Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Review

The protective role of prosaposin and its receptors in the nervous system

*Rebecca C. Meyer, Michelle M. Giddens, Brilee M. Coleman, Randy A. Hall**

Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322, United States

ARTICLE INFO

Article history:
Accepted 10 August 2014
Available online 15 August 2014

Keywords:
Neuroprotection
Neurotrophic
Myelination
Nerve
Lysosome
GPCR

ABSTRACT

Prosaposin (also known as SGP-1) is an intriguing multifunctional protein that plays roles both intracellularly, as a regulator of lysosomal enzyme function, and extracellularly, as a secreted factor with neuroprotective and glioprotective effects. Following secretion, prosaposin can undergo endocytosis via an interaction with the low-density lipoprotein-related receptor 1 (LRP1). The ability of secreted prosaposin to promote protective effects in the nervous system is known to involve activation of G proteins, and the orphan G protein-coupled receptors GPR37 and GPR37L1 have recently been shown to mediate signaling induced by both prosaposin and a fragment of prosaposin known as prosaptide. In this review, we describe recent advances in our understanding of prosaposin, its receptors and their importance in the nervous system.

Contents

*Corresponding author.
E-mail address: rhall@pharm.emory.edu (R.A. Hall).

http://dx.doi.org/10.1016/j.brainres.2014.08.022
0006-8993/

1. Introduction

A number of secreted factors, including a variety of proteins, peptides, bioactive lipids and other types of molecules, are capable of exerting protective actions on neurons and glia in the nervous system. Such protective factors and their receptors have garnered significant research interest for their potential as therapeutic targets in the treatment of neurological damage and disease (Allen et al., 2013; Schulte-Herbruggen et al., 2008). One of the largest and most intensively-studied families of protective factors is the neurotrophins, which includes nerve growth factor, brain-derived neurotrophic factor and several other secreted proteins that act predominantly through receptor tyrosine kinases (Chao, 2003). Other protective factors, including ciliary-derived neurotrophic factor (CNTF) and interleukin-6, signal through cytokine receptors coupled to the JAK/STAT pathway (Bauer et al., 2007). In addition to these distinct classes of neurotrophic factors, there are also a number of unique protective factors that demonstrate potential in the treatment of nervous system injuries and neurological disorders. One such unique factor, which has been studied for more than 20 years in terms of its protective actions on neurons and glia, is prosaposin.

2. The function of prosaposin and the saposins

2.1. *Prosaposin as a lysosomal protein*

Prosaposin was initially identified as the precursor protein for four lysosomal activator proteins known as the saposins A–D (Kishimoto et al., 1992). Saposins were named due to their actions as sphingolipid activator proteins that facilitate the hydrolysis of sphingolipids via lysosomal hydrolases (Kishimoto et al., 1992). Interest in the saposins began in the 1960s with the discovery of saposin B (Mehl and Jatzkewitz, 1964) followed by the discovery of saposin C in 1971 (Ho and O'Brien, 1971). Saposin B, also known as SAP-1, was isolated and cloned in the mid-1980s (Dewji et al., 1986; Isemura et al., 1984). Not long after, the prosaposin precursor protein was sequenced and identified as a homolog of the rat sulfated glycoprotein 1 (SGP-1) (Collard et al., 1988; O'Brien et al., 1988) and the mouse testicular sulfated glycoprotein 1 (Morales et al., 1998).

Each of the four saposins has a distinct role in promoting hydrolysis of sphingolipids, and this facilitation of hydrolysis is thought to be a result of saposin-induced remodeling of lysosomal membranes. Saposins A and C have been shown to enhance β-glucosylceramidase-mediated hydrolysis of glucocerebroside as well as hydrolysis of galactocerebroside (Morimoto et al., 1989; Wenger et al., 1982). Saposin A primarily acts by optimizing the hydrolysis of galactocerebrosides via β-galactosylceramidase (Harzer et al., 1997) whereas saposin C enhances β-glucosidase activity and protects the enzyme from proteolytic degradation (Qi and Grabowski, 1998; Sun et al., 2003). Saposin B enhances hydrolysis of galactocerebroside sulfate (Fischer and Jatzkewitz, 1975), GM1 ganglioside (Inui and Wenger, 1984), and globotriaosylceramide (Gartner et al., 1983; Li et al., 1985), and also promotes glycerolipid hydrolysis (Li et al., 1988). Finally, saposin D has been shown to enhance the hydrolysis of sphingomyelin by sphingomyelin phosphodiesterase (Morimoto et al., 1988). The precise molecular mechanisms by which the saposins promote the lysosomal processing of lipids are still a point of significant research interest, but direct saposin interactions with lipids appear to be important (Kishimoto et al., 1992; Soeda et al., 1993). Saposins may act as "solubilizers", which facilitate the extraction of substrate lipids from lysosomal membranes for presentation to hydrolase enzymes, and/or as "liftases", which enhance enzyme access to substrate lipids via saposin-induced membrane remodeling. Support for the "solubilizer" and "liftase" models comes from a number of studies demonstrating saposin binding to substrate lipids and membrane remodeling induced by saposins (Alattia et al., 2006; Vaccaro et al., 1993; Vogel et al., 1991) Similarly, saposin binding to lipids has been shown to be crucial for lipid loading of CD1, which is necessary for CD1-mediated antigen presentation and immune system recognition of lipid-based antigens on pathogens (Kang and Cresswell, 2004; Leon et al., 2012; Zhou et al., 2004).

Dysfunction or loss of saposins can result in an assortment of lysosomal storage diseases. Saposin A dysfunction has been linked to development of globoid cell leukodystrophy (GLD), also known as Krabbe disease (Matsuda et al., 2001; Spiegel et al., 2005), largely through reports that mice deficient in saposin A also exhibit a Krabbe disease phenotype(Matsuda et al., 2007) and exhibit nervous system deficits including neurological deficits, hindlimb weakness, and a demyelination phenotype (Matsuda et al., 2001). This was further confirmed by a clinical report of abnormal myelination in an infant diagnosed with Krabbe disease and deficient in saposin A (Spiegel et al., 2005). Furthermore, leukocytes from this patient demonstrated an abnormality of galactocerebrosidase activity, which was linked to a three base pair deletion in the saposin A coding region of prosaposin (Spiegel et al., 2005).

Mice deficient in saposin A as well as saposin B display motor deficits including tremor and foot slips and aberrant locomotor activity (Sun et al., 2013). Additionally, these mice

show motor neuron deterioration and accumulation of the autophagy marker LC3-II in the brainstem (Sun et al., 2013), further underscoring the importance of saposin A function. Saposin B deficient mice have a distinct motor neuron deterioration phenotype, which is displayed as a head tremor that manifests itself by 15 months of age (Sun et al., 2008b). Histological analyses of saposin B-deficient brains also have revealed activated microglia and reactive astrocytes, suggesting a proinflammatory response. Furthermore, cells in the brain and kidney accumulate fatty acid sulfatides (Sun et al., 2008b), which is suggestive of a lysosomal storage disorder. In humans, saposin B deficiency is autosomal recessive with afflicted patients developing metachromatic leukodystrophy-like cerebroside sulfate accumulation (Kretz et al., 1990).

Saposin C deficiency can lead to different forms of Gaucher's disease in humans, predominantly type 2 and 3, which are known for neuropathological symptoms (Schnabel et al., 1991; Tamargo et al., 2012; Vaccaro et al., 2010). Similarly, the mouse model of saposin C deficiency mimics type 3 Gaucher's disease (Sun et al., 2010) while the loss of full-length prosaposin (total saposin deficiency) most closely resembles type 2 Gaucher's disease (Hulkova et al., 2001). Finally, the phenotype for saposin D deficient mice is also degenerative in nature with mice developing ataxia at four months of age and progressive degeneration of Purkinje cells resulting in a complete disappearance of Purkinje cells by twelve months of age (Matsuda et al., 2004). A primary function of saposin D is to enhance the hydrolysis of ceramide (Azuma et al., 1994), and thus saposin D deficiency leads to ceramide accumulation, most prominently in the cerebellum (Matsuda et al., 2004). Saposin D deficiency has also been reported in a patient diagnosed with Gaucher's disease (Diaz-Font et al., 2005). Interestingly, total saposin deficiency results in a phenotype that is much more severe than the phenotypes observed upon loss of the individual saposins (Fujita et al., 1996; Hulkova et al., 2001), which suggests that full-length prosaposin possesses biological activities above and beyond the enzymatic actions exhibited by the individual saposins.

2.2. *Prosaposin as a secreted factor*

In addition to serving as a precursor protein for the saposins, full-length prosaposin can also be released as a secreted factor into many secretory fluids including cerebrospinal fluid, semen, milk, pancreatic juice and bile (Hineno et al., 1991). The extracellular presence of full-length prosaposin suggests a discrete function for full-length prosaposin beyond its role as the saposin precursor protein. Indeed, prosaposin has been identified as a neurotrophic factor capable of promoting cell survival, neurite outgrowth and differentiation in a cholinergic cell line (O'Brien et al., 1994, 1995). The neurotrophic sequence of prosaposin was identified as a 12-amino-acid peptide sequence (LIDNNKTEKEIL) located within the saposin C region of prosaposin (O'Brien et al., 1995). In addition to prosaposin, both saposin C and several "prosaptides" (peptides containing the neurotrophic sequence of prosaposin) were found to promote neurite outgrowth, differentiation and cell survival in the same cholinergic cell line (O'Brien et al., 1995). Prosaptides were also shown to be capable of protecting human neuroblastoma cells and hippocampal CA1 neurons *in vitro*

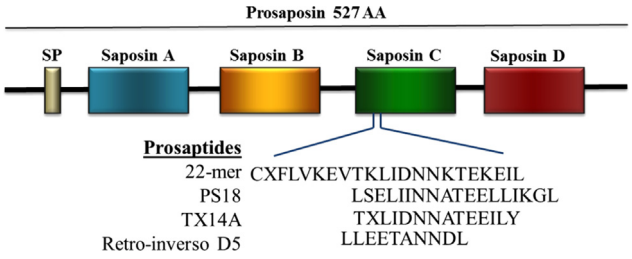

Fig. 1 – Prosaposin and the prosaptides. The region of saposin C that is known to mediate the cytoprotective actions of prosaposin is indicated. The various prosaptides that have been devised to mimic this region are shown in the lower part of the figure. "X" in these sequences denotes D-alanine, and all of the amino acids in the retro-inverso D5 sequence are D-amino acids.

