Review

Molecular Cancer Research

STEAP Proteins: From Structure to Applications in Cancer Therapy

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Abstract

The human 6-transmembrane epithelial antigen of prostate (STEAP) family comprises STEAP1, STEAP2, STEAP3, and STEAP4. All of these proteins are unique to mammals and share an innate activity as metalloreductases, indicating their importance in metal metabolism. Overall, they participate in a wide range of biologic processes, such as molecular trafficking in the endocytic and exocytic pathways and control of cell proliferation and apoptosis. STEAP1 and STEAP2 are overexpressed in several types of human cancers, namely prostate, bladder, colon, pancreas, ovary, testis, breast, cervix, and Ewing sarcoma, but their clinical significance and role in cancer cells are not clear. Still, their localization in the cell membrane and differential expression in normal and cancer tissues make STEAP proteins potential candidates as biomarkers of several cancers, as well as potential targets for new immunotherapeutic strategies for disease attenuation or treatment. This review brings together the current knowledge about each STEAP protein, giving an overview of the roles of this family of proteins in human physiology and disease, and analyzes their potential as immunotherapeutic agents in cancer research. *Mol Cancer Res; 10(5); 573–87. ©2012 AACR.*

Introduction

The 6-transmembrane epithelial antigen of prostate (STEAP) family of proteins includes 4 members, named 6-transmembrane epithelial antigen of prostate 1 to 4 (STEAP1–STEAP4). They all have in common a 6-transmembrane domain, a COOH-terminal domain with significant homology to the yeast FRE family of b-type cytochrome metalloreductases, and an *N*-terminal with homology to the archaeal and bacterial $F_{420}H_2$:$NADP^+$ oxidoreductase (FNO)–binding proteins (Figs. 1–4; ref. 1). STEAP proteins uptake iron and copper because of 2 conserved histidine residues predicted to bind at least an intramembranar heme group (2, 3). The heme-binding 6-transmembrane domain is also present in the Nox and YedZ family, in which only 2 histidine residues are present and received the designation of apoptosis, cancer, and redox-associated transmembrane (ACRATA; ref. 4). In mammals, STEAP proteins contain an exclusive FNO-like domain, enabling them to use intracellular flavin adenine dinucleotide– or flavin mononucleotide–derivate flavins as electron donors for iron and copper reduction (1, 3). Other features common to most STEAP proteins are the presence of the YXXØ consensus sequence (in which Ø is a large hydrophobic amino acid) that is responsible for targeting transmembrane proteins to lysosomes and endosomes, and the Rossman fold (GXGXXG/A motif), a feature of proteins with oxidoreductase and dehydrogenase functions (3, 5). The first role attributed to this family of proteins was their contribution to metal homeostasis by reducing iron and copper, thereby allowing their uptake. The only exception is STEAP1, which does not reduce metals, possibly owing to the absence of the FNO-like domain and the Rossman fold. Nevertheless, the partial colocalization of STEAP1 with transferrin (Tf), transferrin receptor 1 (TfR1), and endosomes specialized in iron uptake suggest that STEAP1 may also have a role in iron metabolism (1). This review updates the available data for the STEAP proteins and discusses their role in pathophysiology, with a particular focus on carcinogenesis.

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Structural Features of STEAP Genes and Proteins

STEAP1 was the first member of the STEAP family to be identified. The STEAP1 gene (Table 1) is located on chromosome 7q21.13 and comprises 10.4 kb, encompassing 5 exons and 4 introns. Transcription of the STEAP1 gene gives rise to 2 different mRNA transcripts of 1.4 kb and 4.0 kb. However, only the 1.4-kb transcript is processed into the mature protein, which contains 339 amino acids with a predicted molecular weight of 36 kilodaltons (6, 7). The 4.0-kb transcript contains a large intron of 2,399 bp, and it is not translated into a mature protein. The protein contains 6-transmembrane domains with the COOH- and *N*-terminals located in the cytosol, and 3 extracellular and 2 intracellular loops (6).

The STEAP2 gene (Table 1), also known as 6-transmembrane protein of the prostate 1 (STAMP1), is located on

Table 1. Characterization of STEAP genes, mRNA transcripts, and proteins

STEAP gene	Chromosome location	Gene size (kb)	Exon/intron	mRNA transcripts (kb)	Amino acids	MW (kilodaltons)	Reference
STEAP 1	7q21.13	10.4	5/4	1.4 4.0	339	36	(6, 9)
STEAP 2	7q21.13	26	6/5	2.2 4 4.5 6.5 (7.5?)	490	56	(7, 8)
STEAP 3	2q14.2	42	6/5	4.3	488	50–55	(3, 9, 10)
STEAP 4	7q21.12	26	5/4	4	459	52	(9, 11)

Abbreviations: MW, molecular weight.

chromosome 7q21.13 and contains 6 exons and 5 introns, corresponding to a full-length cDNA that spans around 26 kb, owing to the large size of intron 2 (12,713 bp; refs. 7, 8). The transcription of STEAP2 seems to be TATA- or CAAT-box independent. Human tissues express 4 different mRNA transcripts, 3 of them resulting from alternative splicing of the last exon, with estimated sizes of 2.2, 4.0, 4.5, and 6.5 kb (7). However, there is some controversy about the size of the larger transcript, which has also been identified as having 7.5 kb (8). No significant rearrangements of the gene have been detected in LNCaP, PC3, and DU145 prostate cancer cell lines, but 4 sites of base variations were identified in prostate cancer cell lines and in normal prostate tissue. These cDNA variations originate from a neutral substitution from CTC to CTT with no amino acid change at codon 272 and 3 missense substitutions: TTT to TGT, leading to Phe17Cys; CGA to CAA, leading to Arg456Gln; and ATG to ATT, resulting in Met475Ile. The open reading frame of the STEAP2 gene is located within the third exon and gives rise to a 490–amino acid protein with a predicted molecular weight of 56 kilodaltons (7, 8).

STEAP3 (Table 1), also known as tumor-suppressor activated pathway-6 (TSAP6) or dudulin-2, is located on chromosome 2q14.2. Human multitissue analysis detected a single STEAP3 transcript of 4.3 kb, which originated from a 42-kb STEAP3 gene with 6 exons and 5 introns (3, 9). The promoter region of the STEAP3 gene contains functional p53-binding sites and interacts with Nix and myt1 proteins, an interaction that increases apoptosis and delays cell-cycle progression (10). STEAP3 is composed of 488 amino acids and 6-transmembrane domains at the COOH-terminal region and a cytoplasmic *N*-terminal oxireductase domain with free access to electrons transported by NAD(P)H, essential for iron and copper uptake (3, 10). The expected molecular weight of this protein is around 50 to 55 kilodaltons, with the detection of 2 isoforms in human epithelial cells from cervical carcinoma, HeLa-39, and HeLa-Tet cell lines, which overexpress STEAP3 (10).

STEAP4 (Table 1), also known as 6-transmembrane protein of prostate 2 (STAMP2), is located on chromosome 7q21, and contains 5 exons and 4 introns. Partly because of the large size of intron 1 (22,516 bp), the genomic sequence comprises around 26 kb. The gene is translated into a single mRNA transcript of 4.0 kb, with a 5′–untranslated region (UTR) of about 1.7 kb. The STEAP4 protein has 495 amino acids and 6-transmembrane regions near the COOH-terminal domain. In the *N*-terminal domain, 3 conserved motifs have been identified, corresponding to a dinucleotide-binding domain, a NADP oxireductase motif, and a motif similar to pyrroline 5-carboxylate reductase (11).

