cells, loss of outer hair cells, exuberant afferent neurite outgrowth, and efferent nerve terminal ending proliferation in Corti's organ; supporting cell disruption and afferent neurite swelling in the ampullae, saccule, and utricle; and finally, deafness and severe vestibular dysfunction [2, 3]. Therefore, prosaposin plays pivotal roles in normal development and homeostasis maintenance in the vestibuloauditory end-organs. Conversely, prosaposin's functional significance in the primary relay nuclei of the vestibuloauditory system in the medulla oblongata, cochlear and vestibular nuclei, still remains unknown. Therefore, in this study, expression patterns of prosaposin and its receptor GPR37 were examined in mouse cochlear and vestibular nuclei by immunohistochemistry.

Five adults (8 weeks old) male ddY mice weighting 35–45 g were obtained from Japan SLC, Inc. (Shizuoka, Japan), housed at 24 ± 2°C under a 12/12-hr light/dark cycle and provided food and water *ad libitum*. After euthanasia with an intraperitoneal Somnopentyl (8×10^{-3} mL/g body weight) injection, animals were transcardially perfused with physiological saline and 4% paraformaldehyde in 0.1 M phosphate-buffer (PB; pH 7.4). The hindbrain was dissected and immersed in the same fixative for 2–3 days. Brain tissue was then transferred to 15% sucrose in 0.1 M PB at 4°C for 1 day, followed by immersion in 30% sucrose in 0.1 M PB at 4°C until the tissue sank in the solution. This cryoprotected tissue was embedded in OCT compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan), frozen at −60°C, cut transversely to 20 µm thickness on a cryostat (CM1510S, Leica Biosystem GmbH., Nussloch, Germany), mounted onto MAS adhesive-coated slides (Matsunami Glass Ind., Ltd., Osaka, Japan), and stored at −25°C until use. For immunohistochemistry, sections were immersed in 0.3% H_2O_2 in methanol at room temperature (RT) for 20 min to eliminate endogenous peroxidase, washed with 0.01 M phosphate-buffered saline (PBS; pH 7.4), and incubated with 2% normal goat serum at RT for 30 min. After rinsing in PBS, the sections were incubated with rabbit anti-prosaposin polyclonal antibody (GTX101064, GeneTex, Irvine, CA, USA) diluted 1:250 or rabbit anti-GPR37 polyclonal antibody (bs-13534R, Bioss Antibodies, Woburn, MA, USA) diluted 1:250 at 4°C for 48 hr. After rinsing in PBS, the sections were incubated with biotinylated goat anti-rabbit IgG (ab6720, Abcam, Cambridge, UK) diluted

*Correspondence to: Saito S: shouichi@gifu-u.ac.jp, Laboratory of Veterinary Anatomy, The Joint Graduate School of Veterinary Sciences, Gifu University, Yanagido 1-1, Gifu-shi, Gifu 501-1193, Japan

1:500 at RT for 60 min. After rinsing in PBS, the sections were incubated with Vectastain ABC Reagent (Vector Laboratories, Newark, CA, USA) at RT for 30 min. Finally, the sections were colorized for 10 min in 0.1 M Tris–HCl buffer (pH 7.4) containing 0.02% diaminobenzidine (DAB) and 0.003% H_2O_2, and then counterstained with hematoxylin, dehydrated, and coverslipped. The antibody specificities were reported previously [33]. For immunofluorescence, the sections were incubated with 2% normal donkey serum at RT for 30 min, and then incubated with guinea pig anti-PGP9.5 polyclonal antibody (GTX10410, GeneTex) diluted 1:500 or goat anti-GFAP polyclonal antibody (GTX89226, GeneTex) diluted 1:500, and anti-prosaposin antibody (GTX101064) diluted 1:250 or anti-GPR37 antibody (bs-13534R) diluted 1:250 at 4°C for 48 hr. After rinsing in PBS, the sections were incubated with Alexa Fluor 488–conjugated donkey anti-guinea pig IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted 1:100 or Alexa Fluor 488–conjugated donkey anti-goat IgG (Jackson ImmunoResearch Laboratories) diluted 1:100, and Alexa Fluor 594–conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories) diluted 1:100 at RT for 90 min. The sections were coverslipped and observed by confocal microscope (LSM710, Carl Zeiss, Obercohen, Germany). The posteroventral part of the cochlear nucleus and the medial part of the vestibular nucleus were mainly observed in this study. The experimental procedures conformed with the Regulations for Animal Experiments in Gifu University, were reviewed by the Committee for Animal Research and Welfare of Gifu University, and finally, were approved by the President of the University (Permission No. 2020-257).

