Gene Section
Review

PSAP (prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy))

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Identity

Hugo: PSAP
Other names: FLJ00245; GLBA; MGC110993; SAP1
Location: 10q22.1
Local order: Between CDH23 (cadherin-like 23; centromeric) and Carbohydrate (chondroitin 6) sulfotransferase 3 (CST3; telomeric).

DNA/RNA

Description

The human PSAP-precursor gene spans approximately 20 kb in length of the long arm of chromosome 10 and consists at least 15 exons. The size of exons range from 57 to 1200 bp and the size of the introns vary from 91 to more than 3800 bp in length. The PSAP gene can be cateogorized as a polycistronic gene. Further analysis of PSAP intronic positions has indicated that it may be evolved from an ancestral gene subjected to two duplication and at least one gene rearrangement.

Transcription

Due to the presence of an alternative splice site in exon 8, PSAP gene could be transcribed into three mRNA isoforms: one with complete exon 8 (9 bases), one without exon 8, and one with downstream 6 bases of exon 8. While all three PSAP mRNAs could be detected in human, mice, and rat, differential expression of PSAP mRNA isoforms has been reported in human and mouse tissues or cell lines. However, in chicken, there are only two mRNA isoforms (+/- exon 8). The exact biological significances of different nucleotide sequences of saposin B domain in
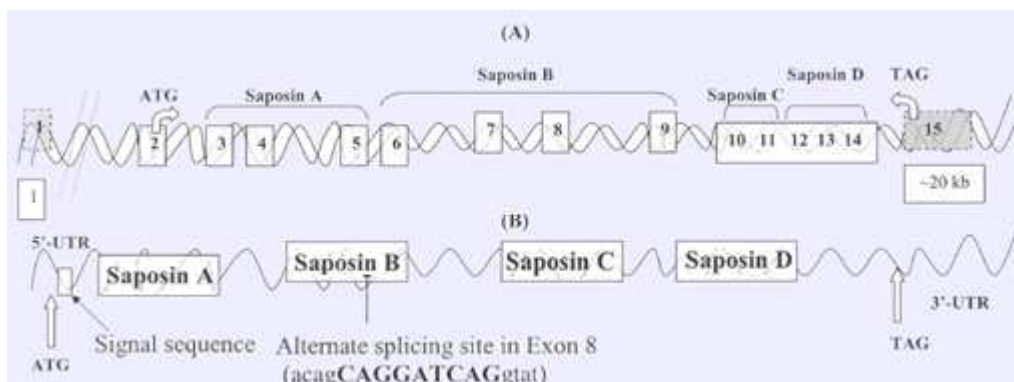
Schematic diagram of the human PSAP gene (A) and cDNA (B). Open squares are exons 2-15 and shaded boxes correspond to untranslated 5' and 3' regions. The signal sequence is located adjacent to ATG and will be removed during transit in the edoplasmic reticulum. Exon 8 of the saposin B domain of prosaposin contain a 9-bp alternate splicing site that might generate three different cDNAs:
a) complete exon 8 (CAG GAT CAG; 3 amino acids),
b) no exon 8, and
c) exon 8 with downstream 6 bases (GAT CAG; 2 amino acids).
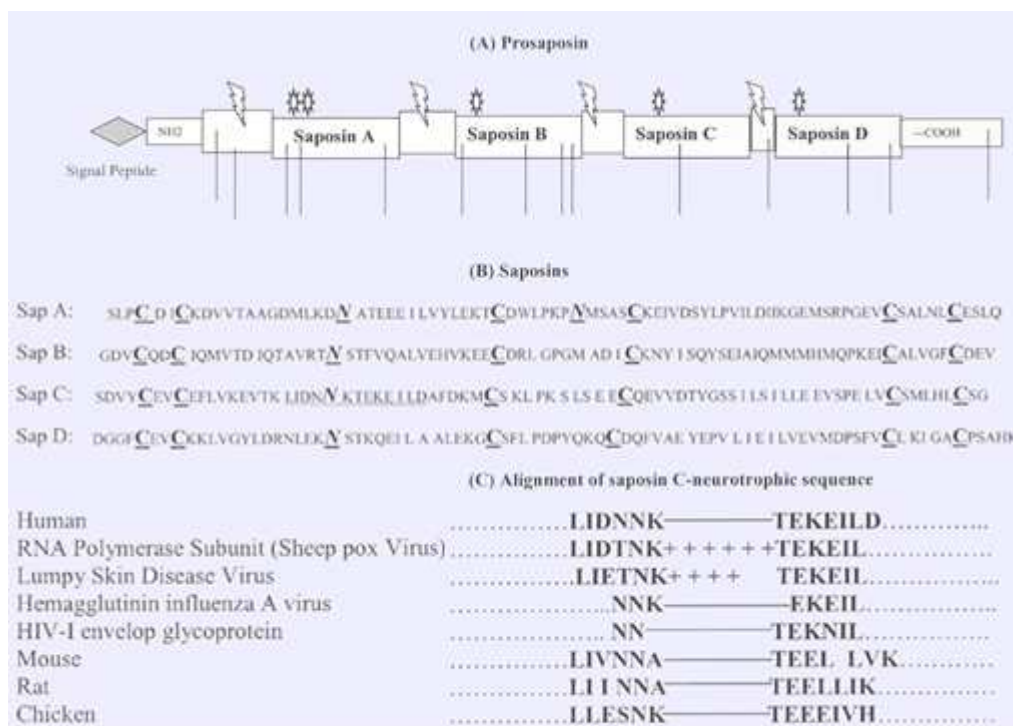
Structure of human PSAP, saposins, and sequence alignment of a known neurotrophic fragment of human saposin C