Tissue Expression and Cellular Localization

STEAP1 is overexpressed in several types of human cancer tissues and cell lines, namely prostate, bladder, colon, pancreas, ovary, testis, breast, cervix, and Ewing sarcoma (Tables 2–4; refs. 6, 12). Among normal tissues, the prostate gland is where STEAP1 expression is more abundant. Other nontumoral human tissues, such as ureter, fallopian tubes, uterus, pituitary, pancreas, stomach, colon, and breast show diffuse and low-intensity staining (6, 12). In the prostate, STEAP1 is primarily expressed in the plasma membrane of the epithelial cells, particularly at cell–cell junctions (6).

STEAP2 was identified as an upregulated gene in normal and malignant prostate cells using suppression subtraction hybridization and cDNA array hybridization (Table 3; ref. 8). Analysis of its expression in several human tissues showed that STEAP2 mRNA expression is more abundant in the prostate (Table 2; ref. 7). STEAP2 is mainly located in epithelial cells of the prostate, particularly in the plasma membrane and Golgi complex, in association with the *trans*-Golgi network (TGN) and early endosomes. Besides the prostate, its expression is also detectable in other normal human tissues, for example, heart, brain, pancreas, ovary, skeletal muscle, mammary gland, testis, uterus, kidney, lung, trachea, and liver (7, 8). In addition, in mouse embryos, STEAP2 has strong expression in the epithelium of the gastroduodenal junction, fetal liver, and in the choroid plexus (1).

STEAP3 is expressed in hematopoietic tissues (Tables 2 and 3), supporting important physiologic functions related to iron metabolism, especially in erythroid precursors. In addition, STEAP3 mRNA has been detected in the fetal liver of mouse embryos and in the bone marrow, placenta, liver,

Table 2. Expression of STEAP mRNA and proteins in normal and cancer tissues

	STEAP 1		STEAP 2		STEAP 3		STEAP 4		Reference
	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	
Normal tissue									
Brain	+	−	++		+		−		(1, 3, 6–8, 11, 14)
Pituitary gland		+							(6)
Fetal liver	++		+		++		+		(1, 3)
Liver	++	−	+		+++		+		(1, 3, 6, 8, 10, 11, 14)
Heart	+		+		+		++		(1, 3, 7, 8, 10, 11, 14)
Trachea			+						(8)
Lungs	+	−	+		+		++		(1, 3, 6, 8, 11, 14, 15)
Thymus	+	−	+				+		(1, 6)
Lymph node		−							(6)
Bone marrow	+	−	+		++		+++		(1, 3, 6, 64)
Thyroid gland		−							(6)
Adipose tissue							+++		(14, 41)
Pancreas	+	+	++		++		++		(1, 3, 6–8, 11, 14)
Spleen	−	−	−		−		−		(3, 6, 8, 14)
Adrenal gland		−							(6)
Stomach	+	+	+				+		(1, 6)
Duodenum	+		++		+		−		(1, 3)
Small intestine			+				+		(8, 11)
Colon	+	+	+		+		−		(1, 3, 6, 8, 11)
Kidney	++	++	+		+		+		(1, 3, 7, 8, 14, 78)
Bladder	+	++							(6, 15, 78)
Breast	++	++	+						(8, 12)
Placenta	+	−	−		++		+++		(1, 3, 6, 8, 10, 11, 14)
Ovary		−	++				−		(6–8, 11)
Fallopian tubes		+							(6)
Uterus		+	+						(6, 8)
Prostate	+++	+++	+++		+		++		(1, 3, 6–8, 11, 15)
Testis		−	+				+		(6, 8, 11, 14)
Ureter		+							(6)
Skeletal muscle	+	−	+		+		+		(1, 3, 6, 8, 10, 11, 14)
Skin		−							(6)
Cancer tissue									
Liver					++	++			(69, 70)
Lungs		++							(15)
Colon		+							(6)
Kidney		++							(78)
Bladder		++							(6)
Breast		++							(12)
Prostate		+++	++		++				(6, 27, 77)
Prostate lymph node metastasis		+++							(15)
Prostate bone metastasis		+++							(15)

NOTE: Low levels, +; medium levels, ++; high levels, +++; not detectable, −.

skeletal muscle, and pancreas of adult mice. A similar distribution of STEAP3 mRNA is seen throughout human tissues, with higher expression levels in the liver and much less expression in the skeletal muscle, fetal liver, pancreas, bone marrow, placenta, and heart (3, 10). The cellular localization of STEAP3 is the plasma membrane, near the nucleus, and in vesicular tubular structures (13).

The STEAP4 gene is mainly expressed in adipose tissue, placenta, bone marrow, lung, pancreas, and heart, followed by prostate, liver, skeletal muscle, pancreas, testis, small intestine, and thymus, with no detectable expression in brain, kidney, spleen, colon, and peripheral blood leukocytes (Tables 2 and 3; refs. 1, 11, 14). The intracellular localization of STEAP4 resembles that of STEAP2; it is found in the

Table 3. Expression of STEAP mRNA and proteins in cell lines

		STEAP 1		STEAP 2		STEAP 3		STEAP 4		
Cell line		**mRNA**	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**	**Reference**
Hepatocellular carcinoma	HepG2							+		(11)
	TTC 1105	−								(75)
Lungs	NCI-H661							−		(11)
Melanoma	Mel 526	+								(75)
	Mel 624	+								
	Mel 888		+							(78)
Myeloid Leukemia	KCL-22	+								(6)
	LTR 6						+			(10)
Lymphoma	Jurkat		−							(78)
	BxPC-3	+	−							(6)
Pancreas	HPAC	++	−							
	Capan-1	++	−							
	Colo 205	++	+							(6)
Colon	CaCo-2	+	+							
	LoVo	++	+							
	T84	++								
	Caki-1		+							(78)
Kidney	SW839		+							
	ACHN		+							
	SMKTP3		−							
	UM-UC-3	++	++							(6, 78)
	5637	+++	++							
	EJ-1		+							(64)
Bladder	T24		+							
	TCCSUP	+								(6)
	HT1197	−								
	SCABER	+								
	J82	−								
	MCF 7[a]	+						−		(6, 11, 81)
	MCF 7-LCC1							−		(11)
	MCF 7-LCC2							−		
Breast	MDA-MB-435[a]	+						−		(6, 11, 82)
	CAMA-1[a]	−								(6, 83)
	DU4475	−								(6)
	SKBr3[a]		−							(70, 85)
	OV-1063	++	++							(6)
Ovary	PA-1	+	+							
	SW 626	++	+							(6, 75)
	CAOV-3	+								(6)
Cervix	HeLa	+	−					−		(6, 11)
	A431	+								(6)
	LNCaP[a]	+++	+++	+++		+		+		(6–8, 11, 77, 84)
	PC3[b]	+	+	+		+		−		
	DU145[b]	+	+	+		−		−		
	CA-HPV10[b]							−		(11, 39, 85)
Prostate	PZ-HPV7[b]							−		
	YPEN-1							−		(11)
	22Rv1[a]			+						(8, 86)

(Continued on the following page)

Table 3. Expression of STEAP mRNA and proteins in cell lines (Cont'd)

		STEAP 1		STEAP 2		STEAP 3		STEAP 4		
Cell line		**mRNA**	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**	**Reference**
	NCI-H660[b]			+						(8, 87)
	NTERA-2	+								(6)
Testis	NCCIT	−								
	TERA-1	+								
	TERA-2	+								
Ewing sarcoma	RD-ES	++	+							(6)
	A 673	++								(75)
	TC 32	++								
Myotube	C2							−		(11)

NOTE: Low levels, +; medium levels, ++; high levels, +++; not detectable, −.
[a]Androgen receptor positive.
[b]Androgen receptor negative.

plasma membrane, near the nuclear region where it colocalizes with the Golgi complex and the TGN and is dispersed in the cytoplasm as bright spots with the appearance of vesicles or tubular-shaped structures associated with vesicular tubular structures, or with reticular shapes associated with the endoplasmic reticulum (11).

