



Serglycin: at the crossroad of inflammation and malignancy

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Serglycin has been initially characterized as an intracellular proteoglycan expressed by hematopoietic cells. All inflammatory cells highly synthesize serglycin and store it in granules, where it interacts with numerous inflammatory mediators, such as proteases, chemokines, cytokines, and growth factors. Serglycin is implicated in their storage into the granules and their protection since they are secreted as complexes and delivered to their targets after secretion. During the last decade, numerous studies have demonstrated that serglycin is also synthesized by various non-hematopoietic cell types. It has been shown that serglycin is highly expressed by tumor cells and promotes their aggressive phenotype and confers resistance against drugs and complement system attack. Apart from its direct beneficial role to tumor cells, serglycin may promote the inflammatory process in the tumor cell microenvironment thus enhancing tumor development. In the present review, we discuss the role of serglycin in inflammation and tumor progression.

Keywords: proteoglycans, serglycin, chondroitin sulfate, inflammation, malignancy

INTRODUCTION

Proteoglycans (PGs) are complex macromolecules consisted of a core protein covalently linked with glycosaminoglycan (GAG) chains named chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), heparin (HP), and heparan sulfate (HS). GAGs are negatively charged polysaccharides comprised of repeating disaccharides of acetylated hexosamines (*N*-acetyl-galactosamine or *N*-acetyl-glucosamine) and mainly by uronic acids (*D*-glucuronic acid or *L*-iduronic acid) being sulfated at various positions. KS is composed of disaccharides containing *N*-acetyl-glucosamine and galactose (1). PGs are synthesized by all cells and distributed in all tissues participating in physiological functions and pathologic conditions. According to their localization they can be divided in three main groups, the cell-surface associated PGs, such as syndecans and glypicans, the matrix secreted PGs (e.g., versican, decorin, perlecan) and the intracellular PGs, with serglycin being the only characterized member of this subfamily to date (1). Numerous studies have demonstrated significant modifications in PG expression in the tumor microenvironment and their contribution to carcinogenesis (1, 2). The type and fine structure of GAG chains attached to PGs are markedly affected in the context of malignant transformation as a result of the altered expression of GAG-synthesizing enzymes (3). Structural modifications of GAGs may facilitate tumorigenesis in various ways, modulating the functions of PGs (3).

Serglycin has been initially considered as a hematopoietic PG present in intracellular secretory compartments. Recent studies demonstrated that serglycin is expressed by a variety of cell types and mediates crucial functions in both normal and pathological conditions (4, 5). Rat L2 yolk sac tumor serglycin was the first

PG gene to be cloned and remains until today the smallest known core protein (18 kDa) (6). In human, serglycin consists of a small core protein (158 amino acids) containing eight serine/glycine repeats (**Figure 1**). Each serine of this repeat region is a potential GAG attachment site. The size of the PG varies according to the GAG chain length, number, and type (7). Serglycin is expressed in all normal hematopoietic cells and hematopoietic tumor cell lines (7–17). In mast cells, eosinophils, neutrophils, and platelets, serglycin is stored together with other bioactive molecules in granules and is secreted upon activation. Serglycin is constitutively secreted by lymphocytes and many hematopoietic tumor cell lines (7, 8, 16). Serglycin is also expressed in non-hematopoietic cells including pancreatic acinar cells (18), chondrocytes (19), smooth muscle cells (20, 21), endothelial cells (20, 22, 23), fibroblasts (20, 24, 25), F9, and NCCIT teratocarcinoma cells (26, 27), murine embryonic stem cells (28), murine uterine deducua cells, parietal endoderm, and fetal liver but not yolk sac hematopoietic cells (29). Recently, it was demonstrated that serglycin is highly expressed by aggressive nasopharyngeal cancer cells (30). The type and sulfation of GAG chains attached to the serglycin core protein, varies between different species and cell types (4, 8, 16, 17, 22, 23, 31–43). For example, serglycin expressed by human mast cells has been shown to contain both HP and CS chains enriched in disulfated disaccharides [being sulfated at C4 and C6 of *N*-acetyl-galactosamine (CS-E)] attached in separate core proteins, whereas mouse mast cells synthesize a hybrid HP/CS-E serglycin. In leukocytes, platelets, myeloma, and endothelial cells, serglycin contains CS chains that are mainly sulfated at C4 of *N*-acetyl-galactosamine (CS-4). Interestingly, the sulfation pattern of CS present in serglycin is regulated during differentiation of

monocytes to macrophages (44, 45). In macrophages serglycin is substituted in a higher degree with CS-E compared to monocytes which contain CS-4 chains. The amount of CS-E expressed by macrophages can be further increased upon activation of differentiated macrophages with phorbol 12-myristate 13-acetate (PMA) (46).