(A) Organization of human PSAP protein. Individual saposin domains and signal peptide are indicated; lightning bolts represent proteolytic cleavage sites in the intersaposin sequences; glycosylation sites and exon-intron boundaries are shown by 8-point stars and vertical lines, respectively.

(B) Amino acid sequence of human saposin A-D. Potential N-Glycosylation type carbohydrate side chain linked to asparagine are indicated with capital letter 'N'. As indicated, each saposin molecule also contains 6 cystein residues positioned at almost similar location. Neurotrophic sequence of saposin C is double-underlined.

(C) Alignment and comparison of neurotrophic sequence of human saposin C with other vertebrates and known viruses. All sequences presented are linear. Each plus sign indicates the presence of a non-fit amino acid.

prosaposin precursor are not known. However, it has been shown that the stability of functionally mature saposin B is not significantly affected by the presence or absence of 6 or 9 base pairs (+3 or +2 amino acids) of exon 8. In addition, mutations in the PSAP gene leading to lack or disruption of saposin B protein in patients showed similar metabolic phenotypes. Taking into consideration that neurotrophic activity of PSAP has been attributed to saposin C domain of the molecule, alternative splicing is not expected to modulate this effect.

Pseudogene

No pseudogene is identified for PSAP.

Protein

Description

Prosaposin is a highly conserved glycoprotein (with approximate molecular weight of 65-72 kDa), and the precursor of 4 small lysosomal proteins (saposin A-D; of 8-13 kDa) which are required for intracellular degradation of certain sphingolipids. Proteolytic cleavage of PSAP precursor mediated by lysosomal cysteine protease-cathepsin D, leads to individual mature saposin proteins (acidic glycoproteins). PSAP is secreted as a full-length protein. However, individual saposin proteins also exist as extracellular mature proteins (e.g., in tissue culture supernatant, serum, prostatic secretions, malignant pleural effusion). Although the origin of mature saposin proteins in the extracellular fluids is not known, it is likely that circulating serum enzymes may participate in proteolytic cleavage of secreted PSAP. Each saposin domain presents with near identical localization of glycosylation sites and cysteine residues. The presence of high percentage homology in amino acid sequences between saposin A and C further indicates that they have originated from a single ancestroral gene at least via duplication and/or gene rearrangement.