In the cochlear nucleus, prosaposin immunoreactivity was observed as dot-like structures in the cytoplasm of broad types of cells (Fig. 1A and 1B). Both the neuronal marker PGP9.5-positive neurons and PGP9.5-negative cells in the cochlear nucleus showed prosaposin immunoreactivity (Fig. 1C–E). Prosaposin immunoreactivity in the vestibular nucleus was similar to that in the cochlear nucleus. Broad types of cells in the vestibular nucleus showed dot-like immunoreactivity of prosaposin (Fig. 1F and 1G). Both PGP9.5-positive and -negative cells showed prosaposin immunoreactivity (Fig. 1H–J). To consider prosaposin immunoreactivity in PGP9.5-negative cells, we performed double-immunofluorescence for prosaposin and the astrocyte marker GFAP. The dot-like immunoreactivity of prosaposin was observed in GFAP-positive cells in both nuclei (Fig. 1K and 1L). On the other hand, GPR37 immunoreactivity was weak and observed sparsely in the cochlear nucleus cells (Fig. 2A and 2B). GPR37 immunoreactivity was found only in PGP9.5-positive cells (Fig. 2C–E). GPR37 immunoreactivity in the vestibular nucleus was weaker and more sparsely distributed than those in the cochlear nucleus (Fig. 2F and 2G). GPR37 immunoreactivity was also found only in PGP9.5-positive cells (Fig. 2H–J) similar to the cochlear nucleus.

Prosaposin is widely distributed in the mammalian brain [20] and is secreted not only from the choroid plexus but also from some neurons, such as hippocampal neurons, via autocrine or paracrine manner [26]. Extracellular prosaposin appears to induce neurotrophic effects via the activation of GPRs, or via endocytosis. Prosaposin is known to activate GPRs, and its neurotrophic effect is suppressed by the treatment of non-hydrolysable GDP analog or pertussis toxin in cultured cells [6, 15]. For endocytosis, endocytic receptors, mannose-6-phosphate receptor (M6PR), sortilin, and low-density lipoprotein receptor-related protein 1 (LRP1) are involved in prosaposin internalization. For M6PR, it is known to mediate endocytosis of exogenous lysosomal molecules [36], and the suppression of M6PR expression reduces the endocytosis of prosaposin [31, 38]. For LRP1, the endocytosis of prosaposin is reduced in cultured cells incubated with an anti-LRP1 antibody, or in LRP1 knockout cells [12]. For sortilin, it can transport extracellular prosaposin in complementary to M6PR or LRP1 pathways [9, 39]. The incorporated prosaposin may facilitate autophagy in neurons. It is known that the disfunction of the endosomal-lysosomal-autophagic pathway causes many neurodegenerative diseases [27], the pathway activating autophagy in neurons is different from that in non-neuronal cells [25], and postmitotic neurons show a higher autophagy basal rate than non-neuronal cells [35]. In the vestibuloauditory system, autophagy suppression inhibits vestibular and spiral ganglion development [1, 22] in which neurons express high-level prosaposin [2, 3, 37]. The dot-like immunoreactivity of prosaposin observed in the cochlear and vestibular nuclei neurons in this study may suggest prosaposin localization in the secretory granules, endosomes, and/or lysosomes of those neurons. These results imply the crucial role of prosaposin in these nuclei similar to that in the related end-organs and ganglions. Prosaposin is also known to activate the glial cells. Prosaposin can prevent cell death and increase sulfatide concentration in Schwann cells and oligodendrocytes [16], activate MAP-kinase and increase sulfatide content in Schwann cells [6, 17], reduce the cell death of Schwann cells by activating PI3K/Akt pathway [7], and protect astrocytes from oxidative stress [23]. In this study, prosaposin immunoreactivity was also found in the glial cells of these nuclei, suggesting that prosaposin is utilized in both neurons and glial cells in these nuclei.