STRUCTURE AND REGULATORY ELEMENTS OF THE HUMAN SERGLYCIN GENE

The human serglycin gene is located in chromosome 10q.22.1 (47, 48) and is consisted by an approximately 1.8 kb of 5'-flanking DNA, three exons, which are separated by two introns of 8.8 kb (intron 1) and 6.7 kb (intron 2) (49, 50). The 5'-untranslated mRNA and the hydrophobic 27 aminoacid signal peptide of the translated protein are encoded in the first exon, whereas the second exon encodes a 49 aminoacid peptide that represents the amino-terminus of the mature serglycin core protein. Finally, the larger exon 3 codes a 82 aminoacid peptide that contains the GAG attachment region, the carboxy-terminus, and the 3'-untranslated mRNA region (49) (Figure 1). An alternative spliced variant of serglycin lacking exon 2 has been detected in neutrophils and in low levels in HL-60 and may be related with the maturation of promyelocytes to form segmented neutrophils (15). Several putative regulatory sites are present in the 5'-flanking region with E-26 specific family of transcription factors (ETS) site (−80) and the cyclic AMP response element (CRE) half site at −70 to be the most important regulatory elements for constitutive expression (51) (Figure 1). The CRE site is also important for the induced expression of serglycin after treatment with PMA and dibutyryl cyclic AMP (dbcAMP) (51). ETS regulatory elements interact with ETS1 and Friend leukemia integration 1 transcription factor proteins (FLI1). The expression of serglycin was shown to be up-regulated in a number of leukemic cell lines, ones that coincidentally have

been shown to express high levels of ETS1 and FLI1 (9, 52). The ETS genes encode transcription factors that play important roles in hematopoiesis, angiogenesis, and organogenesis. In the intron 1 a commonly conserved 70 bp Donehower element is found that may has a *cis*-acting functional role (49). The serglycin gene has also 21 *Alu* elements, with two of them being located in the 5'-flanking region, 8 in the intron 1, and 11 in the intron 2 (49) (Figure 1). *Alu* elements represent one of the most successful of all mobile elements and are primate specific. *Alu* element inserts in or near a gene have the potential to influence expression of that gene in several ways (53). The expression of serglycin in different cell lines depends also on the methylation status and the presence of DNaseI hypersensitivity sites (DHSS) within the serglycin gene (49, 54). Cell-specific DHSS sites have been found in the promoter region, exon 2 and introns of serglycin gene in hematopoietic and endothelial cells (51, 54). These sites are short chromatin regions with disturbed nucleosome formation that have increased sensitivity to factors interacting with DNA and regulate transcription. Several of the DHSS appear to be located well within or very close to *Alu* repeats and this association may play a role in the expression of serglycin (54).

BINDING PARTNERS OF SERGLYCIN

Several studies have demonstrated that serglycin is capable to interact with biological important molecules (summarized in Table 1). The binding is mediated either through GAG chains or core protein or both moieties are required for high affinity binding to serglycin. CS chains of serglycin mediate the binding to CD44 (55), whereas CS-4 chains with a high proportion of 4-sulfated disaccharides (more than 87%) are required for binding to complement components C1q and mannose binding lectin (MBL) (56). Although CS-4 chains are crucial for binding of serglycin to MBL (56) and collagen type I (57), the overall structure may