(Kotani et al., 1996b; O'Brien et al., 1995). There have been multiple prosaptides described in the literature, all of which are variants of the peptide sequence of prosaposin that mediates the protein's cytoprotective actions (Fig. 1).

Studies on human patients with prosaposin mutations suggest that prosaposin may exert neuroprotective and glioprotective actions *in vivo*, although these *in vivo* analyses are complicated by the fact that prosaposin also exerts effects on lysosomal function in addition to its actions as a secreted factor. Several reports describe the postmortem phenotypes of a 16-week old patient and his 20-week old fetal sibling, both of which had a novel mutation in the coding region of the prosaposin gene leading to a complete deficiency of the full-length prosaposin and all four saposins (Bradova et al., 1993; Harzer et al., 1989; Paton et al., 1992). In addition to the lysosomal storage deficits, a striking lack of cortical neurons, myelin and mature oligodendrocytes as well as an increase in reactive astrocytes and microglia were found within the brains of these patients (Hulkova et al., 2001). Additional case studies report similar findings for other patients with prosaposin mutations (Elleder et al., 2005; Kuchar et al., 2009; Sikora et al., 2007). Brain morphogenesis was also altered in most of these cases with the identification of gray matter heterotopias (Elleder et al., 2005; Kuchar et al., 2009). A later study focusing on the neuropathology of three additional cases described an exaggerated sensitivity of the cortical neurons to the loss of prosaposin (Sikora et al., 2007). Whereas non-neuronal cells showed a foamy appearance, characteristic of lysosomal lipid storage and sphingolipid hydrolase insufficiency, neuronal cells contained finely granular lysosomes with compacted inclusions and ubiquitinated proteins (Sikora et al., 2007). These results indicate a distinct phenotype for neuronal cells from prosaposin-deficient patients, above and beyond the pathology that might be expected purely from lysosomal dysfunction. Similar to human patients who do not survive past five months of age, prosaposin-deficient mice have a shortened lifespan and survive for only 30 days (Sun et al., 2008a). These animals have significant lysosomal dysfunction and begin to show neurological deficits around postnatal day 20, with these deficits rapidly progressing until death. Furthermore, mice with mutations in the neurogenic region of saposin C also demonstrate a neurodegenerative phenotype

(Yoneshige et al., 2010), adding to the evidence that prosaposin may exert effects *in vivo* as a secreted neurotrophic factor.

3. Prosaposin release

3.1. *Prosaposin exocytosis following injury/stress*

Like certain other lysosomal proteins, prosaposin can be secreted into the extracellular space, with this secretory process being enhanced under conditions of cellular stress. In models of peripheral nerve cut and crush, for example, prosaposin release is significantly elevated (Hiraiwa et al., 1999). Similarly, total levels of prosaposin mRNA and protein are increased following nerve injury in motoneurons, beginning on the third day following injury and continuing to change until post-injury day 21 (Chen et al., 2008; Unuma et al., 2005). Furthermore, prosaposin expression and release are greatly upregulated in animal models of focal cerebral ischemia (Costain et al., 2010; Hiraiwa et al., 2003; Yokota et al., 2001), and prosaposin release from the retinal pigment epithelial cells in the eye can be elevated by cell stress induced by either light or hydrogen peroxide (Toyofuku et al., 2012).

3.2. *Mechanisms controlling prosaposin release*

As a soluble lysosomal protein, prosaposin must associate with a sorting receptor that can interact with adapter proteins necessary for the formation of cargo vesicles for trafficking to the lysosome (Lobel et al., 1989; Puertollano et al., 2001). Sortilin has been shown to be the key sorting receptor for prosaposin, as cells deficient for sortilin exhibit impaired trafficking of prosaposin to the lysosomal compartment and enhanced release of prosaposin into the extracellular medium (Hassan et al., 2004; Lefrancois et al., 2003; Zeng et al., 2009). The binding of prosaposin to sortilin has been localized to a 17-amino-acid region of the prosaposin carboxyl-terminus (Yuan and Morales, 2010). Additionally, prosaposin must be in its monomeric form to interact with sortilin and be targeted to lysosomes, as it has been shown that prosaposin oligomerization blocks interaction with sortilin and leads to prosaposin secretion (Yuan and Morales, 2011).

Work on retinal pigment epithelial cells has revealed that Sema4A is another prosaposin-interacting protein that blocks prosaposin interaction with sortilin and promotes prosaposin exocytosis (Toyofuku et al., 2012). The association of Sema4A with prosaposin was found to be increased when the cells were exposed to hydrogen peroxide to induce oxidative stress (Toyofuku et al., 2012). These findings reveal a specific molecular mechanism underlying enhanced release of prosaposin following cellular stress.

4. Effects of secreted prosaposin in the nervous system

4.1. *Prosaposin rescues ischemic damage*

Given the aforementioned studies demonstrating that prosaposin expression and release are enhanced following ischemia (Costain et al., 2010; Hiraiwa et al., 2003; Yokota et al., 2001), a number of groups have explored the possibility that prosaposin might protect cells from ischemic damage. For example, the infusion of prosaposin into the lateral ventricles of gerbils prior to the induction of ischemia was found to prevent learning disabilities resulting from ischemic damage (Sano et al., 1994). Similar results were found when an 18-amino-acid prosaptide was infused into the lateral ventricles of gerbils (Kotani et al., 1996b; Morita et al., 2001). These behavioral effects were found to be accompanied by cellular changes consistent with protection of cells from apoptotic death (Morita et al., 2001; Sano et al., 1994). The infusion of the 18-amino-acid prosaptide into the lateral ventricles or the injection of the retro-inverso prosaptide D5 were also able to prevent secondary degeneration in the thalamus following occlusion of the left middle cerebral artery (Igase et al., 1999; Lu et al., 2000). However, the timing, concentration and/or method of delivery of prosaptide treatment may be important for its protective effects, as one study found that peripheral injections of prosaptide D5 exacerbated behavioral deficits in a spinal cord model of ischemia (Lapchak et al., 2000).

4.2. *Prosaposin rescues dopaminergic neurons*

In addition to the protective actions exhibited by prosaposin and prosaptides in ischemic models, prosaptides have also shown protective effects on dopaminergic neurons *in vitro* and in models of Parkinson's disease *in vivo*. For example, prosaptide D5, the aforementioned retro-inverso peptide generated from the neurotrophic sequence of prosaposin that is resistant to protease cleavage and capable of crossing the blood-brain barrier, was found to protect dopaminergic mesencephalon neurons from the neurotoxins MPP^+ *in vitro* and MPTP *in vivo* respectively (Liu et al., 2001). Doses as low as 1 ng/ml prosaptide D5 were found to be capable of rescuing primary dopaminergic neurons from 20 μM MPP^+, whereas inert versions of the peptide had no effect. Similarly, 200 μg/kg prosaptide D5 rescued dopaminergic cells when given every other day for two weeks, 24 h after 1 injection of 40 mg/kg MPTP (Liu et al., 2001). A subsequent study found similar results in cultured SH-SY5Y human neuroblastoma cells when treated with an 18-amino-acid version of prosaptide termed PS18 (Gao et al., 2013). In addition, this group also found that 2 mg/kg PS18 was able to significantly improve behavioral deficits induced by MPTP, rescue dopaminergic neurons, and reduce the reactivity of astrocytes within the substantia nigra and striatum of MPTP-treated mice. Prosaposin treatment was shown in these studies to upregulate the anti-apoptotic factor Bcl-2, down-regulate the pro-apoptotic factor BAX and inhibit MPTP-induced cleavage of caspase-3, suggesting an action on signaling pathways that inhibit apoptosis (Gao et al., 2013). In terms of relevance to human Parkinson's disease, prosaposin was found to be upregulated in the substantia nigra of human Parkinson's patients compared to non-Parkinsonian control patients (Miller et al., 2006).

4.3. *Prosaposin facilitates nerve regeneration and alleviates sensory neuropathy*

Injections of prosaptide TX14(A) were found to shorten recovery time from sciatic nerve crush as well as alleviate thermal hypoalgesia and formalin-evoked hyperalgesia in

diabetic rats (Jolivalt et al., 2008b). Prosaptide TX14(A) was also able to enhance nerve regeneration distance and axonal diameter of the regenerated axons following sciatic nerve injury (Jolivalt et al., 2008b). Prosaposin applied via a collagen-filled nerve guide after sciatic nerve transection in a guinea pig model increased the number of regenerating nerves, for both motor and sensory nerve types (Kotani et al., 1996a).