This study is the first report to show the expression pattern of GPR37 in the vestibuloauditory system. GPR37 was shown to be expressed only in neurons, but it seemed that the number of immunoreactive neuron was small and the immunointensity was weak in GPR37 comparing to those in prosaposin, in the cochlear and vestibular nuclei. Only a subset of neurons may express GPR37 in these nuclei. On the other hand, the immunointensity in the cochlear nucleus neurons appeared to be more intense than that in the vestibular nucleus. The auditory dysfunction was reported to be more severe than the vestibular dysfunction in prosaposin mutation mice [4]. Present study implies the possibility that the difference in severity between auditory and vestibular disfunction may be attributable to the difference in affinity to prosaposin between the auditory and vestibular nuclei, which express different levels of GPR37. However, GPR37 immunoreactivity should be quantified, and GPR37 mRNA expression should be examined by *in situ* hybridization for a more accurate discussion. This study is the first trial to examine GPR37 expression in the vestibuloauditory system; thus, the GPR37 immunoreactive neuron type as well as their precise location in the examined nuclei could not be defined. The cochlear nucleus could be divided into anteroventral, posteroventral, and dorsal parts [8, 32], and the vestibular nucleus into medial, descending, lateral, and superior parts [5], depending on the type of neurons and projections. Currently, we are attempting to identify the precise distribution of GPR37 immunoreactive neurons in these nuclei. In conclusion, this study revealed that both neurons and glial cells express prosaposin, and a small number of neurons also expresses GPR37, in the mouse auditory and vestibular nuclei.