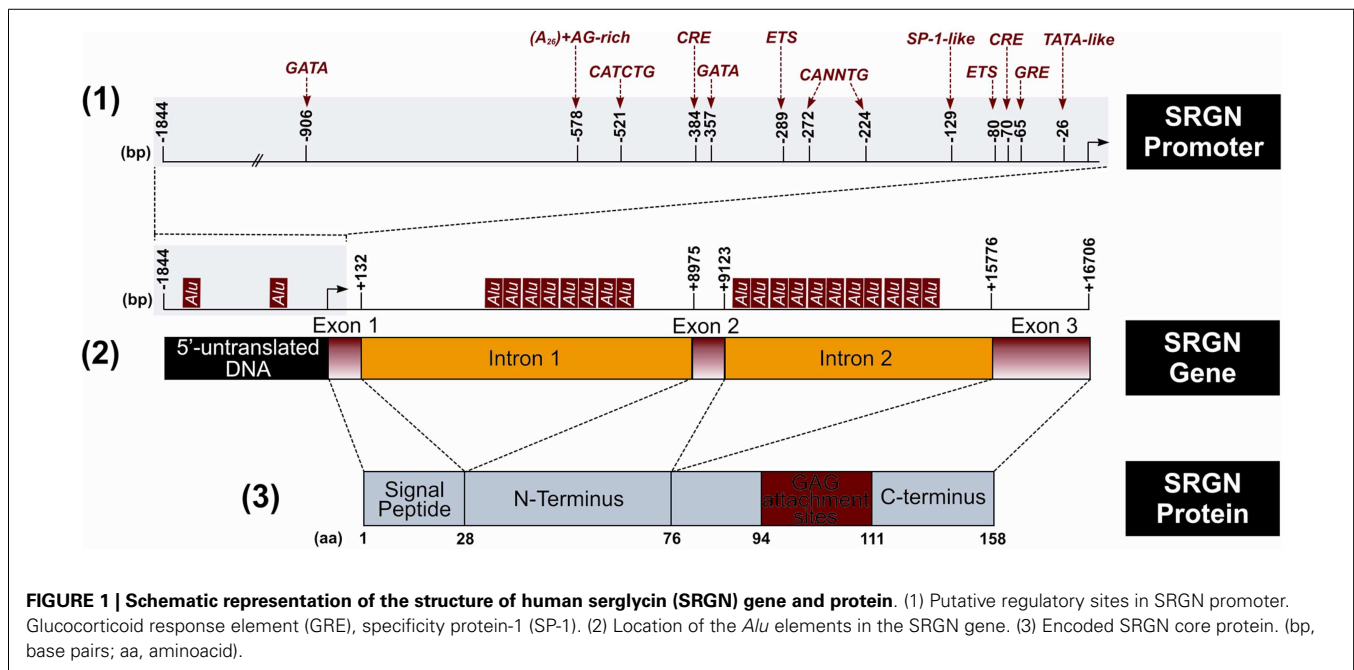


Table 1 | Overview of serglycin binding partners.

Molecule	Interaction type (Reference)	Major functions
CELL-SURFACE RECEPTORS, CHEMOKINES, CYTOKINES, AND COMPLEMENT COMPONENTS		
CD44	Physical association (55)	Cell signaling, adhesion, migration and, lymphocyte activation, hematopoiesis, tumor metastasis
CD53	Computational (experimental knowledge based) (86)	Cell signaling, development, activation, growth, and motility. Activation of leukocytes
HLA-DRA	Computational (experimental knowledge based) (86)	Antigen presentation, immune system response
CXCR4	Computational (experimental knowledge based) (86)	Chemotaxis and activation of leukocytes, hematopoietic stem cell homing, cancer cell metastasis
CXCL1	Colocalization (22)	Chemotaxis, inflammation, angiogenesis, tumor growth
CXCL4	Physical association (60)	Platelet aggregation, chemotaxis, hematopoiesis, angiogenesis, immune system response
CCL3	Physical association (60)	Chemotaxis, inflammation
BMP-like protein	Physical association (60)	Bone and cartilage formation
C1q	Physical association (56)	Complement activation
MBL	Physical association (56)	Complement activation
MATRIX MOLECULES/COMPOUNDS		
Collagen I	Physical association (57)	Matrix organization, cell adhesion
Fibronectin	Physical association (12, 58, 59)	Matrix organization, cell adhesion, and migration
B2M	Computational (experimental knowledge based) (86)	Associates with MHC class I molecules. Implicated in amyloidosis and multiple myeloma
Hydroxyapatite	Physical association (16)	Bone formation
PROTEOLYTIC ENZYME, PORE FORMING PROTEINS		
Lysozyme	Physical association (60)	Anti-microbial activity
GZMB	Physical association (61–63)	Proteolysis, cell death
PRF1	Physical association (61–63)	Pore formation, cell death
Chymases	Physical association (64, 65)	Proteolysis as part of host defense and inflammation
Tryptases	Physical association (65–67)	Proteolysis as part of host defense and inflammation
CPA	Colocalization (68)	Proteolysis as part of host defense and inflammation
Elastase	Colocalization (71)	Proteolysis as part of host defense and inflammation
tPA	Colocalization (23)	Fibrinolysis. Proteolysis in physiological conditions and diseases
MMP13	Physical association (19)	Proteolysis in physiological conditions and diseases
proMMP9	Physical association (73, 74)	Proteolysis in physiological conditions and diseases
NEUROTRANSMITTERS		
Serotonin	Colocalization (69)	Neurotransmission, vasoconstriction, thrombosis
Histamine	Colocalization (69)	Neurotransmission, endothelium permeabilization, inflammation
Dopamine	Colocalization (70)	Neurotransmission
INTRACELLULAR PROTEINS		
CEP70	Physical association (75–77)	Microtubules organization
BAG6	Physical association (75–77)	Quality control of proteins, proteasome assembly and degradation, T cell response
PSRC1	Physical association (75–77)	Mitotic spindle assembly
UBR4	Physical association (75–77)	Ubiquitination, proteasomal, and lysosomal degradation
UBQLN4	Physical association (75–77)	Binds ubiquitinated proteins, proteasomal degradation
SGTA	Physical association (75–77)	Cochaperone, quality control of proteins
ARHGDI1B	Computational (experimental knowledge based) (86)	Cell signaling, proliferation, cytoskeletal organization, and secretion. Cancer invasion and metastasis
LAPTM5	Computational (experimental knowledge based) (86)	Lysosomal destabilization, cell death
ALOX5AP	Computational (experimental knowledge based) (86)	Leukotriene synthesis, inflammation
MSN	Computational (experimental knowledge based) (86)	Links plasma membranes with actin cytoskeleton. Cell signaling and movement
HCLS1	Computational (experimental knowledge based) (86)	Cytoskeleton remodeling, leukocyte chemotaxis, and activation
RAP1B	Computational (experimental knowledge based) (86)	Cell signaling, adhesion, growth, and differentiation

