PROSAPOSIN PRECURSOR PROTEIN: FUNCTIONS AND MEDICAL APPLICATIONS.

REVIEW

HAM D.

Department of Preventive Medicine, Faculty of Medicine, Masaryk University, Brno

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Abstract

Prosaposin is a precursor of four saposins, termed saposin A, B, C, and D, which activate glycosphingolipid hydrolysis. Inherited deficiency of saposins leads to several forms of lysosomal storage disease. Besides the lysosomal function, a secreted form of prosaposin displays several other properties. This precursor was shown in rodents to maintain the nervous system associated with oxidative stress signalling. The sequences of prosaposin were also applied in order to increase the binding of bull sperm to the egg membrane, thereby improving fertilization. Estrogen elevated levels of prosaposin in vitro and in vivo and prosaposin activated the MAPK/Akt signalling cascade, while prosaposin-deficient mice showed involution of the prostate gland. These results led to a hypothesis that this protein may play a role in the pathological alterations of the prostate. Involvement of saposins in lipid presentation to T cells allowed the consideration that these proteins may form links between lipid metabolism and immunological control. Thus, the properties of the multifunctional protein prosaposin summarized in this review could open new avenues for the future pharmaceutical interventions.

Keywords

Cancer, Lysosomes, Prosaposin, Saposin, Prostate

Abbreviations

GSLs, glycosphingolipids; MAPK, mitogen activated protein kinase

Biology of Prosaposin, the Precursor of Four Saposins

Several acid hydrolases involved in the degradation of glycosphingolipids (GSLs) require the assistance of small glycoprotein activator proteins called saposins (saposin A, B, C, or D) (1). These activators are proteolytically cleaved from their prosaposin precursor protein in lysosomes. Deficiency of saposins leads to lysosomal storage diseases. These disorders represent genetically determined metabolic diseases characterized by dysfunction of the lysosomes or lysosomal enzymes. The majority of these diseases have neurological phenotypes resulting from the storage of GSLs in cells of the central nervous system (CNS).
The first activator, saposin B, which activated lysosomal arylsulfatase A necessary for the hydrolytic degradation of GSLs, was identified by Mehl and Jatzkewitz (2). Inherited deficiency of saposin B leads to a lysosomal storage disease, which resembles metachromatic leukodystrophy. This disorder is characterized by the accumulation of sulfatide and also additional glycolipids (3). Deficiency of another activator protein, saposin C, causes an atypical form of Gaucher disease with glucosylceramide accumulation (4). The presence of a specific genetic deficiency of either saposin A or saposin D was not reported. Recently, Matsuda et al. (5) have generated a mouse model lacking the saposin A domain. Mutant mice homozygous for inactivated saposin A slowly developed progressive hind leg paralysis while surviving up to 5 months. The phenotype exhibited infantile globoid cell leukodystrophy similar to, but milder than, that seen in man (Krabbe disease) due to the deficiency of lysosomal galactosylceramidase. Infantile globoid cell leukodystrophy was detected at day 30. This demonstrated that saposin A is required for in vivo degradation of galactosylceramide by galactosylceramidase. The authors suggested that genetic saposin A deficiency might be expected in human patients with undiagnosed late-onset chronic leukodystrophy without galactosylceramidase deficiency. The mutations within the start codon of the whole prosaposin precursor protein result in a complex sphingolipidosis exhibited by lactosylceramide accumulation investigated in two families (6, 7). The rapid and fatal course associated with severe neurovisceral manifestation has been recognized at birth in these disorders. Mutant mice, homozygous for an inactivated gene of the sphingolipid activator protein precursor, exhibit two distinct clinical phenotypes - neonatally fatal and later-onset (8). Progressive neurological signs are developed at around 20 days. At days 35–38, the mice die. This phenotype closely resembles that of the human condition.

The four saposins contain around 80 amino acids and are highly homologous. The saposins are intralysosomal, whereas prosaposin is secreted into the medium of cultured cells or into body fluids (9, 10). It has been shown that a 65 kDa precursor protein was first synthesized and then converted to a secreted form, 70 kDa glycoprotein, by glycosylation (11). The form and amount of secreted prosaposin can vary with the tissue and the cell type (12). Increased quantities of the secreted form of prosaposin were detected in seminal fluid, cerebrospinal fluid, plasma, and milk (10, 13, 14).