In addition to promoting nerve regeneration, prosaptide treatment has also been reported to reduce neuropathic pain. For example, prosaptide TX14(A) was found to reverse thermal hyperalgesia in a sciatic nerve ligation model (Otero et al., 1999). Prosaptide D5 was also able to reverse hyperalgesia in the same model in a manner that was blocked by pertussis toxin treatment, suggesting the activation of G protein pathways by prosaptide (Yan et al., 2000). Additionally, prosaptide TX14(A) was shown to protect diabetic rats against progressive decline in sensory function and also relieve tactile allodynia and paw thermal hyperalgesia (Calcutt et al., 2000). In separate studies, prosaptide TX14(A) was found to protect against TNF-induced hyperalgesia (Wagner et al., 1998), paclitaxel-induced thermal hypoalgeisa (Campana et al., 1998a) and allodynia induced by the HIV envelope glycoprotein gp120 (Jolivalt et al., 2008a). The models of pain were further expanded in a report comparing the ability of prosaptide TX14(A) to mediate protection in a diverse array of pain models (Jolivalt et al., 2006).

4.4. *Prosaposin protects myelinating glial cells*

The aforementioned ability of prosaposin and prosaptides to enhance nerve repair may be mediated via actions on the nerve fibers themselves and/or via actions on myelinating glial cells that surround the nerves. For example, treatment of Schwann cells in culture with prosaposin or prosaptides has been found to promote Schwann cell survival in the face of cellular insults (Campana et al., 1999; Hiraiwa et al., 1997b) and also increase synthesis of sulfatide, a marker for myelin production (Campana et al., 1998b; Hiraiwa et al., 1999). Prosaptide TX14A was also shown to increase sulfatide concentration and reduce cell death, but not promote proliferation, in the CG4 oligodendrocyte cell line and the iSC Schwann cell line (Hiraiwa et al., 1997b). Furthermore, peripheral administration of prosaptide D5 was shown to increase sulfatide concentrations in both the brain and sciatic nerve of developing rats (Hiraiwa et al., 2001). Moreover, prosaptide was shown to increase the expression and enzymatic activity of galactose-1-phosphate uridylyltransferase (GALT), an enzyme predominantly found in myelinating Schwann cells (Hiraiwa et al., 1999). These findings from work on cultures of myelinating glial cells and from *in vivo* studies in rodents are intriguing given that prosaposin-deficient mice and human patients are known to exhibit severe central and peripheral hypomyelination and paucity of mature oligodendrocytes (Fujita et al., 1996; Harzer et al., 1989; Schnabel et al., 1992).

4.5. *Prosaposin is important for cerebellar development and survival*

Multiple studies have identified effects of prosaposin on cerebellar development and cerebellar cell survival. Prosaposin is expressed at high levels in cerebellar regions across diverse species including rat, mouse, and pigeon (Islam et al., 2013; Kondoh et al., 1993; Sun et al., 1994). Furthermore, mice that are hypomorphic for prosaposin exhibit Purkinje cell loss and cerebellar deficits prior to birth (Sun et al., 2008a). Prosaposin mRNA levels reach their peak during the developmental period that marks granule cell proliferation and maturation, suggesting potential effects of prosaposin on granule cell development (Tsuboi et al., 1998). Consistent with this idea, a 22-amino-acid prosaptide was found to promote neurite outgrowth in granule cells (O'Brien et al., 1995). Furthermore, both prosaposin and prosaptide TX14(A) have been shown to rescue cerebellar granule cells from programmed cell death, an effect mediated through the activation of phosphatidylinositide 3-kinases (Tsuboi et al., 1998). Prosaposin may also promote synaptic development and protection in regions of the brain beyond the cerebellum, as treatment with prosaposin has been shown to increase synaptic density in cultured hippocampal neurons (Cove et al., 2006).

4.6. *Prosaposin protects diverse cell types from cellular insults*

In addition to the abilities of prosaposin/prosaptides to exert protective actions on myelinating glial cells and cerebellar neurons, prosaposin/prosaptides have also been shown to exert protective effects on a number of other cell types in culture. For example, prosaposin and prosaptides have been shown to protect pheochromocytoma PC12 cells from various cellular insults (Misasi et al., 2001; Ochiai et al., 2008; Sorice et al., 2008). Additionally, the aforementioned enhancement in prosaposin release induced by oxidative stress in retinal pigment epithelial cells has been shown to lead to prosaposin-mediated protection of photoreceptor cells from oxidative damage (Toyofuku et al., 2012). Prosaposin also protects U937 monocytic cells from tumor necrosis factor-α-induced cell death (Misasi et al., 2004) and cortical astrocytes from death induced by hydrogen peroxide-mediated oxidative stress (Meyer et al., 2013). Finally, in a comprehensive study of the ability of conditioned medium from mouse bone marrow stromal cell-derived neuroprogenitor cells (mMSC-NPCs) to protect neurons from 6-OHDA-induced cell death, prosaposin was identified as the essential component of the mMSC-NPC-conditioned medium that exerted the neuroprotective effects (Li et al., 2010).

5. Receptors controlling prosaposin uptake

5.1. *Importance of prosaposin uptake*

Following secretion into extracellular regions, prosaposin can be re-uptaken by either the same cell or neighboring cells (Hermo et al., 1992; Hiesberger et al., 1998; Igdoura et al., 1993; Vielhaber et al., 1996). The purpose of this endocytic process is unclear. One possibility is that endocytosis may serve to limit the time course of prosaposin action in extracellular spaces, thereby serving as a constraint on the physiological effects of secreted prosaposin described above. Another possibility is that prosaposin uptake may serve as a

mechanism for shuttling gangliosides and/or other membrane lipids from one cell to another. Prosaposin robustly binds to gangliosides and can promote ganglioside transfer from donor liposomes to acceptor erythrocyte ghosts (Hiraiwa et al., 1992). Further work is needed, however, to assess whether prosaposin can actually mediate such ganglioside shuttling between live cells.

5.2. *Prosaposin uptake mediated by LRP1*

The low density lipoprotein receptor-related protein 1 (LRP1) has been shown to mediate most of the uptake of prosaposin into cells (Hiesberger et al., 1998) in a manner that is regulated by the LRP adapter protein GULP (Kiss et al., 2006). A small fraction of prosaposin uptake in certain cells can be mediated by receptors other than LRP1, for example the mannose-6-phosphate receptor (Hiesberger et al., 1998), but nonetheless it is clear that LRP1 mediates the vast majority of prosaposin endocytosis (Hiesberger et al., 1998). LRP1 is one of seven members of the low-density lipoprotein (LDL) receptor family (May et al., 2007). All members of this family share structural similarity but have varied functions. The six other members of this family, in addition to LRP1, are LRP1b, LRP2 (also known as megalin), LDL receptor, very low-density lipoprotein receptor (VLDL receptor), LRP4 (also known as MEGF7) and LRP8 (also known as apolipoprotein E receptor 2) (May et al., 2007). LRP1 was the second receptor in the family to be cloned and has a broad expression pattern throughout the body (Herz et al., 1988). In the brain, LRP1 expression has been identified in neurons and astrocytes (Lillis et al., 2008) as well as microglia (May, 2013). This endocytic receptor appears to function mainly as a trafficking protein and regulator of cell surface receptors and proteases (Boucher et al., 2003; Deane et al., 2008; Herz et al., 1988; Loukinova et al., 2002; Marzolo and Bu, 2009), and in this capacity it has attracted attention due to its ability to influence β-amyloid production (Lillis et al., 2008; Sagare et al., 2012). In addition to their roles in signaling regulation, the LRP receptors also transport diverse cargo including lipoproteins, proteases, vitamins, bacterial proteins, viruses and signaling molecules, recognizing many dozens of individual ligands (Herz and Bock, 2002; Lillis et al., 2005). The number of different ligands bound by LRP1 suggests that this receptor may play a role in numerous cell functions (Lillis et al., 2008; Sagare et al., 2012).

6. Receptors mediating prosaposin signaling via G proteins

6.1. *Prosaposin stimulates ERK and Akt phosphorylation*

Prosaposin and prosaptide treatment can stimulate extracellular signal-regulated kinase (ERK) phosphorylation in a variety of different cell types. ERK1 and ERK2 are widely-expressed protein kinases that mediate pleiotropic cellular effects, with the promotion of cell survival being one prominently-reported effect following transient ERK activation, although prolonged ERK activation can have deleterious consequences (Subramaniam and Unsicker, 2010). In primary Schwann cells, as well as an immortalized Schwann cell line, prosaptide TX14(A) was shown to promote ERK phosphorylation to promote the aforementioned increase in sulfatide synthesis (Campana et al., 1998b). Additionally, prosaptide D5 was found to induce ERK phosphorylation in the immortalized iSC cells (Hiraiwa et al., 2001). In an androgen-independent prostate cancer cell line, prosaptide TX14(A) was shown to induce ERK phosphorylation in a manner that resulted in enhanced proliferation, survival, migration, and invasion of the cancer cells (Koochekpour et al., 2004). In the immortalized PC12 cell line, prosaposin induced cells to enter the S phase of the cell cycle through the ERK signaling pathway (Misasi et al., 2001) and ERK phosphorylation was one of the signaling mechanisms by which prosaposin was found to protect PC12 cells against oxidative cell death (Ochiai et al., 2008). Finally, saposin C was shown to prevent cell death in the monocytic cell line U937 cell line through preventing necrosis and apoptosis, as discussed above, via promotion of both ERK phosphorylation and sphingosine kinase activity (Misasi et al., 2004).