also be important for high affinity binding. This is in agreement with previous studies where the CS-4 chains were essential for binding of serglycin to molecules, such as fibronectin and collagen, chemokine (CXC motif) ligand 4 (CXCL4), chemokine (C-C motif) ligand 3 (CCL3), bone morphogenetic protein (BMP)-like protein, lysozyme, granzyme B (GZMB), perforin (PRF1), and hydroxyapatite although the intact serglycin molecule might interact more efficiently with these molecules (12, 16, 58–63) (**Table 1**). HP or/and CS-E chains of serglycin in mast cells mediate its binding to chymases (64, 65) and tryptases (65–67) (**Table 1**). Serglycin colocalizes with carboxypeptidase A (CPA) (68), serotonin, histamine (69), and dopamine (70) in mast cell granules, CPA in pancreatic acinar cells (18), neutrophil elastase in neutrophils (71) and U937 promonocytes (72), tissue-type plasminogen activator (tPA) (23) and chemokine growth-related oncogene-alpha (GRO- α /CXCL1) in endothelial cells (22). Serglycin is capable to interact with matrix metalloproteinases (MMPs), such as MMP13 (19) and proMMP9 through its core protein (73, 74) (**Table 1**). By using stringent high-throughput yeast two-hybrid system interactions, novel serglycin ligands, such as centrosomal protein 70 kDa (CEP70), BCL2-associated athanogene 6 (BAG6), proline/serine-rich coiled-coil 1 (PSRC1), ubiquitin-protein ligase E3 component n-recognin 4 (UBR4), ubiquitin 4 (UBQLN4), and small glutamine-rich tetratricopeptide repeat (TPR)-containing alpha (SGTA) have been identified (75–77) (**Table 1**). Among them CEP70 and PSRC1 participate in microtubules formation and assembly of mitotic spindle (78, 79), whereas SGTA and BAG6 play key roles in quality control processes for newly synthesized proteins via their ubiquitination and proteasome degradation (80). Recently, it was shown that BAG6 is not only involved in proteasome core particle assembly but also has a key role in efficient regulatory particle assembly by directly associating with precursor regulatory particles (81). UBR4 and UBQLN4 are also involved in the proteasomal degradation of proteins. UBR4 is an E3 ubiquitin-protein ligase that mediates polyubiquitination of low-abundance regulators and selective proteolysis through the proteasome but is also associated with cellular cargoes destined to autophagic vacuoles and degradation through the lysosome (82, 83). UBQLN4 binds either to ubiquitinated or not proteins and promotes their proteasomal degradation (84) but also mediates the recruitment of ubiquitin1 to autophagosomes (85). In another study, Wu et al. (86) constructed a protein functional interaction network that suggests association of serglycin with known and novel proteins, such as Rho GDP dissociation inhibitor (GDI) beta (ARHGDI B), CCL3, lysosomal protein transmembrane 5 (LAPTM5), arachidonate 5-lipoxygenase-activating protein (ALOX5AP), chemokine (C-X-C motif) receptor 4 (CXCR4), beta-2 microglobulin (B2M), moesin (MSN), hematopoietic cell-specific Lyn substrate 1 (HCLS1), GZMB, RAP1B, member of RAS oncogene family, major histocompatibility complex, class II, DR alpha (HLA-DRA) (see **Table 1**). A summary of serglycin interactions is available in <http://www.ebi.ac.uk/Tools/webservices/psicquic/view/main.xhtml?query=srgn>. A large number of candidate molecules might associate with serglycin according to STRING database. A diagram with predicted functional partners for serglycin (confidence score >0.48) is given in **Figure 2** (<http://string-db.org/>).