Prosaposin is the saposin precursor protein with 524 amino acids including a 16 amino acids signal peptide. The full-length precursor molecule contains complex oligosaccharides chains which is probably the result of cotranslational glycosylation of the 53-kDa polypeptide and its later modification within the Golgi system that yield the 70-72 kDa precursor protein. After transport to the lysosome, cathepsin D participates in its proteolytic processing to intermediate molecular forms with 35 to 53 kDa and then to 13-kDa glycoprotein and

finally to the mature 8-11 kDa less or partially glycosylated forms of individual saposin molecules. It is noteworthy that western analysis (using different anti-PSAP monoclonal and polyclonal antibodies) of human seminal fluid and whole cell lysates prepared from a number of malignant prostatic cells and other malignant cell types (e.g., breast, lung) show the presence of multiple bands with approximate molecular weight of 12-, 24-, and 36-kDa. These bands are most probably represent mono-, di-, and tri-saposins and are the result of sequential cleavage of the precursor molecule. Saposins are highly homologous molecules, each with approximately 80 amino acids containing six cysteine residues (forming 3 disulfide bonds and hair-pin structure) and N-glycosylated carbohydrate chains that are highly conserved. PSAP amino acid sequence among various species (e.g., human, rat, mouse, chicken, Zebrafish) reveals evolutionary conservation in terms of saposin domains and the homologus positioning of terminally-situated cysteine residues and an N-linked glycosylated site.

Expression

Prosaposin and individual saposin proteins are expressed by a wide variety of cells types originating from ectodermal, mesodermal, and endodermal germ layers including but not limited to lung, skin, fibroblast, stromal cells, bone, smooth muscle, skeletal muscle, cardiac muscle, placenta, red and white blood cells, pancreas, placenta, lymphoreticular system (spleen, thymus, liver), micro and macrovascular system, genitourinary system (e.g., prostate, testes, seminal vesicle), central and peripheral nervous system, etc. Interestingly, comparative protein expression analysis on normal human adult and fetal tissues has shown elevated levels of PSAP expression in the adult liver and decreased amounts in fetal skeletal muscle. Prosaposin and saposins also present as soluble proteins in extracellular space/fluid including pleural fluid, cerebrospinal fluid, seminal fluid, milk, and serum. PSAP and saposins are predominantly expressed in cells of hematopoietic origin (e.g., red- and white-blood cells) and neuroglial-derived tissues as compared to all other normal cell types in the mammalian system. In malignant cells, compared to their normal cellular counterparts, prosaposin is overexpressed in breast adenocarcinoma cell lines, non small-cell lung adenocarcinoma, neuroblastoma, and schwannoma cell lines. In addition, similar PSAP-overexpression is also detected in glioma cell lines, adult and pediatric brain tumors (e.g., medulloblastoma-, astrocytoma-, glioblastoma multiforme-cell lines), fibrosarcoma, osteosarcoma, and prostate cancer cell lines. In addition, immunoblotting of total protein array derived from different types of tumors (brain, colon, lung, pancreas, rectum, ovary, parotid, skin, bladder, small intestine,

thymus, and uterus) with mouse monoclonal antibodies against PSAP and GAPDH followed by densitometric analysis demonstrated 1.6 to 5-fold increase in PSAP expression in malignant tissues compared to their corresponding normal tissues (Table 1). Most noticeably, PSAP is overexpressed and/or amplified in human prostate cancer tissues, xenografts, and cell lines. Quantitative SNP array hybridization in conjunction with southern hybridization and quantitative real-time PCR demonstrated a frequency of 20.6% for PSAP amplification (4 out of 25 prostate cancer xenografts and metastatic tissues and three out of nine prostate cancer cell lines). Expression of PSAP protein and mRNA in malignant prostate cancer cells is exclusively higher than normal prostate epithelial and stromal cells. Immunoblotting of conditioned media derived from prostate cancer cells shows the presence of PSAP-immunoreactive bands with approximate molecular size of 72-kDa, 140-kDa, and 220-kDa. It is not clear whether or not the 140- and 220-kDa bands represent the dimeric or trimeric form of PSAP. In addition, PSAP mRNA and protein expression is higher in several androgen-independent than the androgen-dependent prostate cancer cell lines. This finding suggests that PSAP expression might be under androgenic, steroid hormone regulation, or feedback control mediated by the hypothalamus-pituitary-gonadal neuroendocrine axis.