Physiologic Roles, Regulation, and Implications in Cancer

STEAP1

Because of its localization on the cell membrane and its predicted secondary structure as a 6-transmembrane protein, it is believed that STEAP1 acts as an ion channel or transporter protein in tight junctions, gap junctions, or in cell adhesion, taking part in intercellular communication. As STEAP1 is overexpressed in cancer, it has been suggested that STEAP1 may facilitate cancer cell proliferation and invasion, perhaps through modulation of concentration of ions such as Na^+, K^+, and Ca^{2+} and small molecules (15–17). Higher levels of voltage-gated Na^+ channels confer a highly invasive phenotype to prostate cancer cells and the presence of these channels seems to be linked with the loss of androgen receptor expression and function and the progression to androgen-independent cell stages (17–20). In addition, modulation of Ca^{2+} and K^+ levels seems to be very important for the progression of prostate tumors toward androgen-insensitive stages, by conferring an apoptotic-resistant cellular phenotype (21–24). Therefore, the relationship between STEAP1 and ion channels should be addressed in the future. STEAP1 also plays an important role in intercellular communication. Blocking STEAP1 with specific monoclonal antibodies in LNCaP cells increases cell death, suggesting that STEAP1 may promote proliferation of cancer cells or prevent apoptosis (15). The pathways underlying these effects are still unknown and need to be studied. On the other hand, STEAP1 seems to facilitate cell growth by raising the intracellular level of reactive oxygen species (ROS), showing that STEAP1 acts both on inter- and intracellular pathways (Fig. 1; ref. 25). 17β-Estradiol seems to be the only known regulator of STEAP1 expression to date. This hormone downregulates STEAP1 expression both *in vivo* in rat mammary glands and *in vitro* in MCF-7 breast cancer cells (12).

STEAP2

STEAP2 works as a shuttle between the Golgi complex and the plasma membrane, moving in both directions, in the endocytic and exocytic pathways, suggesting that it may act as a receptor for endogenous and exogenous ligands, such as lipids and proteins, or as a regulator of protein delivery and sorting mechanisms (7). Taking into account its colocalization with Tf and TfR1, STEAP2 may have a role in the endosomal Tf cycle of erythroid cells, contributing to iron and copper uptake by reducing Fe^{3+} to Fe^{2+} and Cu^{2+} to Cu^+ (Fig. 2). STEAP2 may also regulate iron and copper availability in the choroid plexus, the site of cerebrospinal fluid synthesis and traffic control of molecules and ions between the blood and the cerebrospinal fluid, and in the gastrointestinal tract, through absorption of iron and copper by enterocytes of the proximal duodenum (1, 26).

As prostate cancer progression is androgen dependent and STEAP2 is overexpressed in prostate tissue, the involvement of androgens in the regulation of STEAP2 expression has been investigated. Both *in vivo* and *in vitro* studies suggest that STEAP2 is not an androgen-regulated gene. Androgen-dependent CWR22 tumors (derived from primary human prostate cancer) grown in mice significantly regressed following castration, without any alterations in STEAP2 mRNA expression (7). In addition, LNCaP cells cultured with dihydrotestosterone (DHT) or with a synthetic androgen R1881 did not show any significant differences in STEAP2 expression when compared with nontreated cells (7, 8). Although these results were in agreement with animal experiments, the fact that STEAP2 expression has only been assessed at a single time point of R1881 treatment and only

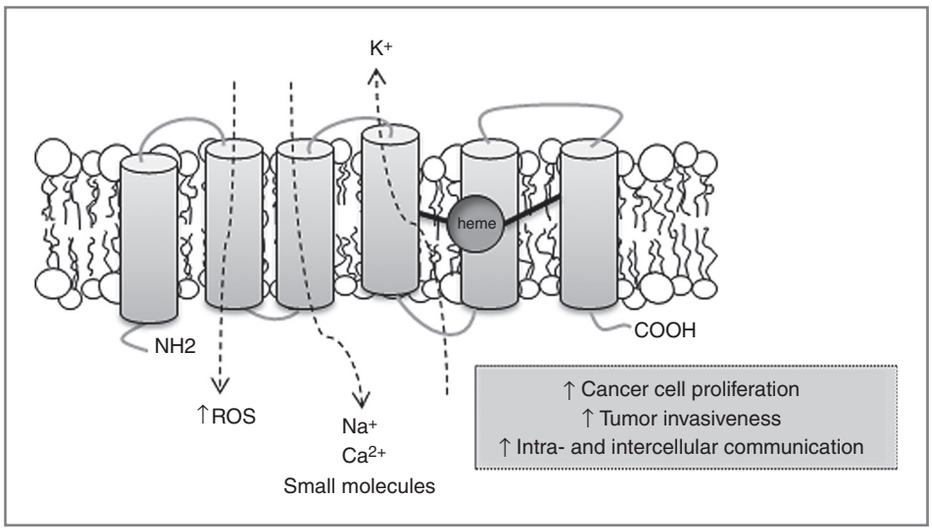

Figure 1. Schematic of STEAP1 protein structure, cellular localization, and physiologic functions. Similar in structure, presenting a 6-transmembrane structure, intracellular COOH- and *N*-terminal, and intramembrane heme group, STEAP1 lacks the innate metalloreductase activity conferred by the presence of the FNO-like domain. STEAP1 actively increases intra- and intercellular communication through the modulation of Na^+, Ca^{2+}, and K^+ concentration, as well as the concentration of small molecules. It stimulates cancer cell proliferation and tumor invasiveness.

using a single concentration of this androgen posed several limitations to the study above. Analyzing STEAP2 expression in response to several androgen concentrations at different time points would give a more comprehensive view of its putative regulation of STEAP2. Furthermore, it would also be of interest to analyze STEAP2 expression at the protein level, as androgens may regulate translation itself. Therefore, until further studies are carried out, it remains unclear if STEAP2 is indeed an androgen-independent gene, especially because STEAP2 expression seems to be responsive to the presence of an active androgen receptor, and this receptor seems to act on the regulation of STEAP2