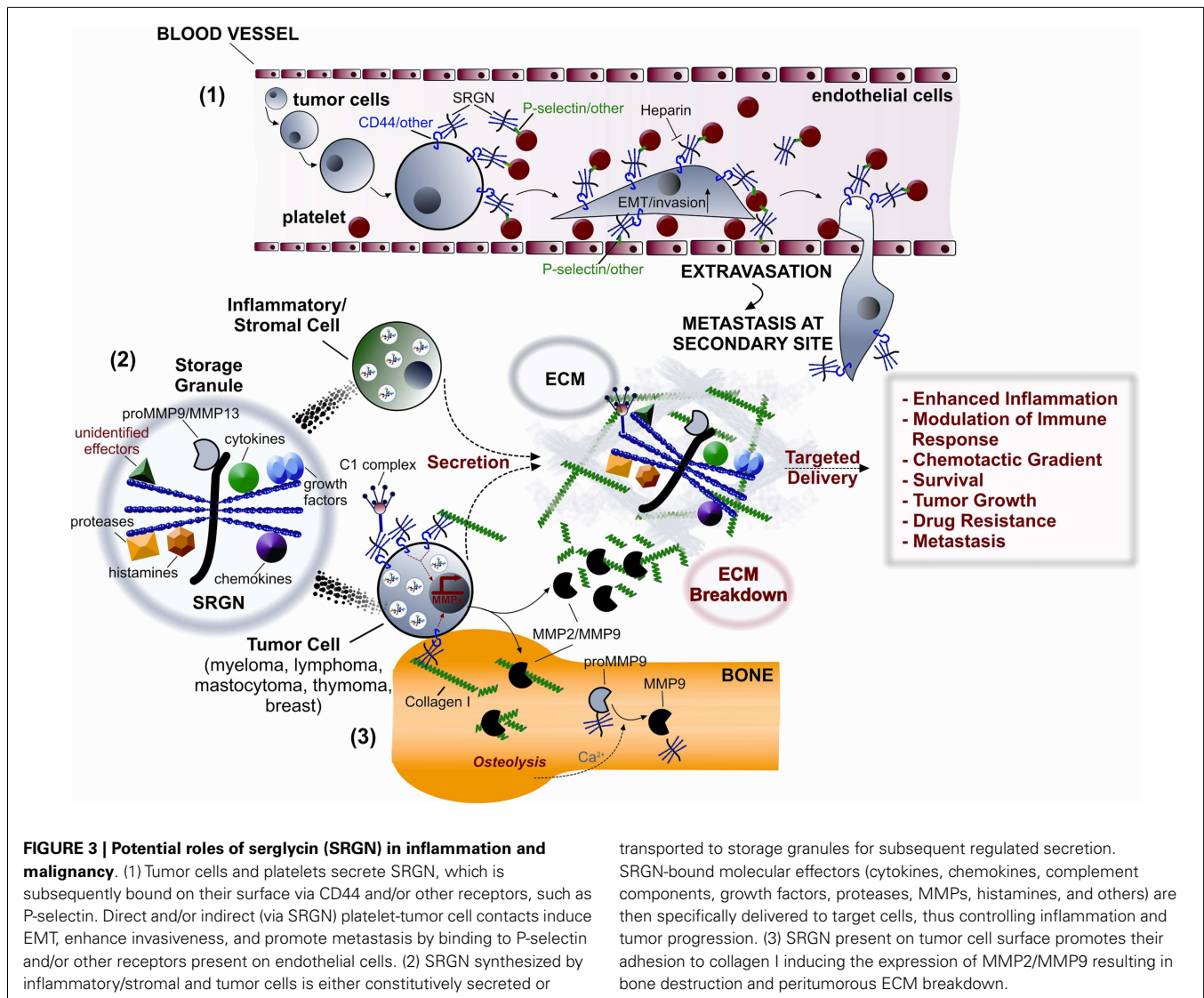
PHYSIOLOGICAL ROLES OF SERGLYCIN IN INFLAMMATION

Serglycin synthesized by inflammatory and stromal cells is secreted either constitutively or in a regulated manner. Serglycin secretion can be induced in several cell types upon external inflammatory stimulation. The biosynthesis of serglycin is up-regulated by liposaccharide (LPS) in macrophages (87), tumor necrosis factor (TNF) in endothelial cells (23) and adipocytes (88) and interleukin 1 β (IL-1 β) in smooth muscle cells (21).