The involvement of pertussis toxin-sensitive GPCR-dependent mechanism for in vitro biological activities of PSAP (or its active molecular derivatives such as saposin C, TX14A) has been demonstrated in a number of cell lines. In addition, using human and mouse fibroblasts and in vivo studies, it has been demonstrated that PSAP entry into the cells is also possible via at least three other independent receptor system including the mannose receptor, mannose-6-phosphate (M-6-P) receptor, and low density lipoprotein receptor-related protein (LRP). Cell type-specific distribution of any of the above receptor systems, their relative abundance, their involvement in various biological activities of soluble PSAP and/or saposin C (e.g., cell signaling, sphingolipid transport), or post-receptor occupancy events require additional studies.

Localisation

Prosaposin exists as a lysosomal, integral membrane, and an intracellular protein. In addition, prosaposin also exist as an integral membrane protein. The relative abundance of prosaposin is believed to be the highest as a secretory (soluble) protein and the lowest as an integral protein. However, it is not clear whether there is a tissue or cell type-specificity (e.g., benign versus malignant cells, epithelial versus stromal cells) for PSAP distribution.

Function

Prosaposin is a dual function molecule; as the precursor of intracellular lysosomal saposin proteins involved in sphingolipid hydrolysis activity and as a secreted soluble protein with neurotrophic activities, including growth, development, and maintenance of the peripheral and central nervous system, nerve regeneration and plasticity, stimulation of neurite outgrowth, stimulation of neuroblastoma cells proliferation, protection from cell-death or apoptosis, and activation of MAPK- and PI3K/Akt-signaling pathways. Column chromatography data indicated the formation of stable complexes between PSAP/saposins and several gangliosides. It has been suggested that PSAP functions as a sphingolipid binding protein and on the cell surface, complex formation between PSAP and gangliosides may suggests a role for this molecule in ganglioside function. Whether or not there is a link between the function of secreted soluble form as a trophic factor and its role as a ganglioside-binding or -career protein remain to be understood. Saposins function as coprotein for intracellular degradation of sphingolipids. Saposin A and C is involved in hydrlysis of glucosylceramide and galactosylceramide. saposin B stimulates galacto-cerebroside sulfate hydrolysis, GM1 ganglioside, and globotriaosylceramide. Saposin C is the activator of sphingomyelin phosphodiesterase. While several members of CD1 proteins are involved in lipid presentation to T cells, prosaposin-deficient mice exhibit certain defects in CD1d-mediated antigenic presentation suggesting that saposins are involved in mobilization of lipid monomers from the lysosomal membrane and their association with CD1d. In addition, prosaposin-deficient fibroblasts transfected with another member of CD1 family (CD1b) also failed to activate lipid-specific T lymphocytes. Upon reconstitution of fibroblasts with saposin C, T-cells response was restored. These findings might be suggestive of potential implications for saposin C or perhaps PSAP in recognition of tumor antigens.

Several reports have identified a number of linear 5-22 amino acid segments called prosaptides (e.g., D5, TX14A) that demonstrate in vitro and/or in vivo neurotrophic activities. These bioactive sequences are located at the downstream region of saposin C domain of PSAP. Prosaptides, saposin C, or PSAP exert their effect at least partially, by binding to a single high-affinity G protein-coupled receptor. This receptor has been partially characterized but not cloned. In malignant cells and tissues, several classic reports have indicated a pluripotent regulatory role for saposin C and PSAP in prostate cancer with potential involvement in prostate carcinogenesis or progression toward metastatic or androgen-independent state.