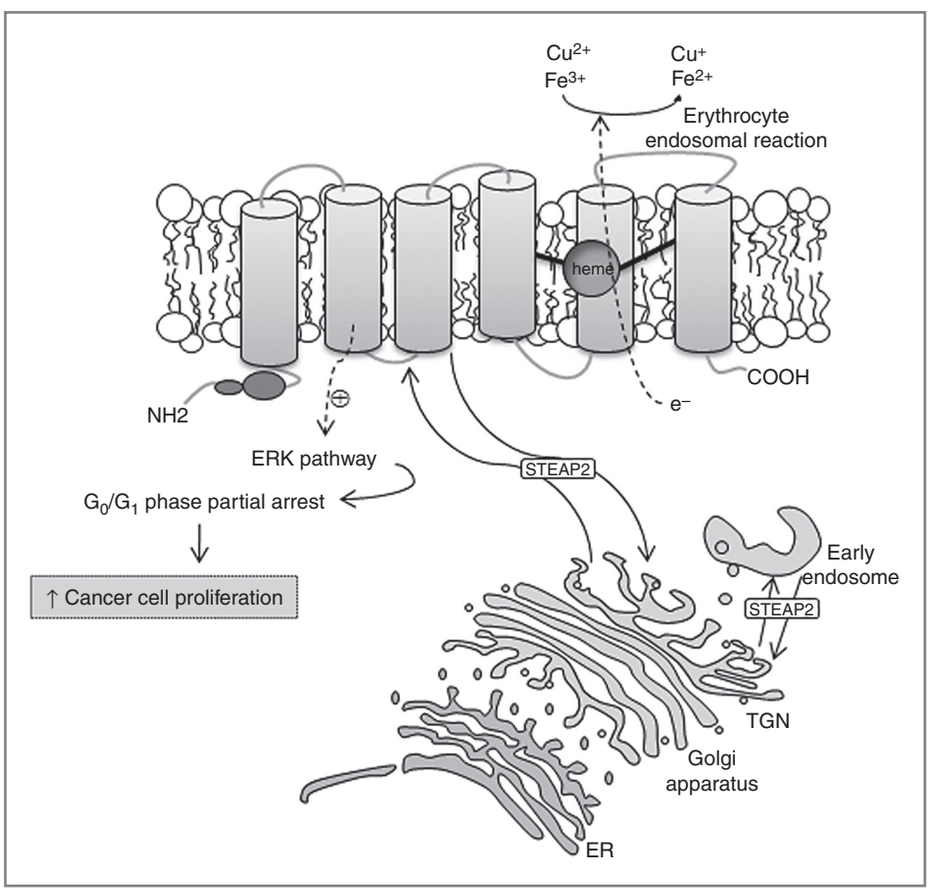

Figure 2. Schematic of STEAP2 protein structure, cellular localization, and physiologic functions. Because of its metalloreductase activity, STEAP2 contributes to proper erythrocyte Fe^{3+} and Cu^{2+} uptake and their reduction to Fe^{2+} and Cu^+, facilitating the progression of the Tf cycle. By activating the ERK pathway, STEAP2 induces partial arrest on the G_0–G_1–cell-cycle phase in cancer cells, thereby increasing cell proliferation and tumor development. STEAP2 can be found in association with early endosomes and the TGN, suggesting that it acts as a receptor for both exogenous and endogenous ligands or as a regulator of protein delivery and sorting mechanisms.

Table 4. Regulation of STEAP proteins and involvement in human diseases

STEAP protein	Regulation	Involvement in disease	Reference
STEAP 1	Androgens (?)	Progression of several cancer types	(6, 12, 15–17, 25)
	17β-E_2	Regulation of the MSC differentiation process (?)	
STEAP 2	Androgens (?)	Prostate cancer progression	(1, 7, 8, 26, 27)
STEAP 3	p53-activated protein	Hepatocellular carcinoma progression (?)	(3, 10, 13, 40)
		Involvement in several types of anemia (?)	
STEAP 4	Androgens	Prostate cancer development and progression	(11, 14, 41–43, 45)
	TNF-α	Obesity-related insulin resistance	
	IL-6	Inflammatory processes	
	Leptin		
	IL-1β		

expression along the progression of prostate cancer. These observations were made in PC3 and DU145 cells, and in 4 relapse derivatives of CWR22 tumors. In PC3 and DU145 cells, STEAP2 expression was not detected, and in CRW22R tumors, the expression of STEAP2 was significant, although lower than in LNCaP cells. Both PC3 and DU145 cell lines represent androgen-independent advanced prostate cancer and do not express androgen receptor, whereas CWR22R tumors do, possibly explaining the differences in STEAP2 expression. Furthermore, as no major genomic rearrangements, mutations, or promoter methylation have been found, loss of STEAP2 expression may be due to a deregulatory mechanism occurring during cancer progression (Table 4; refs. 7, 8).

In vitro and *in vivo* studies show that STEAP2 increases prostate cancer cell proliferation, regulating several genes involved in the cell cycle, causing a partial cell-cycle arrest at the G_0–G_1 phase.

This proliferative activity of STEAP2 seems to be coordinated through the activation of the extracellular signal-regulated kinase (ERK) pathway. Combined with its proliferative features, STEAP2 also acts as a prosurvival factor, as its knockdown increases the number of apoptotic events in prostate cancer cells (Fig. 2; ref. 27). However, the pathways by which STEAP2 inhibits apoptosis are not known and should be explored in the future (Table 4).

STEAP3

STEAP3 was first identified in studies searching for the gene responsible for the hypochromic microcytic anemia in the *nm1054* mouse mutant (Table 4; refs. 3, 5, 28). This autosomal recessive trait that results from a deletion in both STEAP3 alleles is characterized by an inefficient supply of iron to erythrocytes, leading to impairment of hemoglobin synthesis (28). In fact, the anemic phenotype is completely reversed when STEAP3 expression is restored (3).

The role of STEAP3 goes beyond iron metabolism in erythroid precursors (Table 4). It is upregulated upon p53 activation in the LTR6 myeloid leukemia cell line and in MCF7 breast cancer cells, increasing cell death. In addition, 2 other proteins involved in apoptosis interact with STEAP3 both *in vitro* and *in vivo*: the Nix protein, a mitochondrial proapoptotic protein associated with apoptotic cardiomyopathy, terminal erythroid differentiation, reticulocyte maturation, and Parkinson disease; and Myt1 kinase, a regulator of cyclin-dependent kinase activity. Therefore, STEAP3 may be involved in apoptosis and in cell-cycle progression, especially in G_2–M progression (10, 29–33). Nix seems to intensify the apoptotic effect of STEAP3 alone, whereas the interaction between STEAP3 and Myt1 implies modulation of the Myt1 phosphorylation state. When overexpressed, STEAP3 keeps Myt1 dephosphorylated and functional, possibly by recruiting specific phosphatases, by protecting the phosphorylation sites, or by maintaining $p34^{cdc2}$ phosphorylated. Therefore, STEAP3 could be viewed as a positive regulator of Myt1, and together, STEAP3 and Myt1 cause a pronounced effect on the cell cycle, delaying the G_2–M progression (Fig. 3; ref. 10).

STEAP3 also interacts with the translationally controlled tumor protein (TCTP), a Ca^{2+}- and microtubule-binding protein implicated in cell-cycle progression and malignant transformation (Fig. 3; refs. 34, 35). TCTP participates in the inflammatory response triggered by parasites, such as *Plasmodium falciparum* and *Schistosoma mansoni* and has antiapoptotic activity (36–38). TCTP is a nonclassical secreted protein, which is exported to the extracellular milieu independently of the endoplasmic reticulum–Golgi complex pathway. Like TCTP, STEAP3 is also present near the cell nucleus and the plasma membrane, suggesting that in certain circumstances they have simultaneous localization and distribution. In fact, secretion of TCTP is mediated by STEAP3, at least in epithelial and hematopoietic cell lines (293T, HepG2, and K562 cell lines). Moreover, TCTP is also present in exosome preparations derived from the 293T cell line, in which under the influence of STEAP3 overexpression, both endogenous and exogenous TCTP levels are raised (13). The expression of MHC-I was also increased by the overexpression of STEAP3 (13). Exosomes derived from dendritic cells express high levels of MHC-I, MHC-II, and cytosolic proteins likely involved in the function and biogenesis of exosomes (hsc73, annexin II, and Gi2). In addition, they express membrane proteins associated with cell targeting (MFG-E8, Mac-1, and CD9) and T-cell activation (B7.2; ref. 39). Other exosomes derived from