Akt is another protein kinase that typically promotes cell survival (Brunet et al., 2001). Prosaposin and prosaptides can induce Akt phosphorylation in a variety of cells types. In primary Schwann cells, for example, prosaptide TX14(A) was shown to stimulate Akt phosphorylation and promote Schwann cell survival in an manner that was dependent on activation of Akt and phosphatidylinositide 3-kinases, which are upstream activators of Akt (Campana et al., 1999). Furthermore, prosaposin was shown to stimulate the Akt pathway to prevent oxidative cell death in PC12 cells in addition to stimulating signaling through the ERK pathway (Ochiai et al., 2008). Finally, saposin C was shown to activate Akt phosphorylation in a dose-dependent manner in prostate cancer cells, resulting in protection of the cells from caspase activation and apoptosis (Lee et al., 2004).

6.2. *Prosaposin can stimulate G protein-mediated signaling*

Some of the signaling pathways stimulated by prosaposin and prosaptides have been shown to be dependent on G protein activation. In some cases, the evidence has been direct, as for example prosaposin treatment of cells has been shown to promote the activation of Gαi and Gαo G proteins (Hiraiwa et al., 1997a; Meyer et al., 2013; Yan et al., 2000). In other cases, the evidence has been more indirect, as many of the signaling pathways stimulated by prosaposin and prosaptides can be blocked by pertussis toxin, which is an inhibitor of Gαi and Gαo. Notably, analgesia induced by prosaptide is pertussis toxin-sensitive (Yan et al., 2000), as is the ability of prosaposin to induce neurite sprouting (Misasi et al., 1998). Moreover, the aforementioned ability of prosaposin and prosaptides to induce ERK phosphorylation has been shown in multiple cell types to be pertussis toxin-sensitive (Campana et al., 1998b; Lee et al., 2004; Meyer et al., 2013; Misasi et al., 2004).

Building on this earlier literature concerning the ability of prosaposin to stimulate G protein-mediated signaling, recent studies have demonstrated that prosaposin and prosaptide TX14(A) can mediate ERK signaling and cellular protection through stimulation of two orphan G protein-coupled receptors, GPR37 and GPR37L1 (Meyer et al., 2013). These receptors are most similar to endothelin receptors and expressed mainly in the nervous system in both neurons and glia

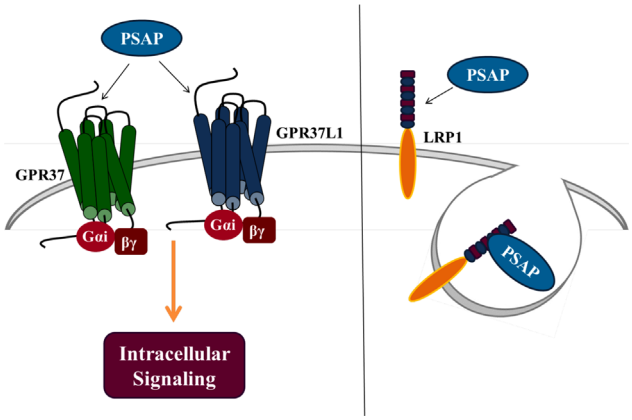

Fig. 2 – Cell surface receptors for prosaposin. Secreted prosaposin ("PSAP") can initiate intracellular signaling via binding to GPR37 and GPR37L1. Uptake of prosaposin from the extracellular space can be mediated by LRP1.

(Cahoy et al., 2008; Donohue et al., 1998; Imai et al., 2001; Leng et al., 1999; Marazziti et al., 1997, 1998; Valdenaire et al., 1998; Zeng et al., 1997). GPR37 is alternatively identified as the Parkin-Associated Endothelin-Like receptor (PAEL-R) (Imai et al., 2001), while GP37L1 is named for its similarity to GPR37. Originally, GPR37 garnered interest due to its identification as a substrate of the E3 ubiquitin ligase parkin (Imai et al., 2001), and much early work on this receptor focused on the propensity of GPR37 to misfold and induce cell stress in the absence of parkin or upon overexpression (Dusonchet et al., 2009; Imai et al., 2001; Kitao et al., 2007; Marazziti et al., 2009; Wang et al., 2008; Yang et al., 2003). These misfolding and trafficking deficits of GPR37 were shown to be rescued by co-expression with interacting partners such as the scaffold protein syntenin-1, which promotes GPR37 trafficking to the plasma membrane (Dunham et al., 2009). Despite the toxicity that has been observed following GPR37 over-expression, GPR37 at physiological expression levels has been shown to protect neuron-like cells from cell death induced by the dopaminergic toxins MPP+, rotenone and 6-OHDA (Lundius et al., 2013). Furthermore, GPR37 and GPR37L1 expression in cultured astrocytes was shown to be necessary for the protection from oxidative stress induced by treatment with prosaposin or prosaptide TX14(A) (Meyer et al., 2013), and plasma membrane-expressed GPR37 was shown to mediate protective effects of extracellular prosaposin in N2a cells (Lundius et al., 2014). Taken together, these findings suggest that while misfolded cytoplasmic GPR37 can be toxic for cells, GPR37 and GPR37L1 expressed in the plasma membrane are cytoprotective and mediate at least some of the well-described protective actions of secreted prosaposin. Thus, GPR37 and GPR37L1 join LRP1 as cell surface receptors for prosaposin (Fig. 2).

7. Integrated model of prosaposin action

Prosaposin has many diverse actions and thus it is challenging at present to synthesize the various physiological effects of this multifunctional protein into a coherent model of

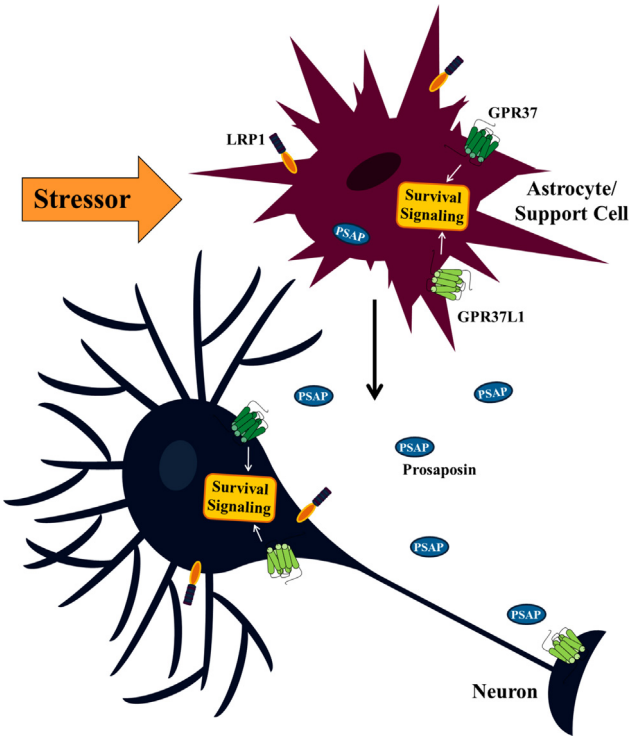

Fig. 3 – Theoretical model of prosaposin function. Prosaposin can be secreted from various cell types in response to cellular stress. Following its release into the extracellular space, prosaposin binds to receptors on nearby neurons and glia to become endocytosed or initiate pro-survival signaling pathways.

prosaposin activity. As a starting point, we propose the following: prosaposin evolved as a key lysosomal enzyme mediating the breakdown of lipids. Like many lysosomal enzymes, such as cathepsins, prosaposin can be secreted following cellular stress, with these secreted lysosomal enzymes having important roles in cleaning up cellular debris following injury (Blott and Griffiths, 2002). However, we propose that secreted prosaposin came to gain an additional role as a signal of cellular injury/inflammation that cues nearby neurons and glia to initiate survival pathways. In this view, GPR37 and GPR37L1 may have evolved as sensors of secreted prosaposin, which thereby makes these receptors sensors of cellular injury/inflammation (Fig. 3). If this model is accurate, it suggests that GPR37 and GPR37L1 may be outstanding targets for novel therapeutics aimed at promoting protection from injuries such as nerve damage or ischemia, since these receptors are part of the normal response of the nervous system to protect itself following injury.

8. Future directions for studies on prosaposin and its receptors

Given that prosaposin and prosaptide have been reported to be protective in animal models of stroke, Parkinson's disease, peripheral nerve damage and demyelination disorders, one important future direction of research in this area will be to

assess which of these protective effects are due to stimulation of GPR37/GPR37L1 and which are due to other effects of secreted prosaposin. If any of the *in vivo* protective effects of prosaposin are indeed confirmed to be dependent on GPR37 and/or GPR37L1, then a further important future direction will be to screen for small molecule agonists and/or positive allosteric modulators for these receptors, as such compounds may have outstanding therapeutic potential due to their abilities to mimic and/or enhance the protective actions of secreted prosaposin. At present, no small molecule ligands are known for either GPR37 or GPR37L1, and thus the pharmacology of these receptors is unexplored terrain that has the potential to yield clinically useful therapeutic drugs. Additionally, there also might be clinical utility in the development of drug-like molecules that either promote prosaposin secretion or slow prosaposin uptake in order to enhance the protective actions of endogenous prosaposin. Such molecules might bind to prosaposin itself or bind to the receptors that control prosaposin uptake, such as LRP1. However, further work will be necessary to understand more about the mechanisms controlling prosaposin release and uptake, with an important issue being how selectively these processes can be modulated. As mentioned earlier, LRP1 has numerous other substrates (Herz and Bock, 2002; Lillis et al., 2005), and therefore it may be challenging to achieve selective modulation of prosaposin levels by targeting LRP1. Finally, future work in this area will seek to disentangle the roles of lysosomal saposins and secreted prosaposin in the pathophysiology of prosaposin deficiency. In addition to providing clinical insights, such efforts will also shed new light on the fundamental biology of this system and thereby contribute to a better overall understanding of prosaposin activity.