Immunohistochemical staining on benign and malignant prostate tissues revealed an intense cytosolic and anti-prosaposin immunoreactivity in tumor cells, stromal, endothelial, and inflammatory mononuclear cells and the intensity of staining was proportional to the overall Gleason's score. PSAP-immunoreactivity was also noticeable as extracellular deposition in hypercellular regions in high-grade prostatic tumors. In addition, PSAP and/or its active molecular derivatives (saposin C or TX14A) stimulate prostate cancer cells growth, motility, and invasion, upregulates uPA/uPAR expression, activates the p42/44 MAPK (Raf-MEK-ERK-RSK-Elk-1 signaling cascade), p38 MAPK, and SAPK/JNK family members of the MAPK superfamily and PI3K/Akt signaling pathways, and protects cells from apoptotic cell-death induction by etoposide via modulation of caspase-3, -7, and -9 expression/activity and/or the PI3K/Akt signaling pathway activation.

Homology

The four saposin A-D proteins share a great deal of homology (~50%) in their amino acids sequences. In addition to these, saposins also contain 6 highly conserved cysteines. Considering all these structural similarities, they differ from each other for their specificity of intracellular or potential extracellular functions. Among fopur saposins, cross-species analysis of saposin sequences, show evolutionary conservation for saposin A, B, and D. However, with the exception to the neurotrophic sequence, saposin C sequence appear to be more species-specific. For example, from the linear human saposin C-neurotrophic sequence (LIDNNKTEKEILD):

(1) LID-NK and TEKEIL is shared with RNA polymerase subunit of sheep Pox virus;
(2) LIÐNK and TEKEL is shared with Lumpy skin disease virus;
(3) NNK and EKEIL is shared with the Hemagglutinin influenza A virus;
(4) NNTEK-IL is shared with HIV-I envelop glycoprotein;
(5) DN---EKEI is shared with Bacillus anthracis; or
(6) LIDN-KT-KEI is shared with flagellar filament outer layer protein precursor (sheet protein) of Lyme disease spirochete.

Although these linearly ordered sequence homologies appear to be remote and partial, but due to the observed profound biological activities of the neurotrophic sequence-derived peptides (in in vitro and in vivo studies) and their relative hydrophilic nature, their presence in pathogenic agents (e.g., HIV virus, anthrax) might have some potential clinical application or might be useful in understanding the mechanism underlying their pathogenicity (with respect to eukaryotic cells).

Mutations

Note: Mutation in PSAP gene in human was reported for the first time in 1990 and so far there are 10 recorded mutations. Seven cases are identified with nucleotide substitutions in the form of missence or

nonsense mutation. Among this three patients with non-sense mutations that led to prosaposin deficiency, 3 cases developed metachromatic leukodystrophy (MLD) phenotype, and one case showed the atypical Gaucher disease. Two cases of PSAP mutation were in the form of nucleotide substitution (splicing type) and both showed clinical characteristics of MLD. In one patient, deletional mutation occurred in the saposin B domain (c.803delG) and led to premature stop codon and total prosaposin deficiency. Interestingly, mutant cDNA was detected in the heterozygous parents who were the careers for the same single base deletion (c.803delG) in exon 9.

Implicated in

Metachromatic Leukodystrophy (MLD), Gaucher Disease, Combined SAP deficiency

Disease

The clinical features in patients with total PSAP deficiency (combined SAP deficiency) are reported to be similar to those in Gaucher disease type 2, which present with acute infantile neuronopathic symptoms, abnormally large size of visceral organs, deteriorating general physical condition, and death in the first two years of life. Saposin A deficiency as a disease entity has not been reported. However, mice with mutation in saposin A demonstrated a phenotype similar to late-onset Krabbe disease. Patients with saposin B

deficiency show similar clinical finding to those with MLD. There are three main types of MLD; Late infantile MLD, Juvenile MLD, and adult MLD. Depending on the patient's age, their clinical signs and symptoms may vary. Saposin C deficient patients present with clinical findings similar to Gaucher disease type 3. Saposin D mutation in a mouse model has shown progressive polyuria and ataxia and accumulation of ceramide in the brain and kidney.

Accumulation of saposins (up to 80-fold) are detected in spleen, liver, and brain of individuals affected with lysosomal storage diseases (LSD) such as Gaucher disease, Niemann-Pick disease (type 1), fucosidosis, Tay-Sachs disease, and Sandhoff disease. Analysis of plasma levels of saposins in patients with LSD disorders has revealed an increase of 59%, 25%, 61%, and 57% above the 95 percentile of control population for saposin A, saposin B, saposin C, and saposin D, respectively.