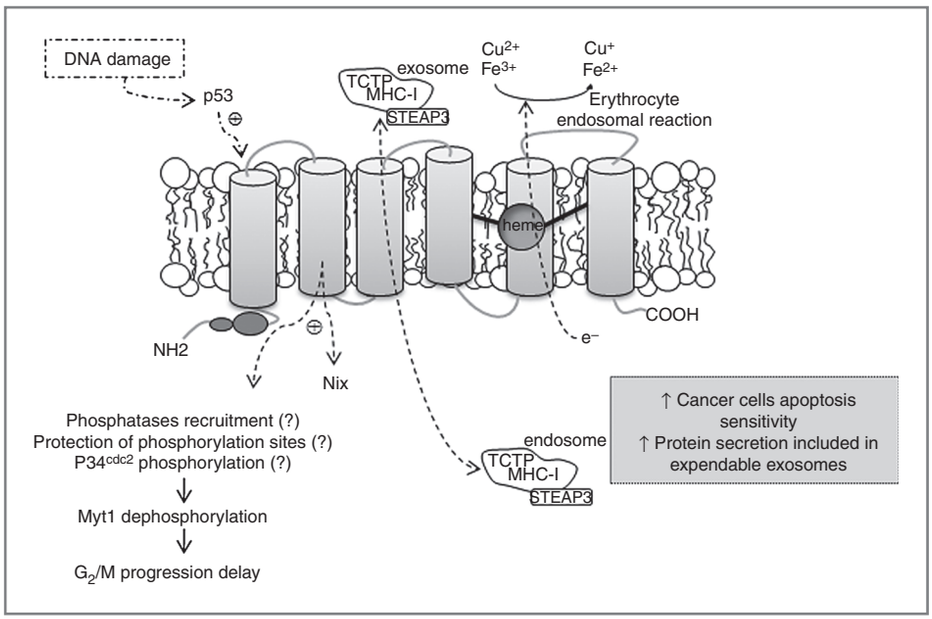

Figure 3. Schematic of STEAP3 protein structure, cellular localization, and physiologic functions. Taking part in the erythrocyte Tf cycle with the reduction and uptake of iron and copper, STEAP3 expression in the endosomal membrane grants an efficient hemoglobin synthesis. Upon p53 activation, upregulation of STEAP3 and its interaction with Nix and Myt1 intensifies cell death events. STEAP3 activates Myt1 through dephosphorylation, by recruiting phosphatases, protecting Myt1 phosphorylation sites, or inducing $p34^{cdc2}$ phosphorylation, thereby preventing cells to proceed adequately into G_2–M phase and leading to apoptosis. STEAP3 colocalizes with TCTP leading to MHC-I and its own overexpression. It takes part in vesicular trafficking and secretion in a way independent of the Golgi complex.

bone marrow erythroblasts and peripheral blood reticulocytes are important for these cells to mature, a process delayed in STEAP3 knockout mice. The induction of DNA damage and the consequent p53 activation in mouse embryo fibroblasts and in adult mice splenocytes stimulated the production of exosomes in a STEAP3-dependent manner. Accordingly, STEAP3 is thought to be the mediator of protein secretion in exosomes that are no longer needed for maturation and survival of the cells. The STEAP3 induction of TCTP secretion could also be seen as a strategy for making cancer cells more sensitive to apoptosis (40). Overall, STEAP3 could have broad functions as a regulator of vesicular trafficking and secretion and in intercellular communication (Table 4).

STEAP4

The localization of STEAP4 also suggests involvement in molecular trafficking (Table 4). STEAP4 moves packaged vesicular tubular structures from the cytoplasm to the periphery of the cell, and its colocalization with the early endosome protein EEA1, in the cell periphery and close to the nucleus, suggests that STEAP4 is either involved in the secretory pathway or in the endocytic pathway (Fig. 4). The link between STEAP4 and the endoplasmic reticulum is not well understood, but STEAP4 may reach the endoplasmic reticulum in an unfolded state and then acquire its active biologic conformation; and/or STEAP4 may actually have a functional role inside the endoplasmic reticulum (11).

STEAP4 has also been associated with obesity, insulin resistance, inflammation, and prostate cancer progression (11, 41–45). The STEAP4 mouse homolog, TNF-α–induced adipose-related protein (TIARP), was first identified as a plasma membrane protein overexpressed in both white and brown adipose tissues, with increased expression during preadipocyte differentiation and strongly induced by TNF-α, interleukin-6 (IL-6), growth hormone, and interleukin-1β (IL-1β). It is clear that TIARP is an important modulator of inflammation and nutrition, implicated in systemic metabolic homeostasis and obesity-related insulin resistance. However, the role of STEAP4 in humans is still uncertain (Fig. 4; refs. 46–51). Unlike TIARP, STEAP4 does not regulate the differentiation of preadipocytes *in vitro* (42). In adipocytes, STEAP4 is present in the plasma membrane and its expression is downregulated in patients who are obese (44). STEAP4 also has an important role in cell viability, proliferation, and apoptosis. The treatment of preadipocytes with an antibody against STEAP4 promotes the appearance of apoptotic cellular morphology, with mitochondrial damage causing swelling and pyknosis (44). STEAP4-induced apoptosis pathways seem to be mediated by caspase-3 and caspase-8. On the other hand, STEAP4 enhances cell proliferation, possibly acting as a signaling molecule. Preadipocyte treatment with anti-STEAP4 antibody decreased the proliferation rate and the number of cells that proceed to the S phase of the cell cycle (44). In primary cultures of adipose tissue, treatment with TNF-α increases its expression in a dose-dependent manner, disclosing a role for STEAP4 in inflammation processes in the adipose tissue. Nevertheless, the effects of TNF-α *in vitro* may not be quite the same as *in vivo*, because patients with higher TNF-α serum levels have lower levels of STEAP4 than controls. TNF-α, along with IL-6, is also responsible for increased STEAP4 expression during adipocyte differentiation and in mature adipocytes. Although the effects are time dependent, long-term exposure of mature adipocytes to IL-6 tends to decrease STEAP4 expression. Leptin, an adipocyte-secreted hormone linked to obesity-related insulin resistance, also regulates STEAP4 expression in mature adipocytes, decreasing both mRNA and protein levels (14, 41, 42). Furthermore, STEAP4 expression is also

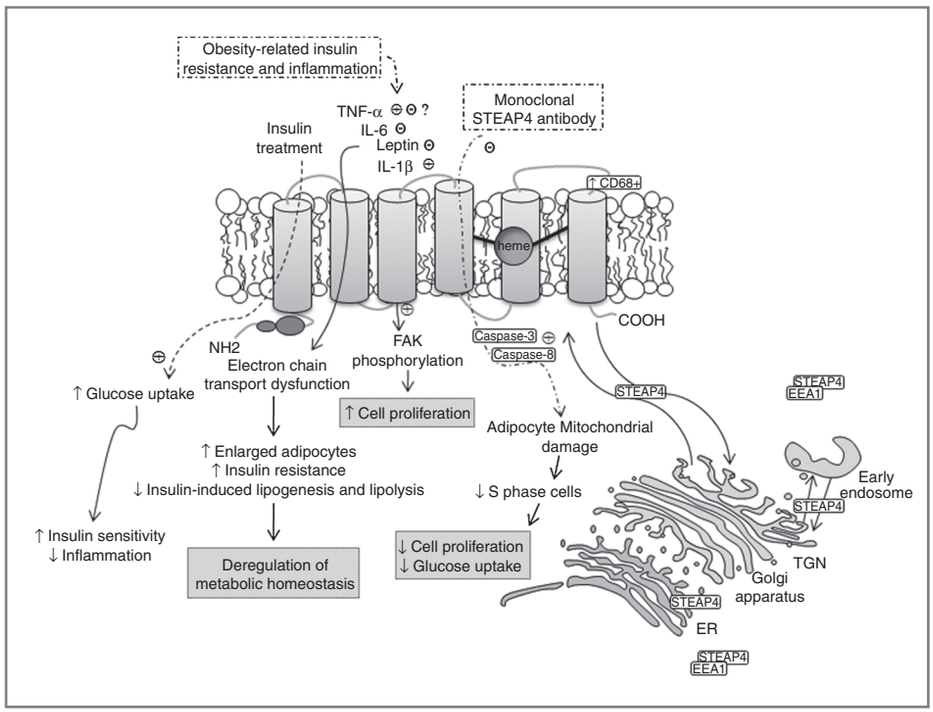

Figure 4. Schematic of STEAP4 protein structure, cellular localization, and physiologic functions. STEAP4 shuttles between the nucleus and the plasma membrane and colocalizes with EEA1, endoplasmic reticulum (ER), TGN, and early endosomes, suggesting its involvement in the endocytic and exocytic pathways. It mediates cell proliferation, apoptosis, glucose uptake, and inflammation in obesity-related insulin resistance cases. Proliferation is induced upon FAK activation. STEAP4 blockage triggers caspase-3 and caspase-8 and causes mitochondrial damage in adipocytes, decreasing cell proliferation and glucose uptake. IL-6 and leptin (repressors) and TNF-α and IL-1β (inductors) regulate STEAP4 actions. The macrophage marker $CD68^+$ colocalizes with the overexpressed STEAP4 in the plasma membrane in patients with rheumatoid arthritis.