Acknowledgments

The authors' research is funded by National Institutes of Health grants R21-NS081461 and R01-NS088413.

REFERENCES

Alattia, J.R., Shaw, J.E., Yip, C.M., Prive, G.G., 2006. Direct visualization of saposin remodelling of lipid bilayers. J. Mol. Biol. 362, 943–953.

Allen, S.J., Watson, J.J., Shoemark, D.K., Barua, N.U., Patel, N.K., 2013. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol. Ther. 138, 155–175.

Azuma, N., O'Brien, J.S., Moser, H.W., Kishimoto, Y., 1994. Stimulation of acid ceramidase activity by saposin D. Arch. Biochem. Biophys. 311, 354–357.

Bauer, S., Kerr, B.J., Patterson, P.H., 2007. The neuropoietic cytokine family in development, plasticity, disease and injury. Nat. Rev. Neurosci. 8, 221–232.

Blott, E.J., Griffiths, G.M., 2002. Secretory lysosomes. Nat. Rev. Mol. Cell Biol. 3, 122–131.

Boucher, P., Gotthardt, M., Li, W.P., Anderson, R.G., Herz, J., 2003. LRP: role in vascular wall integrity and protection from atherosclerosis. Science 300, 329–332.

Bradova, V., Smid, F., Ulrich-Bott, B., Roggendorf, W., Paton, B.C., Harzer, K., 1993. Prosaposin deficiency: further characterization of the sphingolipid activator protein-deficient sibs. Multiple glycolipid elevations (including lactosylceramidosis), partial enzyme deficiencies and ultrastructure of the skin in this generalized sphingolipid storage disease. Hum. Genet. 92, 143–152.

Brunet, A., Datta, S.R., Greenberg, M.E., 2001. Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr. Opin. Neurobiol. 11, 297–305.

Cahoy, J.D., Emery, B., Kaushal, A., Foo, L.C., Zamanian, J.L., Christopherson, K.S., Xing, Y., Lubischer, J.L., Krieg, P.A., Krupenko, S.A., Thompson, W.J., Barres, B.A., 2008. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J. Neurosci. 28, 264–278.

Calcutt, N.A., Freshwater, J.D., O'Brien, J.S., 2000. Protection of sensory function and antihyperalgesic properties of a prosaposin-derived peptide in diabetic rats. Anesthesiology 93, 1271–1278.

Campana, W.M., Eskeland, N., Calcutt, N.A., Misasi, R., Myers, R.R., O'Brien, J.S., 1998a. Prosaptide prevents paclitaxel neurotoxicity. Neurotoxicology 19, 237–244.

Campana, W.M., Hiraiwa, M., O'Brien, J.S., 1998b. Prosaptide activates the MAPK pathway by a G-protein-dependent mechanism essential for enhanced sulfatide synthesis by Schwann cells. FASEB J. 12, 307–314.

Campana, W.M., Darin, S.J., O'Brien, J.S., 1999. Phosphatidylinositol 3-kinase and Akt protein kinase mediate IGF-I- and prosaptide-induced survival in Schwann cells. J. Neurosci. Res. 57, 332–341.

Chao, M.V., 2003. Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat. Rev. Neurosci. 4, 299–309.

Chen, J., Saito, S., Kobayashi, N., Sato, K., Terashita, T., Shimokawa, T., Mominoki, K., Miyawaki, K., Sano, A., Matsuda, S., 2008. Expression patterns in alternative splicing forms of prosaposin mRNA in the rat facial nerve nucleus after facial nerve transection. Neurosci. Res. 60, 82–94.

Collard, M.W., Sylvester, S.R., Tsuruta, J.K., Griswold, M.D., 1988. Biosynthesis and molecular cloning of sulfated glycoprotein 1 secreted by rat Sertoli cells: sequence similarity with the 70-kilodalton precursor to sulfatide/GM1 activator. Biochemistry 27, 4557–4564.

Costain, W.J., Haqqani, A.S., Rasquinha, I., Giguere, M.S., Slinn, J., Zurakowski, B., Stanimirovic, D.B., 2010. Proteomic analysis of synaptosomal protein expression reveals that cerebral ischemia alters lysosomal Psap processing. Proteomics 10, 3272–3291.

Cove, J., Morales, C.R., Baranes, D., 2006. SGP-1 increases dendritic and synaptic development dependent on synaptic activity. Neurosci. Res. 56, 372–385.

Deane, R., Sagare, A., Zlokovic, B.V., 2008. The role of the cell surface LRP and soluble LRP in blood-brain barrier Abeta clearance in Alzheimer's disease. Curr. Pharm. Des. 14, 1601–1605.

Dewji, N., Wenger, D., Fujibayashi, S., Donoviel, M., Esch, F., Hill, F., O'Brien, J.S., 1986. Molecular cloning of the sphingolipid activator protein-1 (SAP-1), the sulfatide sulfatase activator. Biochem. Biophys. Res. Commun. 134, 989–994.

Diaz-Font, A., Cormand, B., Santamaria, R., Vilageliu, L., Grinberg, D., Chabas, A., 2005. A mutation within the saposin D domain in a Gaucher disease patient with normal glucocerebrosidase activity. Hum. Genet. 117, 275–277.

Donohue, P.J., Shapira, H., Mantey, S.A., Hampton, L.L., Jensen, R.T., Battey, J.F., 1998. A human gene encodes a putative G protein-coupled receptor highly expressed in the central nervous system. Brain Res. Mol. Brain Res. 54, 152–160.

Dunham, J.H., Meyer, R.C., Garcia, E.L., Hall, R.A., 2009. GPR37 surface expression enhancement via N-terminal truncation or protein-protein interactions. Biochemistry 48, 10286–10297.