The generation of serglycin^{-/-} mice has demonstrated a wide impact of serglycin on the functional properties of immune cells. In inflammatory cells serglycin is localized in secretory granules and vesicles participating in crucial roles in intracellular storage and secretion of bioactive molecules (**Figure 3**). In mast cells, serglycin is implicated in the storage of granule-localized proteases such as chymases, tryptases and CPA (34, 68), histamine, serotonin, and dopamine in mast cells (69, 70). Serglycin forms complexes with mast cell proteases modulating their activities. Several studies have addressed functions linked to HP, the major GAG component of serglycin, in mast cells. HP/chymase complexes bind to HP-binding substrates of the enzyme thus presenting them to chymase and enhancing their proteolysis (89). HP on serglycin was shown to significantly block the inhibition of chymase by natural inhibitors such as alpha 1-protease inhibitor, alpha 1-antichymotrypsin, alpha 2-macroglobulin, and soybean trypsin inhibitor (90, 91). HP is also involved in the formation of active tryptase tetramers (92, 93). It is suggested that serglycin apart from its protective role for partner molecules is also linked to transport to target sites, where proteases are released to perform their functions (94).

Serglycin mediates the storage of GZMB but not of granzyme A in cytotoxic T lymphocytes (CTLs) (95). It is also essential for the storage of elastase in azurophil granules of neutrophils. In contrast, serglycin is not involved in the storage of other granule components such as cathepsin G and proteinase 3 in these cells (71). Similarly, knockdown of serglycin in platelets results in defective storage of CXCL4, CXCL7, and platelet-derived growth factor (PDGF) (96). The impaired storage of mast cell proteases, neutrophil elastase, GZMB in CTLs and CXCL4 in platelets was not due to altered mRNA levels coding for these molecules, suggesting a crucial role for serglycin on storage rather than mRNA expression. The ability of serglycin to promote storage of granule components is suggested to be due to specific electrostatic interactions between the sulfated GAG chains and the basically charged regions of secretory granule components (95). The stable expression of granule components followed by their diminished protein levels in serglycin knockdown cells and the association of serglycin with SGTA, BAG6, UBR4, and UBQLN4, which are involved in quality control during biosynthesis and degradation of proteins, might suggest a regulatory role for serglycin in cytoplasmic quality control machinery.

Serglycin is also involved in apoptosis and immune regulation. Diminished storage of mast cell proteases in serglycin^{-/-} mice resulted in reduced sensitivity of mast cells to apoptosis as a consequence of the reduced granule damage, reduced release of proteases into the cytosol, and defective caspase-3 activation (97). Mast cells lacking serglycin expression preferentially died by necrosis rather than apoptosis and the necrotic phenotype of



transported to storage granules for subsequent regulated secretion. SRGN-bound molecular effectors (cytokines, chemokines, complement components, growth factors, proteases, MMPs, histamines, and others) are then specifically delivered to target cells, thus controlling inflammation and tumor progression. (3) SRGN present on tumor cell surface promotes their adhesion to collagen I inducing the expression of MMP2/MMP9 resulting in bone destruction and peritumorous ECM breakdown.

binding to C1q and MBL. CS-4 chains with a high proportion of 4-sulfated disaccharides are required for the interactions with complement proteins (56). CS-E and in a lower extent HP compete with CS-4 chains of serglycin for binding to C1q, whereas only CS-E competes for binding to MBL. Serglycin secreted by various immune cells that carries CS-E or/and HP is more likely capable to regulate the activity of both pathways (56).

In a recent study serglycin was found to be among the most abundantly expressed genes in epicardial adipose tissue and was up-regulated with pro-inflammatory genes such as IL-1 β , IL-6, IL-8, and chemokine receptor 2 (CCR2) (88). In human umbilical vein endothelial cells (HUVECs) the storage of CXCL1 in secretory vesicles to the apical side was partly depended on serglycin, whereas upon stimulation with IL-1 β an increased colocalization of the two molecules inside the vesicles before secretion was observed suggesting a possible involvement of serglycin in inflammatory conditions (22). The presence of serglycin in platelets may be also important for these cells to fully display their

pro-inflammatory properties. In platelets, serglycin associates with fibronectin, PDGF, CXCL7, CXCL4, RANTES/CCL5, and CCL3 and its absence leads to reduced storage of PDGF, CXCL4, and CXCL7 in α -granules, contributing to defective platelet aggregation and leukocyte activation (96). Serglycin expression is elevated in the dermal vessels in dermatomyositis, a chronic inflammatory disease of the skin (101). In addition, mRNA expression of serglycin is induced upon UVB radiation and IL-1 α exposure in cultured human dermal fibroblasts suggesting that serglycin may participate in dermal inflammation (25).

EXPRESSION AND BIOLOGICAL ROLES OF SERGLYCIN IN MALIGNANCIES

Although the main function of serglycin in the biology of immune cells is the proper formation of secretory granules and vesicles as well as the storage and secretion of several components in the extracellular matrix (ECM), the role of serglycin in malignancies is rather intriguing. Serglycin seems to participate in tumor

development in a manner that at least partially requires interactions between tumor cells and their microenvironment (102) (**Figure 3**).