induced by another proinflammatory cytokine, IL-1β. In human mesenchymal stem cell (MSC)–derived adipocytes, IL-1β treatment significantly increased STEAP4 mRNA and protein levels in a time-dependent manner. Overall, the effects of proinflammatory cytokines on STEAP4 could potentially indicate that STEAP4 has a protective role, restraining inflammation and insulin resistance (42). Correspondingly, STEAP4 induces glucose uptake after insulin treatment in human mature adipocytes, indicating that STEAP4 augments insulin sensitivity. The same happens in preadipocyte cultures, in which lower glucose uptake upon treatment with anti-STEAP4 antibody and insulin stimulation can be observed (42, 44). One hypothesis for the effects of STEAP4 in adipose tissue is that, similar to other members of its family, STEAP4 has an *N*-terminal domain with NADP-oxireductase activity that allows cellular uptake of iron and copper. Both are essential for glucose and lipid metabolism and could be associated with electron transport in mitochondria and regulation of ROS-related pathologic pathways, which are major players in obesity and obesity-related insulin resistance (52–54). Alteration of STEAP4 expression could eventually lead to dysfunction of these 2 processes and, consequently, disruption of metabolic homeostasis (1, 11, 14, 41, 44, 52, 55). Likewise, inflammation occurring in obesity and rheumatoid arthritis has TNF-α as a critical player (Fig. 4; refs. 56–58). The colocalization of STEAP4 with $CD68^+$ in the joints of patients with rheumatoid arthritis and its overexpression in the synovia of patients with rheumatoid arthritis and osteoarthritis indicate the role of STEAP4 in these diseases (43). Still, further studies are required to support the involvement of STEAP4 in the inflammatory process in the joints of patients with rheumatoid arthritis. In addition, STEAP4 may have a physiologic function in other organs such as placenta, lung, and heart, where its expression is more abundant (Table 4).

The mouse counterpart of STEAP4 has been associated with the appearance of a metabolic syndrome (MetS) phenotype in the TIARP knockout mouse, but the role of STEAP4 in MetS in humans is not clear (51). Two inconclusive epidemiology studies were conducted with the aim of establishing a correlation between STEAP4 gene polymorphisms and MetS associated with insulin resistance (59, 60). Of the several single-nucleotide polymorphisms (SNP) identified in regions that included introns, exons, and UTRs of the STEAP4 gene, only 3 were chosen for genotyping: 224 A/G (rs1981529, Gly75Asp) at exon 2; 364 G/A (rs34741656, Ala122Thr) at exon 2; and 7414 G/A (rs8122) at 3′-UTR. The polymorphism at exon 2 was previously identified as being a missense mutation. Although the first 2 had been initially described by Miot and colleagues (59) as having no effect on the prevalence or incidence of MetS, it was seen later that the rs8122 and rs1981529 SNPs had significant levels of association with MetS, in females but not in males (59, 60). Also, 2 common STEAP4 gene haplotypes identified as H1 (rs8122-rs1981529-rs34741656, G-A-G) and H2 (rs8122-rs1981529-rs34741656, A-G-G) were significantly associated with MetS phenotype in females. The reason why these associations were only found in the female population is still unknown (60). To continue exploring the effect of STEAP4 in MetS, $CD14^+$ monocytes were analyzed in the peripheral blood of 97 unrelated Chinese subjects (48 with MetS and 49 controls) to determine the relation between

cardiovascular alterations of patients with MetS and STEAP4 expression. The results showed that mRNA STEAP4 levels were lower in patients with MetS, and more significantly in women. Downregulation of STEAP4 was then positively correlated with high-density lipoprotein and negatively with body mass index, low-density lipoprotein, insulin, and fasting blood glucose, thereby suggesting that diminished STEAP4 levels may play a role in obesity, dyslipidemia, and hyperglycemia, all risk factors for MetS development. Furthermore, STEAP4 downregulation was also associated with 2 cardiac malfunctions that are repercussions of MetS; carotid atherosclerosis and left ventricular diastolic function (61).

As STEAP4 is expressed in the prostate, the role of androgens as possible regulators of its expression has been investigated (Table 4). Exposing LNCaP cells to DHT, testosterone, and to the synthetic androgen R1881 for different time periods increased the expression of STEAP4 mRNA in dose- and time-dependent manners, indicating that STEAP4 is an androgen-regulated gene (11). Further analysis of several other cell lines showed that STEAP4 mRNA expression is absent in prostate cell lines that do not express androgen receptor (PC3, DU145, CA-HPV10, PZ-HPV7, and YPEN-1), breast cancer cell lines (MCF7, MCF7-LCC1, MCF7-LCC2, and MB435), the hepatocellular carcinoma cell line HepG2, C2 myotube cell line, and HeLa cervical carcinoma cell line. As STEAP4 mRNA has only been found in androgen-dependent and androgen receptor–expressing LNCaP cells and not in the androgen receptor–negative prostate cancer cell lines, an active androgen receptor seems to be required. In addition, there seems to be a possible involvement of STEAP4 in prostate carcinogenesis due to the higher abundance of STEAP4 in prostate cancer samples compared with normal tissue (11). However, the pathway by which the active androgen receptor regulates STEAP4 expression needs to be elucidated.

The differential expression of STEAP4 in androgen receptor–positive and androgen receptor–negative prostate cancer cell lines, possibly associated with progression to a more aggressive phenotype, was attributed to an epigenetic mechanism (45). In LNCaP and DU145 cell lines, CpG islands were detected next to the STEAP4 gene promotor region, in the 5′-upstream sequence and part of the first exon, but were only methylated in the DU145 cell line, especially at the 5′-upstream sequence. Methylation reversal allowed an increase in both mRNA and protein expression in DU145 cells. Therefore, it seems that methylation of the STEAP4 promotor region is associated with the absence of STEAP4 expression in prostate cancer cells. Nevertheless, CpG methylations may not be the only epigenetic alterations occurring in the STEAP4 gene, and, therefore, histone modifications and interaction with transcription factors should also be evaluated, as well as its significance in prostate cancer development (45). Following the observed differential expression of STEAP4 between androgen receptor–positive and androgen receptor–negative prostate cancer cell lines, the potential role of STEAP4 in cancer progression was examined (11). In a colony formation assay using adherent cell-culture conditions, overexpression of STEAP4 in transfected PC3, DU145, and COS-1 originated larger and higher number of colonies compared with controls transfected with the empty vector (11). In contrast, in soft agar cultures, the number of colonies formed by 239T cells transfected with STEAP4 decreased in comparison to controls, and inhibition was reversed by adding a monoclonal antibody against STEAP4, indicating an inhibitory effect of STEAP4 in the proliferation rate of anchorage-independent cell cultures. The ability of STEAP4 to inhibit anchorage-independent cell proliferation was linked to the phosphorylation of focal adhesion kinase (FAK), a molecule implicated in carcinogenesis (Fig. 4; refs. 45, 62). STEAP4 interacts with FAK in immunoprecipitation assays, and 239T cells overexpressing STEAP4 show reduced FAK phosphorylation compared with mocked transfected cells, which in turn had time-dependent increased phosphorylation of FAK. Therefore, it is plausible to attribute the inhibition of cell growth in suspension to an inadequate activation of FAK by STEAP4. Concurring with the proliferative effect of STEAP4 in adherent cell cultures is the fact that these cells showed higher levels of FAK phosphorylation. However, the mechanism by which STEAP4 regulates FAK phosphorylation remains unclear. STEAP4 itself is predicted to have several putative phosphorylation sites that could be associated with its functions and signaling pathways in cancer cells (45).