The current strategies for the treatment of GSL lysosomal storage diseases involve enzyme replacement therapy, substrate deprivation or gene therapy. In enzyme replacement therapy, the defective enzyme is compensated by intravenously administered purified enzyme to patients. This therapy has proven to be successful in Type I Gaucher disease (15). However, this approach is only useful for the systemic, non-CNS storage disorders, since glycoprotein enzymes or drugs for substrate deprivation do not cross the blood-brain barrier in the GSL storage diseases with a neurological phenotype. Therefore, applications of gene therapy in neurological disorders have the potential for proving this approach.
The mechanism of action of saposins was extensively studied. The recent model of lysosomal hydrolysis suggests that GSLs reach the lysosomes, where they are digested. GSLs are transported in the form of intra-lysosomal/intra-endosomal vesicles (16) after endocytosis from the plasma membrane. The final step of GSLs proteolysis is the hydrolysis of ceramide into sphingosine and fatty acid by acid ceramidase. The recently resolved crystal structure of saposin B revealed two dimeric chains resembling a “shell-like” structure for lipid binding (17). Each chain consists of four alpha-helices. The inner surface of the “shell” is hydrophobic while the outer area is negatively charged. It was proposed that this structure extracts target lipids from membranes into the inner cavity by a conformational change. Cerebroside sulphate head groups in a soluble saposin B-lipid complex would then form an arylsulfatase A – substrate complex. It has been questioned why, despite the very close homology, each saposin activates a different hydrolase. This was partially answered by stating that the potential differences at the tertiary or quaternary structural level and altered electrostatic potential at the molecular surface between saposins could affect binding to lipids (18). Resolving the three-dimensional structure of saposin C revealed five alpha-helices without a conformational change prior to lipid binding (19). Thus, solving the crystal structures of other two saposins (A and D) will provide further insight into the mechanism of lipid binding by these activators.

Besides the lysosomal function, secreted prosaposin revealed several other functions. Prosaposin has been shown to be involved in maintenance of the nervous system, in oxidative stress signalling, fertilization, and possibly in cancer development. Surprisingly, a new function of saposins in lipid presentation to T cells through CD1 proteins has recently been reported (20, 21). Continued investigation on prosaposin may lead to the resolution of these newly discovered functions and a mechanism of action of this multifunctional protein. Following the characteristics of the secreted prosaposin precursor protein could provide not only a review of its discussed properties, but also their relevance to the future therapeutical interventions.

**THE ROLE OF PROSAPOSIN IN THE NERVOUS SYSTEM**

Prosaposin is abundant in the brain and localized exclusively in certain neurons and nerve fibres (22). This precursor has been identified as a neurotrophic factor in murine neuroblastoma (NS20Y) cells that promoted neurite outgrowth, enhanced choline acetyltransferase activity in human neuroblastoma (SK-N-MC) cells, and prevented the death of neural cells (23). Neurotrophic activity resides in an amino-terminal 12-residue peptide comprising the hydrophilic region of the human saposin C domain. Furthermore, an 18-mer peptide encompassing the hydrophilic sequence of rat saposin C domain protected hippocampal CA1 neurons against lethal forebrain ischemia (24) and also protected neurons against chronic hypoxic stress (25). Although the molecular mechanism of 18-mer peptide neurotrophic effects is unknown, it has been proposed that the peptide acts as an antioxidant agent. Secreted
prosaposin provided also trophic support for the repair of the injured nerve (26). Recently, the level of prosaposin mRNA was elevated by more than 400% over controls in an ischemic brain (27). This observation suggests the role of prosaposin in a signalling response to oxidative stress.

Prosaposin and a 14-mer peptide that contain the neurotrophic region of prosaposin partly prevented apoptosis of primary Schwann cells induced by serum withdrawal. The signalling mechanism responsible for the survival of Schwann cells has been elucidated and indicates that phosphatidylinositol 3-kinase (PI3K) and its target Akt activity was increased. This indicated that prosaposin activates the PI3K/Akt pathway to induce the survival of Schwann cells (28). The same group (29) has proposed a new role of prosaposin for mitogen activated protein kinase (MAPK) in signal transduction. Prosaposin rapidly stimulated protein tyrosine phosphorylation in neuronal PC12 cells, Schwann cells and oligodendrocytes (30), and increased phosphorylation of MAPK. Further studies revealed that prosaposin exerted its trophic effect by binding to a high-affinity receptor, which activated a pertussis-sensitive G-protein (30). In this regard, enhanced synthesis of sulfatide was implicated in myelin lipid synthesis. Thus, these observations proposed that prosaposin might be useful as a therapeutic agent in the treatment of peripheral neuropathies.