Serglycin with different GAG chains and sulfation patterns is expressed in numerous human hematopoietic and non-hematopoietic tumors (8–17, 26, 27, 30, 55, 103). Interestingly, the megakaryocytic tumor cells synthesize a hybrid CS/HS serglycin (12). The expression levels of serglycin may vary during hematopoietic cell differentiation (9, 14, 15) and is constitutively secreted in the ECM in hematopoietic and solid tumors (16, 30). Serglycin has been proposed as a selective biomarker for acute myeloid leukemia compared to Philadelphia chromosome-negative chronic myeloproliferative disorders since it is highly expressed only by leukemic blasts of patients with acute myeloid leukemia and not in acute lymphocytic leukemia (10). Expression of serglycin has been demonstrated in a variety of lymphoma, myeloma, mastocytoma, and thymoma cells. In these cells the presence of CS-4 or CS-6 side chains of serglycin are required for binding to CD44, whereas serglycin carrying HS or HP is not capable for binding to CD44 (17, 55). Secreted serglycin in the tumor microenvironment may interact with CD44 on tumor cells triggering CD44 signaling (**Figure 3**). CD44 is involved in cell–cell and cell–matrix interactions and signals through several pathways by binding via its cytoplasmic domain to multiple cell membrane and intracellular functional proteins thus regulating cancer cells' epithelial to mesenchymal transition (EMT), migration, metastasis, proliferation, apoptosis, and resistance (104, 105). Importantly, CD44 has been recognized as a cancer stem cell marker for a variety of tumor types.

The expression of serglycin was also confirmed in several MM cell lines (16). Serglycin is the major PG synthesized by MM cells and is constitutively secreted to the culture medium. Interestingly, serglycin is also localized on the cell surface where it is attached through its CS-4 chains. Serglycin levels are elevated in the bone marrow aspirates of patients with newly diagnosed MM, suggesting a potent correlation of serglycin accumulation with disease progression (16). Serglycin present on myeloma cell surface promotes the adhesion of myeloma cells to collagen I. The adhesion of myeloma cells to collagen I but also the interaction of soluble collagen I with myeloma cells via cell-surface serglycin enhances the biosynthesis and secretion of MMP2 and MMP9, which are involved in bone destruction (57) (**Figure 3**). The regulatory role of serglycin in the biosynthesis and secretion of proteases has been also shown in other cellular systems (23, 106). Serglycin is colocalized with tPA in secretory vesicles in HUVEC (23), whereas Madin-Darby canine kidney cells stably transfected with serglycin express elevated levels of MMP9 and urokinase plasminogen activator (uPA) both at mRNA and protein levels (106). The release of proteolytic enzymes by tumor cells or stromal cells and the regulation of their activity in the tumor microenvironment are crucial for tumor progression and tumor-induced bone disease (107, 108). In addition, secreted serglycin was found to influence the bone mineralization process through inhibition of the crystal growth rate of hydroxyapatite, thus providing another possible explanation for impaired bone formation and loss of bone mass commonly seen in MM patients (16). Serglycin synthesized and secreted by human acute monocytic leukemia cell line THP1 as

well as serglycin isolated from myeloma cells forms complexes with proform of MMP9 (proMMP9) *in vivo* and *in vitro* (73, 74). Both the hemopexin-like (PEX) domain and the fibronectin-like (FnII) module of the enzyme are involved in the formation of the heteromer. The formation of heteromers alters the mode of activation of proMMP9 and the interaction of the enzyme with its substrates (73, 109). Another study supports the direct protein–protein interaction between serglycin and MMPs (19). It has been shown that serglycin is colocalized with MMP13 in cytoplasmic granules in chondrocytes interacting with a fragment of C-terminal domain of MMP13 that comprises the hinge and PEX domains (19). ProMMP9 in the heteromer is activated in the presence of Ca^{2+} , although this cation stabilizes MMP9 without activating the single proenzyme. Ca^{2+} induces the cleavage of both the C-terminal PEX domain of the enzyme and the core protein of PG and the release of the activated enzyme. MM located within bone marrow and solid tumors, which metastasize in the bones, induce bone destruction and release Ca^{2+} . The accumulation of serglycin within bone marrow in MM may be involved in the formation of heteromers with proMMP9 in the ECM triggering a Ca^{2+} -induced activation of the enzyme (**Figure 3**).