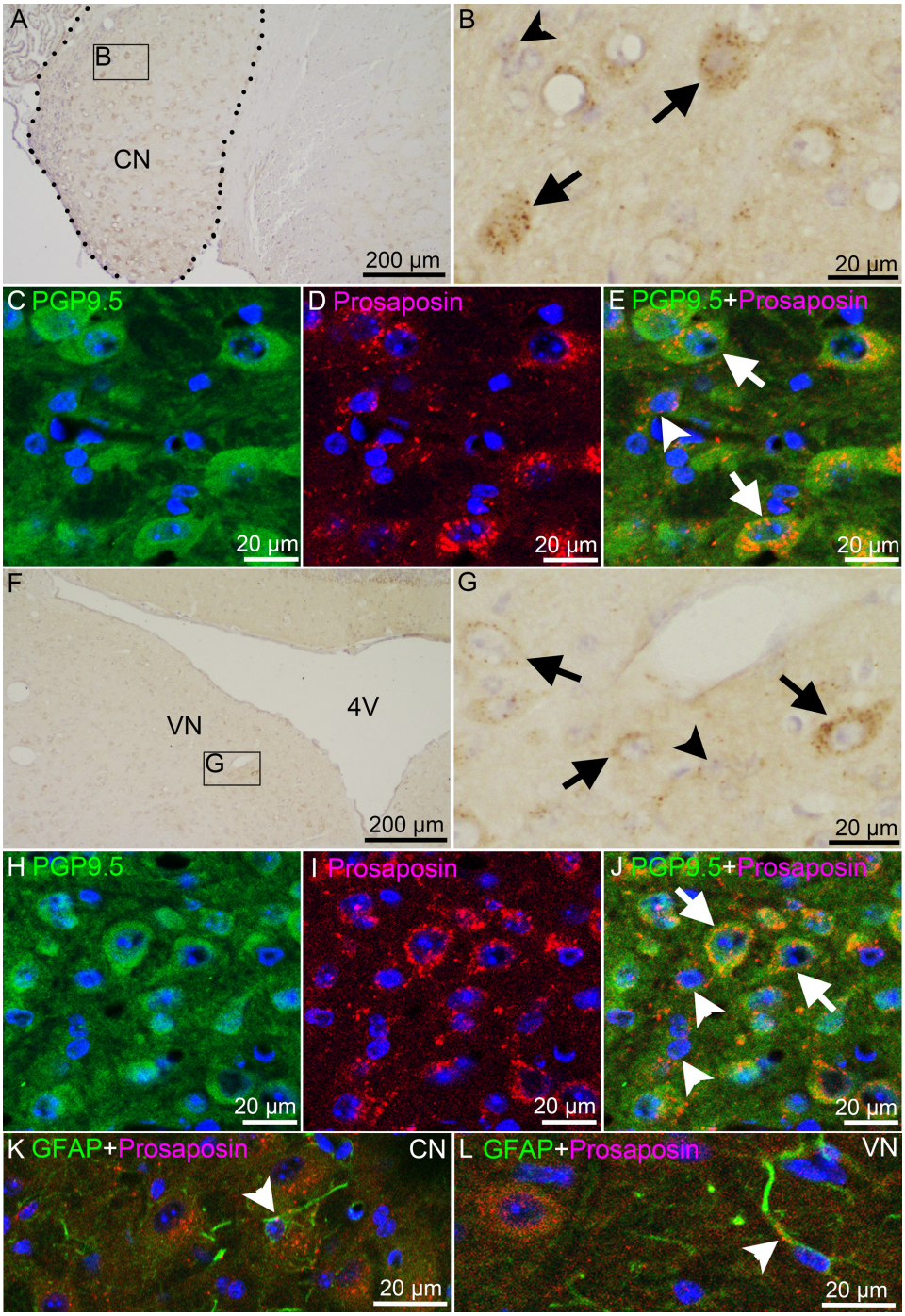

Fig. 1. Prosaposin immunoreactivity in the cochlear (CN) and vestibular nuclei (VN). (**A**) Prosaposin immunoreactivity with hematoxylin counterstaining in CN. Immunoreactivity was widely distributed in CN (circled by dashed line). (**B**) High-magnification squared in (A). The dot-like immunostaining pattern in the cell body was observed in the large- (arrows) and small-sized cells (arrowhead). (**C**) Neuronal marker PGP9.5 immunoreactivity in CN. Only neurons were stained by anti-PGP9.5 antibody. (**D**) Prosaposin immunoreactivity in CN. (**E**) Double immunofluorescence staining of PGP9.5 and prosaposin in CN. Prosaposin immunoreactivities were found in PGP9.5-positive (arrows) and PGP9.5-negative (arrowhead) cells. (**F**) Prosaposin immunoreactivity in VN near the lateral fourth ventricle (4V). Immunoreactivity was widely distributed in VN. (**G**) High magnification squared in (F). Prosaposin immunoreactivity in VN was similar to that in CN. Arrows indicate that the large cells contained dot-like prosaposin immunoreactivities in soma, and arrowhead indicates small-sized cell showing prosaposin immunoreactivity. (**H**) PGP9.5 immunoreactivity in VN. (**I**) Prosaposin immunoreactivity in VN. (**J**) Double immunofluorescence staining of PGP9.5 and prosaposin in VN. Prosaposin immunoreactivities were found in PGP9.5-positive (arrows) and PGP9.5-negative (arrowheads) cells. (**K**) Double immunofluorescence staining of GFAP and prosaposin in CN. Arrowhead indicates prosaposin immunoreactivity in GFAP-positive cell. (**L**) Double immunofluorescence staining of GFAP and prosaposin in VN. Arrowhead indicates prosaposin immunoreactivity in GFAP-positive cell.