STEAP Proteins as Biomarkers of Disease

Initial studies on STEAP1 expression during the development of prostate cancer did not find any significant alterations along the different cancer stages (6). However, a later analysis of prostate cancer cases, benign prostate hyperplasia, and nonprostatic malignancies disclosed a negative correlation between STEAP1 expression and histologic grading of prostate cancer cells (63). In addition, STEAP1 protein expression is higher in primary colon and bladder cancer when compared with colon and bladder cell lines derived from metastatic cancers (6). So, more studies are required to clarify the association of STEAP1 expression with histologic grading in different cancer types, to understand the clinical significance of STEAP1 and its importance in cancer progression, particularly in prostate cancer initiation, development, and metastasis. Recently, STEAP1 mRNA has been identified by real-time PCR in serum of patients with cancer (64). This highly sensitive and specific method allowed the distinction between 50 patients bearing pancreatic, bladder, breast, prostate, colon-rectal, lung, or stomach tumors and healthy subjects. External factors like age, histologic type, and clinical stage of cancer and therapy were evaluated, but none of them had a direct influence on STEAP mRNA levels. Therefore, the idea that STEAP1 may be a useful marker for several types of cancer, as well as its potential for cancer diagnosis, was reinforced (64–66).

Although STEAP1 and STEAP2 have been almost exclusively associated with prostate cancer, both are also

differentially expressed in murine and human MSCs, and in human bone marrow cells (67). As MSCs can differentiate into several types of cells that compose bone marrow and, due to the cell-surface localization of STEAP1 and STEAP2, these epithelial antigens could also be useful markers for isolation and purification of MSCs, distinguishing between normal and abnormal populations of bone marrow cells. In addition, its possible involvement in the regulation of the differentiation process of the MSCs should be further investigated (68).

Expression of STEAP2 also differs between normal and prostate cancer tissue. *In situ* hybridization analysis of 18 normal and 25 tumoral prostate sections showed that STEAP2 expression is about 2.5-fold higher in prostate cancer cells than in normal cells, suggesting that STEAP2 could be implicated in prostate cancer progression (7). Moreover, the immunohistochemical analysis of 17 benign and 67 cancer specimens of prostate tissue was in agreement with mRNA expression data (27). Despite the higher levels of STEAP2 mRNA and protein in prostate cancer, the only study that attempted to establish an association of STEAP2 expression with the Gleason score of prostate tumors was unsuccessful (27). However, this question should be further analyzed as the expression of ERK, 1 of the proteins that may mediate the proliferative and antiapoptotic functions of STEAP2, augments from normal to benign prostate hyperplasia and prostate cancer stages (27). If ERK expression is associated with advanced stages of prostate cancer and mediates STEAP2 functions, is STEAP2 expression really independent of the tumor grade? Nonetheless, STEAP2 may be a good and useful marker for the detection of prostate cancer progression.

STEAP3 could be used as a marker of the transition from cirrhosis to hepatocellular carcinoma (HCC; refs. 69, 70). Of the different causes that lead to hepatocellular carcinoma, hepatitis B and C virus, chronic alcohol abuse, and cirrhosis are the most common (71). It is known that HCC is associated with inhibition of transcription of a wide range of genes. These events in the adult liver also occur in the late fetal liver development, implying that some genetic changes are common to these conditions. Another common feature of these 2 liver stages is that active proliferation becomes more prominent than apoptosis, a consequence of the downregulation of proapoptotic genes. STEAP3 is one of these genes, and its expression is remarkably diminished in HCC nodules compared with cirrhotic peritumoral tissues. STEAP3 mRNA levels in peritumoral cirrhosis are significantly lower than in healthy liver (70). Its expression is dependent on tumor differentiation stage, with lower levels of protein associated with moderately or poorly differentiated tumors. In HCC-free cirrhosis, STEAP3 protein expression levels are notably increased when compared with healthy liver. Overall, STEAP3 expression is associated with the liver health status, that is, if the tissue is healthy or cirrhotic, if HCC is present or not. In the case of HCC-free cirrhosis, STEAP3 expression also differs according to the stage of differentiation. Therefore, analysis of STEAP3 expression levels could help identify the transition from HCC-free cirrhosis into a cancer-developing liver. Therefore, the use of STEAP3 as a new marker for hepatic carcinogenesis should be taken into account (69, 70). Furthermore, as STEAP3 is highly expressed in fetal liver and has an important role in hemoglobin production in erythroid precursors, it could be of interest to investigate whether STEAP3 could be considered as a marker of viability for fetal development and for other types of anemia besides the recessively inherited hypochromic microcytic anemia (3).

No studies have been conducted with STEAP4 to establish its usefulness as a biomarker of disease.

Its involvement in prostate cancer progression and clinical significance should be further clarified, especially in the androgen-dependent phase of tumor development. The possibility that STEAP4 could be a new prostate cancer biomarker of great importance for early detection of disease is reasonable due to its androgen-regulation dependence. The role of STEAP4 as a biomarker of inflammation in a variety of tissues, in obesity, and other metabolic and cardiac disorders should also be of interest, as well as understanding the clinical significance of STEAP4 overexpression in placenta and lung.

STEAP Proteins as Immunotherapeutic Targets

STEAP1 has been considered to be a good target for T-cell–based immunotherapy, with applications in prostate, colon, pancreas, bladder, Ewing sarcoma, breast, testicular, ovarian, and melanoma cancers, as it has the required features of tumor-associated antigens (TAA), specifically, cell-surface localization, high expression levels in several types of tumors, especially in the prostate, and absence of expression in vital organs (6, 72). Good immunotherapy techniques require the increase of expression or cross-presentation of self-peptides to naïve T cells. Therefore, the ultimate purpose of tumor immunotherapy is the production of an effective vaccine containing epitopes that elicit both $CD8^+$– and $CD4^+$–T-cell immune responses, leading to tumor regression. This vaccine should be administrated to patients with cancer without using invasive techniques (73, 74).

The identification of STEAP1 epitopes has been directed toward prostate, renal, and bladder cancers with some success. The first STEAP1 epitopes used to trigger an antitumor immune response were $STEAP_{292}$ (MIAVFLPIV), a naturally processed peptide, and its modified version, $STEAP_{292.2L}$ (MLAVFLPIV). Their selection was based on their strong binding to HLA-A*0201 molecules and ability to elicit a sustained cytotoxic T-lymphocyte (CTL) response. In fact, $STEAP_{292}$, and especially $STEAP_{292.2L}$, induced naïve $CD8^+$ T cells into CTL capable of recognizing peptide-loaded cells with high specificity. Moreover, CTL induced by $STEAP_{292.2L}$ peptide not only recognized peptide-loaded cells, but also tumor cells from prostate, colon, bladder, Ewing sarcoma, melanoma, and embryonic rhabdomyosarcoma that expressed STEAP1 (75). Two additional nonameric STEAP1 epitopes ($STEAP_{86–94}$ and