Dusonchet, J., Bensadoun, J.C., Schneider, B.L., Aebischer, P., 2009. Targeted overexpression of the parkin substrate Pael-R in the nigrostriatal system of adult rats to model Parkinson's disease. Neurobiol. Dis. 35, 32–41.
Elleder, M., Jerabkova, M., Befekadu, A., Hrebicek, M., Berna, L., Ledvinova, J., Hulkova, H., Rosewich, H., Schymik, N., Paton, B. C., Harzer, K., 2005. Prosaposin deficiency – a rarely diagnosed, rapidly progressing, neonatal neurovisceral lipid storage disease. Report of a further patient. Neuropediatrics 36, 171–180.
Fischer, G., Jatzkewitz, H., 1975. The activator of cerebroside sulphatase. Purification from human liver and identification as a protein. Hoppe Seylers Z. Physiol. Chem. 356, 605–613.
Fujita, N., Suzuki, K., Vanier, M.T., Popko, B., Maeda, N., Klein, A., Henseler, M., Sandhoff, K., Nakayasu, H., 1996. Targeted disruption of the mouse sphingolipid activator protein gene: a complex phenotype, including severe leukodystrophy and wide-spread storage of multiple sphingolipids. Hum. Mol. Genet. 5, 711–725.
Gao, H.L., Li, C., Nabeka, H., Shimokawa, T., Saito, S., Wang, Z.Y., Cao, Y.M., Matsuda, S., 2013. Attenuation of MPTP/MPP(+) toxicity *in vivo* and *in vitro* by an 18-mer peptide derived from prosaposin. Neuroscience 236, 373–393.
Gartner, S., Conzelmann, E., Sandhoff, K., 1983. Activator protein for the degradation of globotriaosylceramide by human alpha-galactosidase. J. Biol. Chem. 258, 12378–12385.
Harzer, K., Paton, B.C., Poulos, A., Kustermann-Kuhn, B., Roggendorf, W., Grisar, T., Popp, M., 1989. Sphingolipid activator protein deficiency in a 16-week-old atypical Gaucher disease patient and his fetal sibling: biochemical signs of combined sphingolipidoses. Eur. J. Pediatr. 149, 31–39.
Harzer, K., Paton, B.C., Christomanou, H., Chatelut, M., Levade, T., Hiraiwa, M., O'Brien, J.S., 1997. Saposins (sap) A and C activate the degradation of galactosylceramide in living cells. FEBS Lett. 417, 270–274.
Hassan, A.J., Zeng, J., Ni, X., Morales, C.R., 2004. The trafficking of prosaposin (SGP-1) and GM2AP to the lysosomes of TM4 Sertoli cells is mediated by sortilin and monomeric adaptor proteins. Mol. Reprod. Dev. 68, 476–483.
Hermo, L., Morales, C., Oko, R., 1992. Immunocytochemical localization of sulfated glycoprotein-1 (SGP-1) and identification of its transcripts in epithelial cells of the extratesticular duct system of the rat. Anat. Rec. 232, 401–422.
Herz, J., Hamann, U., Rogne, S., Myklebost, O., Gausepohl, H., Stanley, K.K., 1988. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. EMBO J. 7, 4119–4127.
Herz, J., Bock, H.H., 2002. Lipoprotein receptors in the nervous system. Annu. Rev. Biochem. 71, 405–434.
Hiesberger, T., Huttler, 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.
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.
Hiraiwa, M., Soeda, S., Kishimoto, Y., O'Brien, J.S., 1992. Binding and transport of gangliosides by prosaposin. Proc. Natl. Acad. Sci. USA 89, 11254–11258.
Hiraiwa, M., Campana, W.M., Martin, B.M., O'Brien, J.S., 1997a. Prosaposin receptor: evidence for a G-protein-associated receptor. Biochem. Biophys. Res. Commun. 240, 415–418.
Hiraiwa, M., Taylor, E.M., Campana, W.M., Darin, S.J., O'Brien, J.S., 1997b. 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.
Hiraiwa, M., Campana, W.M., Mizisin, A.P., Mohiuddin, L., O'Brien, J.S., 1999. Prosaposin: a myelinotrophic protein that promotes expression of myelin constituents and is secreted after nerve injury. Glia 26, 353–360.
Hiraiwa, M., Campana, W.M., Wang, C.Y., Otero, D.A., O'Brien, J.S., 2001. A retro-inverso Prosaptide D5 promotes a myelination process in developing rats. Brain Res. Dev. Brain Res. 128, 73–76.
Hiraiwa, M., Liu, J., Lu, A.G., Wang, C.Y., Misasi, R., Yamauchi, T., Hozumi, I., Inuzuka, T., O'Brien, J.S., 2003. Regulation of gene expression in response to brain injury: enhanced expression and alternative splicing of rat prosaposin (SGP-1) mRNA in injured brain. J. Neurotrauma. 20, 755–765.
Ho, M.W., O'Brien, J.S., 1971. Gaucher's disease: deficiency of 'acid'-glucosidase and reconstitution of enzyme activity *in vitro*. Proc. Natl. Acad. Sci. USA 68, 2810–2813.
Hulkova, H., Cervenkova, M., Ledvinova, J., Tochackova, M., Hrebicek, M., Poupetova, H., Befekadu, A., Berna, L., Paton, B. C., Harzer, K., Boor, A., Smid, F., Elleder, M., 2001. A novel mutation in the coding region of the prosaposin gene leads to a complete deficiency of prosaposin and saposins, and is associated with a complex sphingolipidosis dominated by lactosylceramide accumulation. Hum. Mol. Genet. 10, 927–940.
Igase, K., Tanaka, J., Kumon, Y., Zhang, B., Sadamoto, Y., Maeda, N., Sakaki, S., Sakanaka, M., 1999. An 18-mer peptide fragment of prosaposin ameliorates place navigation disability, cortical infarction, and retrograde thalamic degeneration in rats with focal cerebral ischemia. J. Cereb. Blood Flow Metab. 19, 298–306.
Igdoura, S.A., Hermo, L., Rosenthal, A., Morales, C.R., 1993. Nonciliated cells of the rat efferent ducts endocytose testicular sulfated glycoprotein-1 (SGP-1) and synthesize SGP-1 derived saposins. Anat. Rec. 235, 411–424.
Imai, Y., Soda, M., Inoue, H., Hattori, N., Mizuno, Y., Takahashi, R., 2001. An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. Cell 105, 891–902.
Inui, K., Wenger, D.A., 1984. Biochemical, immunological, and structural studies on a sphingolipid activator protein (SAP-1). Arch. Biochem. Biophys. 233, 556–564.
Isemura, S., Saitoh, E., Sanada, K., 1984. Isolation and amino acid sequence of SAP-1, an acidic protein of human whole saliva, and sequence homology with human gamma-trace. J. Biochem. 96, 489–498.
Islam, M.R., Abdullah, J.M., Atoji, Y., 2013. Distribution of prosaposin mRNA in the central nervous system of the pigeon (*Columba livia*). Anat. Histol. Embryol. 42, 257–265.
Jolivalt, C.G., Ramos, K.M., Herbetsson, K., Esch, F.S., Calcutt, N.A., 2006. Therapeutic efficacy of prosaposin-derived peptide on different models of allodynia. Pain 121, 14–21.
Jolivalt, C.G., Dacunha, J.M., Esch, F.S., Calcutt, N.A., 2008a. Central action of prosaptide TX14(A) against gp120-induced allodynia in rats. Eur. J. Pain. 12, 76–81.
Jolivalt, C.G., Vu, Y., Mizisin, L.M., Mizisin, A.P., Calcutt, N.A., 2008b. Impaired prosaposin secretion during nerve regeneration in diabetic rats and protection of nerve regeneration by a prosaposin-derived peptide. J. Neuropathol. Exp. Neurol. 67, 702–710.
Kang, S.J., Cresswell, P., 2004. Saposins facilitate CD1d-restricted presentation of an exogenous lipid antigen to T cells. Nat. Immunol. 5, 175–181.
Kishimoto, Y., Hiraiwa, M., O'Brien, J.S., 1992. Saposins: structure, function, distribution, and molecular genetics. J. Lipid Res. 33, 1255–1267.
Kiss, R.S., Ma, Z., Nakada-Tsukui, K., Brugnera, E., Vassiliou, G., McBride, H.M., Ravichandran, K.S., Marcel, Y.L., 2006. The lipoprotein receptor-related protein-1 (LRP) adapter protein

GULP mediates trafficking of the LRP ligand prosaposin, leading to sphingolipid and free cholesterol accumulation in late endosomes and impaired efflux. J. Biol. Chem. 281, 12081–12092.
Kitao, Y., Imai, Y., Ozawa, K., Kataoka, A., Ikeda, T., Soda, M., Nakimawa, K., Kiyama, H., Stern, D.M., Hori, O., Wakamatsu, K., Ito, S., Itohara, S., Takahashi, R., Ogawa, S., 2007. Pael receptor induces death of dopaminergic neurons in the substantia nigra via endoplasmic reticulum stress and dopamine toxicity, which is enhanced under condition of parkin inactivation. Hum. Mol. Genet. 16, 50–60.
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.
Koochekpour, S., Sartor, O., Lee, T.J., Zieske, A., Patten, D.Y., Hiraiwa, M., Sandhoff, K., Remmel, N., Minokadeh, A., 2004. Prosaptide TX14A stimulates growth, migration, and invasion and activates the Raf-MEK-ERK-RSK-Elk-1 signaling pathway in prostate cancer cells. Prostate 61, 114–123.
Kotani, Y., Matsuda, S., Sakanaka, M., Kondoh, K., Ueno, S., Sano, A., 1996a. Prosaposin facilitates sciatic nerve regeneration *in vivo*. J. Neurochem. 66, 2019–2025.
Kotani, Y., Matsuda, S., Wen, T.C., Sakanaka, M., Tanaka, J., Maeda, N., Kondoh, K., Ueno, S., Sano, A., 1996b. A hydrophilic peptide comprising 18 amino acid residues of the prosaposin sequence has neurotrophic activity *in vitro* and *in vivo*. J. Neurochem. 66, 2197–2200.
Kretz, K.A., Carson, G.S., Morimoto, S., Kishimoto, Y., Fluharty, A.L., O'Brien, J.S., 1990. Characterization of a mutation in a family with saposin B deficiency: a glycosylation site defect. Proc. Natl. Acad. Sci. USA 87, 2541–2544.
Kuchar, L., Ledvinova, J., Hrebicek, M., Myskova, H., Dvorakova, L., Berna, L., Chrastina, P., Asfaw, B., Elleder, M., Petermoller, M., Mayrhofer, H., Staudt, M., Krageloh-Mann, I., Paton, B.C., Harzer, K., 2009. Prosaposin deficiency and saposin B deficiency (activator-deficient metachromatic leukodystrophy): report on two patients detected by analysis of urinary sphingolipids and carrying novel PSAP gene mutations. Am. J. Med. Genet. A 149A, 613–621.
Lapchak, P.A., Araujo, D.M., Shackelford, D.A., Zivin, J.A., 2000. Prosaptide exacerbates ischemia-induced behavioral deficits *in vivo*; an effect that does not involve mitogen-activated protein kinase activation. Neuroscience 101, 811–814.
Lee, T.J., Sartor, O., Luftig, R.B., Koochekpour, S., 2004. Saposin C promotes survival and prevents apoptosis via PI3K/Akt-dependent pathway in prostate cancer cells. Mol. Cancer 3, 31.
Lefrancois, S., Zeng, J., Hassan, A.J., Canuel, M., Morales, C.R., 2003. The lysosomal trafficking of sphingolipid activator proteins (SAPs) is mediated by sortilin. EMBO J. 22, 6430–6437.
Leng, N., Gu, G., Simerly, R.B., Spindel, E.R., 1999. Molecular cloning and characterization of two putative G protein-coupled receptors which are highly expressed in the central nervous system. Brain Res. Mol. Brain Res. 69, 73–83.
Leon, L., Tatituri, R.V., Grenha, R., Sun, Y., Barral, D.C., Minnaard, A.J., Bhowruth, V., Veerapen, N., Besra, G.S., Kasmar, A., Peng, W., Moody, D.B., Grabowski, G.A., Brenner, M.B., 2012. Saposins utilize two strategies for lipid transfer and CD1 antigen presentation. Proc. Natl. Acad. Sci. USA 109, 4357–4364.
Li, N., Sarojini, H., An, J., Wang, E., 2010. Prosaposin in the secretome of marrow stroma-derived neural progenitor cells protects neural cells from apoptotic death. J. Neurochem. 112, 1527–1538.
Li, S.C., Kihara, H., Serizawa, S., Li, Y.T., Fluharty, A.L., Mayes, J.S., Shapiro, L.J., 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.
Li, S.C., Sonnino, S., Tettamanti, G., Li, Y.T., 1988. Characterization of a nonspecific activator protein for the enzymatic hydrolysis of glycolipids. J. Biol. Chem. 263, 6588–6591.
Lillis, A.P., Mikhailenko, I., Strickland, D.K., 2005. Beyond endocytosis: LRP function in cell migration, proliferation and vascular permeability. J. Thromb. Haemost. 3, 1884–1893.
Lillis, A.P., Van Duyn, L.B., Murphy-Ullrich, J.E., Strickland, D.K., 2008. LDL receptor-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies. Physiol. Rev. 88, 887–918.
Liu, J., Wang, C.Y., O'Brien, J.S., 2001. Prosaptide D5, a retro-inverso 11-mer peptidomimetic, rescued dopaminergic neurons in a model of Parkinson's disease. FASEB J. 15, 1080–1082.
Lobel, P., Fujimoto, K., Ye, R.D., Griffiths, G., Kornfeld, S., 1989. Mutations in the cytoplasmic domain of the 275 kd mannose 6-phosphate receptor differentially alter lysosomal enzyme sorting and endocytosis. Cell 57, 787–796.
Loukinova, E., Ranganathan, S., Kuznetsov, S., Gorlatova, N., Migliorini, M.M., Loukinov, D., Ulery, P.G., Mikhailenko, I., Lawrence, D.A., Strickland, D.K., 2002. Platelet-derived growth factor (PDGF)-induced tyrosine phosphorylation of the low density lipoprotein receptor-related protein (LRP). Evidence for integrated co-receptor function betwenn LRP and the PDGF. J. Biol. Chem. 277, 15499–15506.
Lu, A.G., Otero, D.A., Hiraiwa, M., O'Brien, J.S., 2000. Neuroprotective effect of retro-inverso prosaptide D5 on focal cerebral ischemia in rat. Neuroreport 11, 1791–1794.
Lundius, E.G., Stroth, N., Vukojevic, V., Terenius, L., Svenningsson, P., 2013. Functional GPR37 trafficking protects against toxicity induced by 6-OHDA, MPP+ or rotenone in a catecholaminergic cell line. J. Neurochem. 124, 410–417.
Lundius, E.G., Vukojevic, V., Hertz, E., Stroth, N., Cederlund, A., Hiraiwa, M., Terenius, L., Svenningsson, P., 2014. GPR37 protein trafficking to the plasma membrane regulated by prosaposin and GM1 gangliosides promotes cell viability. J. Biol. Chem. 289, 4660–4673.
Marazziti, D., Golini, E., Gallo, A., Lombardi, M.S., Matteoni, R., Tocchini-Valentini, G.P., 1997. Cloning of GPR37, a gene located on chromosome 7 encoding a putative G-protein-coupled peptide receptor, from a human frontal brain EST library. Genomics 45, 68–77.
Marazziti, D., Gallo, A., Golini, E., Matteoni, R., Tocchini-Valentini, G.P., 1998. Molecular cloning and chromosomal localization of the mouse Gpr37 gene encoding an orphan G-protein-coupled peptide receptor expressed in brain and testis. Genomics 53, 315–324.
Marazziti, D., Di Pietro, C., Golini, E., Mandillo, S., Matteoni, R., Tocchini-Valentini, G.P., 2009. Induction of macroautophagy by overexpression of the Parkinson's disease-associated GPR37 receptor. FASEB J. 23, 1978–1987.
Marzolo, M.P., Bu, G., 2009. Lipoprotein receptors and cholesterol in APP trafficking and proteolytic processing, implications for Alzheimer's disease. Semin. Cell Dev. Biol. 20, 191–200.
Matsuda, J., Vanier, M.T., Saito, Y., Tohyama, J., Suzuki, K., 2001. A mutation in the saposin A domain of the sphingolipid activator protein (prosaposin) gene results in a late-onset, chronic form of globoid cell leukodystrophy in the mouse. Hum. Mol. Genet. 10, 1191–1199.
Matsuda, J., Kido, M., Tadano-Aritomi, K., Ishizuka, I., Tominaga, K., Toida, K., Takeda, E., Suzuki, K., Kuroda, Y., 2004. Mutation in saposin D domain of sphingolipid activator protein gene causes urinary system defects and cerebellar Purkinje cell degeneration with accumulation of hydroxy fatty acid-containing ceramide in mouse. Hum. Mol. Genet. 13, 2709–2723.