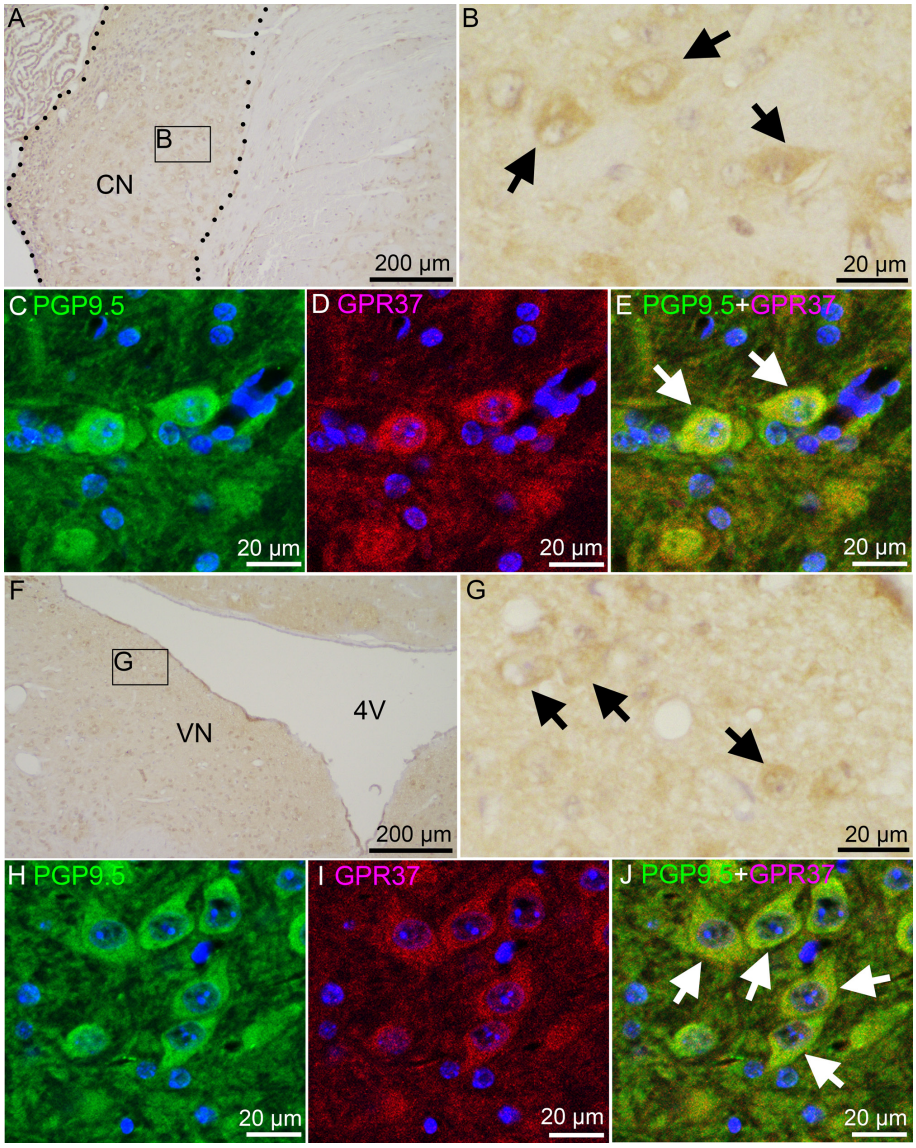

Fig. 2. G protein-coupled receptor 37 (GPR37) immunoreactivity in the cochlear (CN) and vestibular nuclei (VN). (**A**) GPR37 immunoreactivity was weak and found only in a small number of cells in CN (circled by dashed line). (**B**) High magnification squared in (A). Arrows indicate GPR37 immunoreactive cell in CN. (**C**) PGP9.5 immunoreactivity in CN. (**D**) GPR37 immunoreactivity in CN. (**E**) Double immunofluorescence staining of PGP9.5 and GPR37 in CN. GPR37 immunoreactivities were found only in PGP9.5-positive cells (arrows). (**F**) GPR37 immunoreactivity was very weak and found only in a small number of cells in VN. (**G**) High magnification squared in (F). Arrow indicates GPR37 immunoreactive cell in VN. (**H**) PGP9.5 immunoreactivity in VN. (**I**) GPR37 immunoreactivity in VN. (**J**) Double immunofluorescence staining of PGP9.5 and GPR37 in VN. GPR37 immunoreactivities were found only in PGP9.5-positive cells (arrows).

CONFLICT OF INTEREST. The authors declare no competing interests.

ACKNOWLEDGMENTS. Funding from the HPC Corporation (Nagoya, Japan) and the CMC Corporation (Kyoto, Japan) is gratefully acknowledged. The sponsors' involvement was only financial for encouraging the basic sciences.

REFERENCES

1. Aburto MR, Sánchez-Calderón H, Hurlé JM, Varela-Nieto I, Magariños M. 2012. Early otic development depends on autophagy for apoptotic cell clearance and neural differentiation. *Cell Death Dis* **3**: e394. [Medline] [CrossRef]
2. Akil O, Chang J, Hiel H, Kong JH, Yi E, Glowatzki E, Lustig LR. 2006. Progressive deafness and altered cochlear innervation in knock-out mice lacking prosaposin. *J Neurosci* **26**: 13076–13088. [Medline] [CrossRef]
3. Akil O, Lustig LR. 2012. Severe vestibular dysfunction and altered vestibular innervation in mice lacking prosaposin. *Neurosci Res* **72**: 296–305. [Medline] [CrossRef]