$STEAP_{262–270}$) were found to be HLA-A*0201–restricted epitopes. Both can be found in human and mouse, but with slightly different constitutions; human $STEAP_{86–94}$ differs at position 9 from the mouse peptide (FLYTLLRE**V**→-FLYTLLRE**I**) and human $STEAP_{262–270}$ differs at position 6 from the mouse peptide (LLLGT**I**HAL→LLLGT**V**HAL). The latter has been initially identified as a TAA of STEAP3 (76, 77). Despite the differences mentioned, both $STEAP_{86–94}$ and $STEAP_{262–270}$ peptides are immunogenic *in vivo*, in HLA-A*0201 transgenic mice (HHD), and *in vitro*, in peptide-specific human $CD8^+$ T cells from healthy donors. Furthermore, $STEAP_{86–94}$ and $STEAP_{262–270}$ from human and mouse $CD8^+$ T cells were able to recognize STEAP1 expressed in human tumor cells in an HLA-A*0201–restricted manner. Also, specific CTL-expressing $STEAP_{86–94}$ were amplified *ex vivo,* from the peripheral blood of 3 out of 5 patients with non–small cell lung carcinoma and 2 out of 3 patients with prostate cancer, reinforcing the protective role of STEAP1. However, the contribution of tumor cells expressing STEAP1 to the observed immunologic response generated by the naturally processed STEAP1, as well as the quantitative and qualitative discrimination of the T cells expressing $STEAP_{86–94}$ and $STEAP_{262–270}$ remain to be identified (76). As a TAA, STEAP1 was also thought to be able to trigger an immune response to eliminate a tumor by specifically eliciting $CD4^+$ helper T cells. Consequently, 2 specific synthetic STEAP1 peptides, $STEAP_{102–116}$ (HQQYFYKIPILVINK) and $STEAP_{192–206}$ (LLNWAYQQVQQNKED), which strongly bind to different classes of HLA-DR, can also be presented by those different classes to $CD4^+$ helper T cells (74, 78). These naturally processed epitopes are both presented to $CD4^+$ helper T cells by tumor cells such as PC3 prostate cells, m697 melanoma cells, and Epstein Barr virus–transformed lymphoblastoid cell lines, but only $STEAP_{102–116}$ is presented to $CD4^+$ helper T cells by antigen-presenting cells (APC; ref. 74). The reactive $CD4^+$ helper T cells present in the different types of cancer may play a crucial role in the immune response that follows their activation; these cells would initiate the immune response sending the appropriate signals to APCs and CTL, resulting in CTL expansion, maturation, costimulation, and generation of memory CTL populations (79). In fact, both of these peptides were identified as being processed endogenously, through direct presentation of STEAP1 peptides by HLA-DR molecules, and exogenously, by APCs that process STEAP1 peptides derived from cell lysates, as seen *in vitro* with renal and bladder cancer (78).

Application of therapeutic vaccination, using STEAP1 as a target, that efficiently attenuates or even stops cancer progression is still in its early steps. No commercial formulations are available, particularly in prostate cancer, as the efficacy of the developed vaccines relies on the immunosuppressive state of the patients included in clinical trials and their tumor microenvironment, which prevents triggering of an immune response. Two studies that applied a mouse STEAP1 DNA prime/Venezuelan equine encephalitis virus-like replicon particles boost vaccine showed the efficacy of this therapeutic strategy against prostate cancer (73, 80). The capacity of this vaccine to trigger an immune response could be seen by the increasing number of $CD8^+$ and $CD4^+$ T cells and by the production of cytokines such as TNF-α, IFN-α, IL-2, and IL-12 following vaccination of C57BL/6 mice injected with a cell line derived from the transgenic adenocarcinoma mouse model, TRAMPC-2 (73). Protection against prostate cancer dramatically increases when vaccination occurs in mice mimicking earlier stages of cancer. Immunosuppressive mechanisms that lead to a reduction of Th1 and Th2 function, reduction of proinflammatory cytokines, and increased expression of immunosuppressive factors activated during prostate cancer progression tend to interfere with the efficacy of the vaccine (80). Avoiding the establishment of an immunosuppressive tumor microenvironment seems, therefore, to be the key to the success of therapeutic vaccination in later cancer stages. Thus, all together, these observations offer new possibilities for novel immunotherapeutic strategies, not only directed toward prevention but also for the treatment of patients with cancer.

The ability of STEAP3 to elicit an immune response was assessed *in vitro* in resensitized splenocytes against peptide-loaded RMA-*S*-HHD-B7.1 cell line and against peptide-loaded dendritic cells. STEAP3 binds to HLA-A2.1 molecules in a restricted and stable manner, and the activated CTL cells promote a dose-dependent peptide-specific cell lysis. Using the HLA-A2.1–positive LNCaP and the HLA-A2.1–negative PC3 cell lines as targets for peptide-loaded RMA-*S*-HHD-B7.1 cells, the lysis pattern promoted by the stimulation of CTL confirmed that STEAP3 is indeed presented by MHC-I molecules expressed on the tumor cell surface and that it is processed inside the cell. As an MHC-I–presented peptide on tumor cells *in vitro*, it was assumed that *in vivo*, STEAP3 could have antitumor reactivity. In fact, subcutaneous administration of autologous CTL cells around a previously induced tumor led to an expressive tumor regression (77). The confirmation that human CTL precursors specific for STEAP3 have the ability to initiate an antitumor response in healthy and prostate cancer–bearing individuals highlights the potential of $STEAP_{262–270}$ for immunotherapeutic procedures against tumors in which STEAP3 is expressed (77). Together with the STEAP3 coexistence with MHC-I and MHC-II in exosomes derived from dendritic cells and its dependence on p53, its use for exosomal immunotherapy could be the next step.

Although no studies have been done to establish an immunotherapeutic strategy using STEAP2 or STEAP4, their contribution to molecular trafficking and involvement in prostate cancer progression make them promising targets. The unique features of STEAP4 could also be directed toward adipose tissue to prevent inflammation and reduce insulin resistance, both associated with obesity (44). In addition, the FNO-like domain, which is only absent in STEAP1, could also be seen as a potential target in STEAP2, STEAP3, and STEAP4, with the advantage of its exclusive presence in this family of proteins in mammals. Overall,

future immunotherapeutic strategies may include all STEAP family members, with promising results. Furthermore, their use as vaccine components could also lead to a major improvement in prostate cancer, anemia, metabolic disorders, and antiinflammatory therapies, in which these STEAP proteins are overexpressed.

Conclusions and Future Prospects

All members of the STEAP family share common features in their structure and act as metalloreductases in human cells, with the exception of STEAP1, because of the absence of the FNO-like domain and Rossman fold. Overall, STEAP1, STEAP2, STEAP3, and STEAP4 are overexpressed in several human cancers, and some studies suggest that these proteins, particularly STEAP1 and STEAP2, increase the proliferation of cancer cells, suggesting their potential as therapeutic targets. Still, the mechanisms by which each STEAP protein contributes to cell proliferation and cancer progression in different organs and tissues remain largely elusive. Thus, more data are required to clarify the role of STEAP proteins in the cell cycle, proliferation, and apoptosis. Furthermore, the regulation of STEAP expression should also be addressed, particularly in those tissues in which STEAP is overexpressed in malignancies. These studies should encompass detailed analysis of hormones, particularly androgens in the prostate, and cytokines, and the analysis of the epigenetic mechanisms involved in STEAP regulation, not only in animal and cellular models, but also in a clinical context in patients with cancer.

The fact that STEAP proteins, particularly STEAP1 and STEAP2, localize at the cell membrane, are overexpressed in cancer tissues, and are absent in vital organs, underlines their potential as biomarkers of disease and as potential immunotherapeutic targets against prostate, bladder, kidney, and liver cancer. More studies to establish clear associations of STEAP expression with clinical data, and the effects on cancer treatments, in animal models and in patients with cancer should be entailed to provide further support for their use as biomarkers of disease and to ascertain their immunotherapeutic potential.

A comprehensive analysis of the functions and clinical importance of STEAP in cancer is still in its early days, but the information gathered to date is promising and definitively encourages further research exploring the expression, regulation, and role of STEAP proteins in cancer pathophysiology, diagnosis, and therapeutic approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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