Matsuda, J., Yoneshige, A., Suzuki, K., 2007. The function of sphingolipids in the nervous system: lessons learnt from mouse models of specific sphingolipid activator protein deficiencies. J. Neurochem. 103 (Suppl. 1), S32–S38.
May, P., Woldt, E., Matz, R.L., Boucher, P., 2007. The LDL receptor-related protein (LRP) family: an old family of proteins with new physiological functions. Ann. Med. 39, 219–228.
May, P., 2013. The low-density lipoprotein receptor-related protein 1 in inflammation. Curr. Opin. Lipidol. 24, 134–137.
Mehl, E., Jatzkewitz, H., 1964. [A cerebrosidesulfatase from swine kidney]. Hoppe Seylers Z. Physiol. Chem. 339, 260–276.
Meyer, R.C., Giddens, M.M., Schaefer, S.A., Hall, R.A., 2013. GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. Proc. Natl. Acad. Sci. USA 110, 9529–9534.
Miller, R.M., Kiser, G.L., Kaysser-Kranich, T.M., Lockner, R.J., Palaniappan, C., Federoff, H.J., 2006. Robust dysregulation of gene expression in substantia nigra and striatum in Parkinson's disease. Neurobiol. Dis. 21, 305–313.
Misasi, R., Sorice, M., Garofalo, T., Griggi, T., Campana, W.M., Giammatteo, M., Pavan, A., Hiraiwa, M., Pontieri, G.M., O'Brien, J.S., 1998. Colocalization and complex formation between prosaposin and monosialoganglioside GM3 in neural cells. J. Neurochem. 71, 2313–2321.
Misasi, R., Sorice, M., Di Marzio, L., Campana, W.M., Molinari, S., Cifone, M.G., Pavan, A., Pontieri, G.M., O'Brien, J.S., 2001. Prosaposin treatment induces PC12 entry in the S phase of the cell cycle and prevents apoptosis: activation of ERKs and sphingosine kinase. FASEB J. 15, 467–474.
Misasi, R., Garofalo, T., Di Marzio, L., Mattei, V., Gizzi, C., Hiraiwa, M., Pavan, A., Grazia Cifone, M., Sorice, M., 2004. Prosaposin: a new player in cell death prevention of U937 monocytic cells. Exp. Cell Res. 298, 38–47.
Morales, C.R., Hay, N., El-Alfy, M., Zhao, Q., 1998. Distribution of mouse sulfated glycoprotein-1 (prosaposin) in the testis and other tissues. J. Androl. 19, 156–164.
Morimoto, S., Martin, B.M., Kishimoto, Y., O'Brien, J.S., 1988. Saposin D: a sphingomyelinase activator. Biochem. Biophys. Res. Commun. 156, 403–410.
Morimoto, S., Martin, B.M., Yamamoto, Y., Kretz, K.A., O'Brien, J.S., Kishimoto, Y., 1989. Saposin A: second cerebrosidase activator protein. Proc. Natl. Acad. Sci. USA 86, 3389–3393.
Morita, F., Wen, T.C., Tanaka, J., Hata, R., Desaki, J., Sato, K., Nakata, K., Ma, Y.J., Sakanaka, M., 2001. Protective effect of a prosaposin-derived, 18-mer peptide on slowly progressive neuronal degeneration after brief ischemia. J. Cereb. Blood Flow Metab. 21, 1295–1302.
O'Brien, J.S., Kretz, K.A., Dewji, N., Wenger, D.A., Esch, F., Fluharty, A.L., 1988. Coding of two sphingolipid activator proteins (SAP-1 and SAP-2) by same genetic locus. Science 241, 1098–1101.
O'Brien, J.S., Carson, G.S., Seo, H.C., Hiraiwa, M., Kishimoto, Y., 1994. Identification of prosaposin as a neurotrophic factor. Proc. Natl. Acad. Sci. USA 91, 9593–9596.
O'Brien, J.S., Carson, G.S., Seo, H.C., Hiraiwa, M., Weiler, S., Tomich, J.M., Barranger, J.A., Kahn, M., Azuma, N., Kishimoto, Y., 1995. Identification of the neurotrophic factor sequence of prosaposin. FASEB J. 9, 681–685.
Ochiai, T., Takenaka, Y., Kuramoto, Y., Kasuya, M., Fukuda, K., Kimura, M., Shimeno, H., Misasi, R., Hiraiwa, M., Soeda, S., 2008. Molecular mechanism for neuro-protective effect of prosaposin against oxidative stress: its regulation of dimeric transcription factor formation. Biochim. Biophys. Acta 1780, 1441–1447.
Otero, D.A., Conrad, B., O'Brien, J.S., 1999. Reversal of thermal hyperalgesia in a rat partial sciatic nerve ligation model by Prosaptide TX14(A). Neurosci. Lett. 270, 29–32.
Paton, B.C., Schmid, B., Kustermann-Kuhn, B., Poulos, A., Harzer, K., 1992. Additional biochemical findings in a patient and fetal sibling with a genetic defect in the sphingolipid activator protein (SAP) precursor, prosaposin. Evidence for a deficiency in SAP-1 and for a normal lysosomal neuraminidase. Biochem. J. 285 (Pt 2), 481–488.
Puertollano, R., Aguilar, R.C., Gorshkova, I., Crouch, R.J., Bonifacino, J.S., 2001. Sorting of mannose 6-phosphate receptors mediated by the GGAs. Science 292, 1712–1716.
Qi, X., Grabowski, G.A., 1998. Acid beta-glucosidase: intrinsic fluorescence and conformational changes induced by phospholipids and saposin C. Biochemistry 37, 11544–11554.
Sagare, A.P., Deane, R., Zlokovic, B.V., 2012. Low-density lipoprotein receptor-related protein 1: a physiological Abeta homeostatic mechanism with multiple therapeutic opportunities. Pharmacol. Ther. 136, 94–105.
Sano, A., Matsuda, S., Wen, T.C., Kotani, Y., Kondoh, K., Ueno, S., Kakimoto, Y., Yoshimura, H., Sakanaka, M., 1994. Protection by prosaposin against ischemia-induced learning disability and neuronal loss. Biochem. Biophys. Res. Commun. 204, 994–1000.
Schnabel, D., Schroder, M., Sandhoff, K., 1991. Mutation in the sphingolipid activator protein 2 in a patient with a variant of Gaucher disease. FEBS Lett. 284, 57–59.
Schnabel, D., Schroder, M., Furst, W., Klein, A., Hurwitz, R., Zenk, T., Weber, J., Harzer, K., Paton, B.C., Poulos, A., et al., 1992. Simultaneous deficiency of sphingolipid activator proteins 1 and 2 is caused by a mutation in the initiation codon of their common gene. J. Biol. Chem. 267, 3312–3315.
Schulte-Herbruggen, O., Jockers-Scherubl, M.C., Hellweg, R., 2008. Neurotrophins: from pathophysiology to treatment in Alzheimer's disease. Curr. Alzheimer Res. 5, 38–44.
Sikora, J., Harzer, K., Elleder, M., 2007. Neurolysosomal pathology in human prosaposin deficiency suggests essential neurotrophic function of prosaposin. Acta Neuropathol. 113, 163–175.
Soeda, S., Hiraiwa, M., O'Brien, J.S., Kishimoto, Y., 1993. Binding of cerebrosides and sulfatides to saposins A-D. J. Biol. Chem. 268, 18519–18523.
Sorice, M., Molinari, S., Di Marzio, L., Mattei, V., Tasciotti, V., Ciarlo, L., Hiraiwa, M., Garofalo, T., Misasi, R., 2008. Neurotrophic signalling pathway triggered by prosaposin in PC12 cells occurs through lipid rafts. FEBS J. 275, 4903–4912.
Spiegel, R., Bach, G., Sury, V., Mengistu, G., Meidan, B., Shalev, S., Shneor, Y., Mandel, H., Zeigler, M., 2005. A mutation in the saposin A coding region of the prosaposin gene in an infant presenting as Krabbe disease: first report of saposin A deficiency in humans. Mol. Genet. Metab. 84, 160–166.
Subramaniam, S., Unsicker, K., 2010. ERK and cell death: ERK1/2 in neuronal death. FEBS J. 277, 22–29.
Sun, Y., Witte, D.P., Grabowski, G.A., 1994. Developmental and tissue-specific expression of prosaposin mRNA in murine tissues. Am. J. Pathol. 145, 1390–1398.
Sun, Y., Qi, X., Grabowski, G.A., 2003. Saposin C is required for normal resistance of acid beta-glucosidase to proteolytic degradation. J. Biol. Chem. 278, 31918–31923.
Sun, Y., Jia, L., Williams, M.T., Zamzow, M., Ran, H., Quinn, B., Aronow, B.J., Vorhees, C.V., Witte, D.P., Grabowski, G.A., 2008a. Temporal gene expression profiling reveals CEBPD as a candidate regulator of brain disease in prosaposin deficient mice. BMC Neurosci. 9, 76.
Sun, Y., Witte, D.P., Ran, H., Zamzow, M., Barnes, S., Cheng, H., Han, X., Williams, M.T., Skelton, M.R., Vorhees, C.V., Grabowski, G.A., 2008b. Neurological deficits and glycosphingolipid accumulation in saposin B deficient mice. Hum. Mol. Genet. 17, 2345–2356.
Sun, Y., Liou, B., Ran, H., Skelton, M.R., Williams, M.T., Vorhees, C.V., Kitatani, K., Hannun, Y.A., Witte, D.P., Xu, Y.H., Grabowski, G.A., 2010. Neuronopathic Gaucher disease in the mouse: viable combined selective saposin C deficiency and mutant