THE ROLE OF PROSAPOSIN IN FERTILIZATION

Another function of prosaposin is related to the alteration of fertility. Prosaposin is found in luminal fluids from the rat rete testis, efferent ducts, and epididymis (31, 32). In addition, this protein is associated with rat spermatids (31, 32). Prosaposin facilitated the binding of sperm to the avian perivitelline membrane through a 60-amino acid sequence epitope, which is conserved among chicken, humans, rats, and mice (33). On this basis, a peptide technology was developed that uses synthetic FertPlus to increase binding of sperm to the egg membrane. It was demonstrated that the pregnancy rate for thawed sperm from bulls was increased when the bull sperm was exposed to the chemically synthesized peptide containing the A-B sequence of prosaposin (34). The mechanism of action is unknown, but it was suggested that the primary role was increasing the number of sperm binding to the outer egg layer, zona pellucida. These data also imply a possibility of using FertPlus to improve the fertility of some subfertile males.

PROSAPOSIN AND CANCER DEVELOPMENT

The prosaposin level was increased in vivo and in vitro as a result of estrogen stimulation. A cancer cell line MCF-7 cultured in medium supplemented with 17β-estradiol (0.1–100 nM) dependently increased secretion of prosaposin into media. Secretion of prosaposin was accompanied by procathepsin D, another lysosomal protein (35). The authors suggested that prosaposin, together with other lysosomal
proteins such as procathepsin D, may be involved in eliminating barriers to tumour metastasis by facilitating hydrolysis of membrane glycolipids. Ovine prosaposin mRNA levels in the endometrial epithelium increased in response to elevated levels of estrogen (36). Furthermore, the up-regulation of prosaposin has been shown to downregulate integrin receptors in T47D and MCF7 breast cancer cell lines, thus impairing the metastasis capacity of tumour cells (37).

The results on the involution of the prostate gland in prosaposin-deficient mice may imply the importance of this protein in the pathological alteration of the prostate (38, 39). A morphometric analysis of the male reproductive organs of prosaposin knockout mice showed a reduction in size and weight of testes, epididymis, seminal vesicles, and prostate gland when compared to controls (38, 39). The diameter of the tubuloalveolar glands of the prostate was smaller and lined by epithelial cells, which were shorter in these mice. The lining epithelium of the efferent duct was ciliated, in contrast to heterozygous or normal mice with non-ciliated epithelia. This was not observed in other glands of homozygous mutant mice (pancreas or submandibular glands) and covering epithelia (small intestine), which were fully differentiated. The effect of prosaposin deletion on the prostate appeared to be correlated with the reduced proliferation and differentiation of this epithelium. Ablation of the prosaposin gene was associated with the inactivation of MAPK and Akt pathways in the prostate. However, these observations were performed in mice at the prepubertal age since prosaposin-deficient mice die from neurological defects. In order to prolong the life of the animals, we have generated transgenic mice carrying the prosaposin-human neurofilament light chain gene promoter cDNA. Introducing prosaposin cDNA only into the nervous system of mice by crossing prosaposin heterozygous mutants with mice bearing prosaposin under control of the neurofilament promoter (40) could generate offspring with the precursor expressed only in the nervous system. Future experiments carried out on such mice would allow clarification of the effect of the prosaposin deletion gene in the prostate of adult animals. Most recently, immunostaining of malignant tissues and an androgen – independent prostate cancer cell line displayed increased reactivity against prosaposin (41).

In reference to the novel function of saposins in lipid presentation to T cells (20, 21), further investigation may elucidate an unknown link between lipid metabolism and immunity, thus providing insight into the mechanism of the potential role of prosaposin in cancer. This may also allow design of new pharmaceutical avenues that would affect not only immunological control over this process, but possibly over cancer development, too.

A c k n o w l e d g e m e n t

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