4. Akil O, Sun Y, Vijayakumar S, Zhang W, Ku T, Lee CK, Jones S, Grabowski GA, Lustig LR. 2015. Spiral ganglion degeneration and hearing loss as a consequence of satellite cell death in saposin B-deficient mice. *J Neurosci* **35**: 3263–3275. [Medline] [CrossRef]
5. Barmack NH. 2003. Central vestibular system: vestibular nuclei and posterior cerebellum. *Brain Res Bull* **60**: 511–541. [Medline] [CrossRef]
6. Campana WM, Hiraiwa M, O'Brien JS. 1998. Prosaptide activates the MAPK pathway by a G-protein-dependent mechanism essential for enhanced sulfatide synthesis by Schwann cells. *FASEB J* **12**: 307–314. [Medline]
7. Campana WM, Darin SJ, O'Brien JS. 1999. Phosphatidylinositol 3-kinase and Akt protein kinase mediate IGF-I- and prosaptide-induced survival in Schwann cells. *J Neurosci Res* **57**: 332–341. [Medline] [CrossRef]
8. Di Bonito M, Studer M. 2017. Cellular and molecular underpinnings of neuronal assembly in the central auditory system during mouse development. *Front Neural Circuits* **11**: 18. [Medline] [CrossRef]
9. Du H, Zhou X, Feng T, Hu F. 2022. Regulation of lysosomal trafficking of progranulin by sortilin and prosaposin. *Brain Commun* **4**: fcab310. [Medline] [CrossRef]
10. Harzer K, Paton BC, Christomanou H, Chatelut M, Levade T, Hiraiwa M, O'Brien JS. 1997. Saposins (sap) A and C activate the degradation of galactosylceramide in living cells. *FEBS Lett* **417**: 270–274. [Medline] [CrossRef]
11. Harzer K, Hiraiwa M, Paton BC. 2001. Saposins (sap) A and C activate the degradation of galactosylsphingosine. *FEBS Lett* **508**: 107–110. [Medline] [CrossRef]
12. Hiesberger T, Hüttler S, Rohlmann A, Schneider W, Sandhoff K, Herz J. 1998. Cellular uptake of saposin (SAP) precursor and lysosomal delivery by the low density lipoprotein receptor-related protein (LRP). *EMBO J* **17**: 4617–4625. [Medline] [CrossRef]
13. Hineno T, Sano A, Kondoh K, Ueno S, Kakimoto Y, Yoshida K. 1991. Secretion of sphingolipid hydrolase activator precursor, prosaposin. *Biochem Biophys Res Commun* **176**: 668–674. [Medline] [CrossRef]
14. Hiraiwa M, O'Brien JS, Kishimoto Y, Galdzicka M, Fluharty AL, Ginns EI, Martin BM. 1993. Isolation, characterization, and proteolysis of human prosaposin, the precursor of saposins (sphingolipid activator proteins). *Arch Biochem Biophys* **304**: 110–116. [Medline] [CrossRef]
15. Hiraiwa M, Campana WM, Martin BM, O'Brien JS. 1997. Prosaposin receptor: evidence for a G-protein-associated receptor. *Biochem Biophys Res Commun* **240**: 415–418. [Medline] [CrossRef]