glucocerebrosidase (V394L) mice with glucosylsphingosine and glucosylceramide accumulation and progressive neurological deficits. Hum. Mol. Genet. 19, 1088–1097.
Sun, Y., Zamzow, M., Ran, H., Zhang, W., Quinn, B., Barnes, S., Witte, D.P., Setchell, K.D., Williams, M.T., Vorhees, C.V., Grabowski, G.A., 2013. Tissue-specific effects of saposin A and saposin B on glycosphingolipid degradation in mutant mice. Hum. Mol. Genet. 22, 2435–2450.
Tamargo, R.J., Velayati, A., Goldin, E., Sidransky, E., 2012. The role of saposin C in Gaucher disease. Mol. Genet Metab. 106, 257–263.
Toyofuku, T., Nojima, S., Ishikawa, T., Takamatsu, H., Tsujimura, T., Uemura, A., Matsuda, J., Seki, T., Kumanogoh, A., 2012. Endosomal sorting by Semaphorin 4A in retinal pigment epithelium supports photoreceptor survival. Genes Dev. 26, 816–829.
Tsuboi, K., Hiraiwa, M., O'Brien, J.S., 1998. Prosaposin prevents programmed cell death of rat cerebellar granule neurons in culture. Brain Res. Dev. Brain Res. 110, 249–255.
Unuma, K., Chen, J., Saito, S., Kobayashi, N., Sato, K., Saito, K., Wakisaka, H., Mominoki, K., Sano, A., Matsuda, S., 2005. Changes in expression of prosaposin in the rat facial nerve nucleus after facial nerve transection. Neurosci. Res. 52, 220–227.
Vaccaro, A.M., Tatti, M., Ciaffoni, F., Salvioli, R., Maras, B., Barca, A., 1993. Function of saposin C in the reconstitution of glucosylceramidase by phosphatidylserine liposomes. FEBS Lett. 336, 159–162.
Vaccaro, A.M., Motta, M., Tatti, M., Scarpa, S., Masuelli, L., Bhat, M., Vanier, M.T., Tylki-Szymanska, A., Salvioli, R., 2010. Saposin C mutations in Gaucher disease patients resulting in lysosomal lipid accumulation, saposin C deficiency, but normal prosaposin processing and sorting. Hum. Mol. Genet. 19, 2987–2997.
Valdenaire, O., Giller, T., Breu, V., Ardati, A., Schweizer, A., Richards, J.G., 1998. A new family of orphan G protein-coupled receptors predominantly expressed in the brain. FEBS Lett. 424, 193–196.
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.
Vogel, A., Schwarzmann, G., Sandhoff, K., 1991. Glycosphingolipid specificity of the human sulfatide activator protein. Eur. J. Biochem. 200, 591–597.
Wagner, R., Myers, R.R., O'Brien, J.S., 1998. Prosaptide prevents hyperalgesia and reduces peripheral TNFR1 expression following TNF-alpha nerve injection. Neuroreport 9, 2827–2831.
Wang, H.Q., Imai, Y., Inoue, H., Kataoka, A., Iita, S., Nukina, N., Takahashi, R., 2008. Pael-R transgenic mice crossed with parkin deficient mice displayed progressive and selective catecholaminergic neuronal loss. J. Neurochem. 107, 171–185.
Wenger, D.A., Sattler, M., Roth, S., 1982. A protein activator of galactosylceramide beta-galactosidase. Biochim. Biophys. Acta 712, 639–649.
Yan, L., Otero, D.A., Hiraiwa, M., O'Brien, J.S., 2000. Prosaptide D5 reverses hyperalgesia: inhibition of calcium channels through a pertussis toxin-sensitive G-protein mechanism in the rat. Neurosci. Lett. 278, 120–122.
Yang, Y., Nishimura, I., Imai, Y., Takahashi, R., Lu, B., 2003. Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila. Neuron 37, 911–924.
Yokota, N., Uchijima, M., Nishizawa, S., Namba, H., Koide, Y., 2001. Identification of differentially expressed genes in rat hippocampus after transient global cerebral ischemia using subtractive cDNA cloning based on polymerase chain reaction. Stroke 32, 168–174.
Yoneshige, A., Suzuki, K., Matsuda, J., 2010. A mutation in the saposin C domain of the sphingolipid activator protein (Prosaposin) gene causes neurodegenerative disease in mice. J. Neurosci. Res. 88, 2118–2134.
Yuan, L., Morales, C.R., 2010. A stretch of 17 amino acids in the prosaposin C terminus is critical for its binding to sortilin and targeting to lysosomes. J. Histochem. Cytochem. 58, 287–300.
Yuan, L., Morales, C.R., 2011. Prosaposin sorting is mediated by oligomerization. Exp. Cell Res. 317, 2456–2467.
Zeng, J., Racicott, J., Morales, C.R., 2009. The inactivation of the sortilin gene leads to a partial disruption of prosaposin trafficking to the lysosomes. Exp. Cell Res. 315, 3112–3124.
Zeng, Z., Su, K., Kyaw, H., Li, Y., 1997. A novel endothelin receptor type-B-like gene enriched in the brain. Biochem. Biophys. Res. Commun. 233, 559–567.
Zhou, D., Mattner, J., Cantu 3rd, C., Schrantz, N., Yin, N., Gao, Y., Sagiv, Y., Hudspeth, K., Wu, Y.P., Yamashita, T., Teneberg, S., Wang, D., Proia, R.L., Levery, S.B., Savage, P.B., Teyton, L., Bendelac, A., 2004. Lysosomal glycosphingolipid recognition by NKT cells. Science 306, 1786–1789.