Total prosaposin deficiency leads to a lethal phenotype in both man and mice. Mice with homozygous inactivation of prosaposin gene showed similar clinicopathologic pictures to the human patient with total PSAP deficiency. Among these features was intrauterine or early neonatal death in PSAP-/- mice. In other mice, severe developmental abnormalities in the nervous system and male reproductive system was detected. Neuroembryological developmental abnormalities presented as muscular weakness, trembling or shakiness of head, and ataxia of the limb and progressed to severe weakness and shaking of head

Table 1. PSAP overexpression in human tumor tissues

Tissue	Diagnosis	Differentiation	Fold increase	Age	Sex
Brain	Glioblastoma Multiforme	Poorly	2.4	33	F
Colon	Adenocarcinoma	Moderately	1.7	58	M
Lung	Squamous Cell Carcinoma	Poorly	2.2	47	F
Lung	Squamous Cell Carcinoma	Well	3.2	72	M
Lung	Squamous Cell Carcinoma	Moderately	5.0	46	F
Pancreas	Adenocarcinoma	Moderately	2.0	61	M
Pancreas	Adenocarcinoma	N/A	4.7	60	M
Rectum	Adenocarcinoma	Moderately	1.6	66	M
Ovary	Thecoma	N/A	3.1	22	F
Parotid	Adenocarcinoma	N/A	1.8	47	M
Skin	Melanoma	N/A	2.3	25	M
Bladder	Transitional Cell Carcinoma	Moderately	2.0	50	F
Small Intestine	Leiomyoma	N/A	2.1	57	M
Small Intestine	Non-Hodgkin's Lymphoma	N/A	1.6	45	M
Thymus	Malignant Thymoma	Moderately	1.7	79	F
Thymus	Thymoma (mixed)	N/A	1.5	45	M
Uterus	Endometrial Adenocarcinoma	N/A	2.3	49	F

Table 1: PSAP overexpression in Human tumor tissues (Koochekpour et al. unpublished observation).

and trunk and after 4 weeks they developed seizures and persistant tonic epilepsy and finally died at the age of 35 days. Evidence of lysosomal storage disease was detected by abnormal accumulation of ceramide in brain, liver, and kidney, and storage of gangliosides and ceramide and hypomyelination of the brain. Gross pathological features were also detected in the male reproductive organ including atrophy of prostate gland, testes, epididymis, seminal vesicle, and reduced spermatogenesis. Microscopic examination of the involuted prostate, seminal vesicles, and epididymis revealed the presence of rudimentary undifferentiated epithelial cells. In spite of these abnormal findings, the testosterone level was normal or even elevated.

Oncogenesis

Overall the expression and biofunctional significances of prosaposin and saposins in cancer are largely unknown. The observation of PSAP overexpression in squamous cell carcinoma of lung, melanoma of skin, ovarian carcinoma, transitional cell carcinoma of the bladder, leiomyoma or non-hodgkin's lymphoma of the small intestine, malignant thymoma, glioblastoma multiforme, and adenocarcinoma of the colon, pancreas, parotid, and endometrium is a strong indication of the potential involvement of PSAP at least in human carcinogenesis (Table 1). Most convincingly, available in vitro data indicate its potential significance and involvement in prostate carcinogenesis and its progression toward metastatic and/or androgen-independent status. Probably the most important finding was the genomic amplification and/or overexpression of PSAP in androgen-independent prostate cancer cell lines and punch biopsy samples of xenografts and lymph node metastases obtained from patients with hormone-refractory androgen-dependent or independent prostate cancer. Immunohistochemical staining has demonstrated the relative abundance of immunoreactive-PSAP in high Gleason grade tumors as compared to the low-grade tumors or benign prostatic hyperplasia. Although PSAP appears to function as a protooncogene in prostate cancer cells, direct experimental evidence is not available at this time.

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