16. Hiraiwa M, Taylor EM, Campana WM, Darin SJ, O'Brien JS. 1997. Cell death prevention, mitogen-activated protein kinase stimulation, and increased sulfatide concentrations in Schwann cells and oligodendrocytes by prosaposin and prosaptides. *Proc Natl Acad Sci USA* **94**: 4778–4781. [Medline] [CrossRef]
17. Hiraiwa M, Campana WM, Mizisin AP, Mohiuddin L, O'Brien JS. 1999. Prosaposin: a myelinotrophic protein that promotes expression of myelin constituents and is secreted after nerve injury. *Glia* **26**: 353–360. [Medline] [CrossRef]
18. Kishimoto Y, Hiraiwa M, O'Brien JS. 1992. Saposins: structure, function, distribution, and molecular genetics. *J Lipid Res* **33**: 1255–1267. [Medline] [CrossRef]
19. Klein A, Henseler M, Klein C, Suzuki K, Harzer K, Sandhoff K. 1994. Sphingolipid activator protein D (sap-D) stimulates the lysosomal degradation of ceramide in vivo. *Biochem Biophys Res Commun* **200**: 1440–1448. [Medline] [CrossRef]
20. Kondoh K, Sano A, Kakimoto Y, Matsuda S, Sakanaka M. 1993. Distribution of prosaposin-like immunoreactivity in rat brain. *J Comp Neurol* **334**: 590–602. [Medline] [CrossRef]
21. Li SC, Kihara H, Serizawa S, Li YT, Fluharty AL, Mayes JS, Shapiro LJ. 1985. Activator protein required for the enzymatic hydrolysis of cerebroside sulfate. Deficiency in urine of patients affected with cerebroside sulfatase activator deficiency and identity with activators for the enzymatic hydrolysis of GM1 ganglioside and globotriaosylceramide. *J Biol Chem* **260**: 1867–1871. [Medline] [CrossRef]
22. Magariños M, Pulido S, Aburto MR, de Iriarte Rodríguez R, Varela-Nieto I. 2017. Autophagy in the vertebrate inner ear. *Front Cell Dev Biol* **5**: 56. [Medline] [CrossRef]
23. Meyer RC, Giddens MM, Schaefer SA, Hall RA. 2013. GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. *Proc Natl Acad Sci USA* **110**: 9529–9534. [Medline] [CrossRef]
24. Meyer RC, Giddens MM, Coleman BM, Hall RA. 2014. The protective role of prosaposin and its receptors in the nervous system. *Brain Res* **1585**: 1–12. [Medline] [CrossRef]
25. Mitra S, Tsvetkov AS, Finkbeiner S. 2009. Protein turnover and inclusion body formation. *Autophagy* **5**: 1037–1038. [Medline] [CrossRef]
26. Nabeka H, Saito S, Li X, Shimokawa T, Khan MSI, Yamamiya K, Kawabe S, Doihara T, Hamada F, Kobayashi N, Matsuda S. 2017. Interneurons secrete prosaposin, a neurotrophic factor, to attenuate kainic acid-induced neurotoxicity. *IBRO Rep* **3**: 17–32. [Medline] [CrossRef]
27. Nixon RA, Yang DS, Lee JH. 2008. Neurodegenerative lysosomal disorders: a continuum from development to late age. *Autophagy* **4**: 590–599. [Medline] [CrossRef]
28. O'Brien JS, Kishimoto Y. 1991. Saposin proteins: structure, function, and role in human lysosomal storage disorders. *FASEB J* **5**: 301–308. [Medline] [CrossRef]
29. O'Brien JS, Carson GS, Seo HC, Hiraiwa M, Kishimoto Y. 1994. Identification of prosaposin as a neurotrophic factor. *Proc Natl Acad Sci USA* **91**: 9593–9596. [Medline] [CrossRef]
30. O'Brien JS, Carson GS, Seo HC, Hiraiwa M, Weiler S, Tomich JM, Barranger JA, Kahn M, Azuma N, Kishimoto Y. 1995. Identification of the neurotrophic factor sequence of prosaposin. *FASEB J* **9**: 681–685. [Medline] [CrossRef]
31. Qian M, Sleat DE, Zheng H, Moore D, Lobel P. 2008. Proteomics analysis of serum from mutant mice reveals lysosomal proteins selectively transported by each of the two mannose 6-phosphate receptors. *Mol Cell Proteomics* **7**: 58–70. [Medline] [CrossRef]
32. Rubel EW, Fritzsch B. 2002. Auditory system development: primary auditory neurons and their targets. *Annu Rev Neurosci* **25**: 51–101. [Medline] [CrossRef]
33. Sarkar S, Homma T, Onouchi S, Shimizu Y, Shiina T, Nabeka H, Matsuda S, Saito S. 2021. Expression of the G protein-coupled receptor (GPR) 37 and GPR37L1 in the mouse digestive system. *J Vet Med Sci* **83**: 1–8. [Medline] [CrossRef]
34. Schulze H, Kolter T, Sandhoff K. 2009. Principles of lysosomal membrane degradation: Cellular topology and biochemistry of lysosomal lipid degradation. *Biochim Biophys Acta* **1793**: 674–683. [Medline] [CrossRef]
35. Son JH, Shim JH, Kim KH, Ha JY, Han JY. 2012. Neuronal autophagy and neurodegenerative diseases. *Exp Mol Med* **44**: 89–98. [Medline] [CrossRef]
36. Stein M, Zijderhand-Bleekemolen JE, Geuze H, Hasilik A, von Figura K. 1987. Mr 46,000 mannose 6-phosphate specific receptor: its role in targeting of lysosomal enzymes. *EMBO J* **6**: 2677–2681. [Medline] [CrossRef]
37. Terashita T, Saito S, Miyawaki K, Hyodo M, Kobayashi N, Shimokawa T, Saito K, Matsuda S, Gyo K. 2007. Localization of prosaposin in rat cochlea. *Neurosci Res* **57**: 372–378. [Medline] [CrossRef]
38. Vielhaber G, Hurwitz R, Sandhoff K. 1996. Biosynthesis, processing, and targeting of sphingolipid activator protein (SAP)precursor in cultured human fibroblasts. Mannose 6-phosphate receptor-independent endocytosis of SAP precursor. *J Biol Chem* **271**: 32438–32446. [Medline] [CrossRef]
39. Zhou X, Sun L, Bastos de Oliveira F, Qi X, Brown WJ, Smolka MB, Sun Y, Hu F. 2015. Prosaposin facilitates sortilin-independent lysosomal trafficking of progranulin. *J Cell Biol* **210**: 991–1002. [Medline] [CrossRef]