Matrix secreted and cell-surface associated serglycin protects myeloma cells from complement system attack induced by immunotherapy, therefore promoting the survival of myeloma cells (56). Serglycin inhibits specifically the classical and the lectin pathways via binding to C1q and MBL and do not interfere with the alternative pathway (56) (**Figure 3**). The intact serglycin molecule is required for binding to MBL, whereas binding to C1q is mediated exclusively by CS-4 side chains. A similar mode of inhibition of the classical and lectin pathways was demonstrated for secreted serglycin by aggressive breast cancer cells (110). It has been shown previously that complement components such as C1q, C3, C3a, C4, C5, and the membrane attack complex (MAC) are deposited in the inflammatory tumor microenvironment. The assumption has been made is that these activated complement proteins play a role in tumor defense directly through complement-dependent cytotoxicity and indirectly through antibody-dependent cell-mediated cytotoxicity (111). The mechanism of complement activation in cancer is known to involve mainly the classical and the lectin pathways (111). Complement is activated by factors present on tumor cells or induced by treatment of tumor cells with therapeutic antibodies. Malignant cells express a variety of complement inhibitors, which all attenuate complement cytotoxicity (111). The inhibition of complement is also a great limitation during immunotherapy against several types of cancer. These data suggest a role for serglycin as a modulator of immune system response in the tumor microenvironment that may protect tumor cells from complement attack. Another great limitation in the treatment of malignancies is the development of drug resistance. Serglycin is among genes that over-expressed in six tumor cell lines of hematopoietic origin that resist in doxorubicin, methotrexate, cisplatin, and vincristine treatment compared to the drug sensitive parental cell lines (112). The implication of serglycin in drug resistance is of great interest and the mechanism of action is yet unknown.

Only few studies have demonstrated expression of serglycin in non-hematological tumors. Increased expression of serglycin in patients with hepatocellular and nasopharyngeal carcinoma

was correlated with unfavorable prognosis and represented an independent unfavorable prognostic indicator for overall survival and recurrence as well as disease free and distant metastasis free survival (30, 103). Metastatic nasopharyngeal carcinoma cells highly express and secrete in the culture medium serglycin, which promotes motility, invasion, and metastasis (30). The overexpression of serglycin is also associated with EMT in nasopharyngeal cancer cells. The functions of serglycin were dependent on the fully glycosylated molecule. Treatment of cancer cells with exogenously added glycanated serglycin promoted cancer cell metastasis and invasion, whereas non-glycosylated core protein of serglycin failed to induce cell motility (30). *In vitro*, serglycin was highly expressed and secreted by aggressive tumor cell lines (110). In invasive MDA-MB-231 breast cancer cells serglycin represents the major PG type and is abundantly expressed and secreted in the culture medium (110). Furthermore, serglycin is highly expressed by other aggressive breast cancer cells, which also belong to the Basal B subgroup, and they show mesenchymal phenotype, enhanced invasive properties and enriched expression of EMT transcriptional drivers (113). These cells exhibit an EMT gene signature and are found to resemble breast cancer stem cells, being CD44^{high}CD24^{low} (114). Interestingly, stable overexpression of intact serglycin gene and a truncated form of serglycin lacking GAG attachment sites in low aggressive MCF-7 breast cancer cells demonstrated that serglycin promotes breast cancer cell anchorage-independent growth, migration, and invasion. The tumor promoting properties of serglycin are dependent on the overexpression and secretion of glycanated serglycin (110). Therefore, the specific structure of CS-4 present on serglycin is important for serglycin functions in breast cancer. Altered biosynthesis of CS chains has been demonstrated in various cancer types. Specific structures of CS influence various biological processes during tumor growth and spread (115). CHST11 gene that specifically mediates 4-O sulfation of CS is highly expressed in MDA-MB-231 breast cancer cells and breast cancer tissues. CS-4 chains mediate the binding of breast cancer cells to P-selectin and facilitate the formation of metastasis (116).

INFLAMMATION AUGMENTS MALIGNANCY: POSSIBLE ROLES OF SERGLYCN

During tumor development, cancer cells produce cytokines and chemokines that attract and activate inflammatory cells, endothelial cells, fibroblasts, and platelets to secrete growth factors, cytokines, and chemokines. Serglycin may regulate the biosynthesis, secretion, and targeted delivery of many inflammatory mediators, which can act in various cell types in paracrine and autocrine manner in multiple ways to enhance inflammatory process and support tumor growth and metastasis (Figure 3).

Tumors are often infiltrated by immune cells, such as T lymphocytes, mast cells, and macrophages, which are recruited to the site by chemokines and cytokines secreted by the various cells in the tumor milieu. Macrophages are attracted by responding to CCL2, IL-4, IL-10, and IL-13, acquire an activated phenotype with pro-tumorigenic properties, releasing a variety of chemokines, cytokines, growth factors, and proteases (117). Mast cells infiltration is occurred in the tumor microenvironment in a number of human malignancies in response to tumor-derived chemoattractants such as CCL2 and CCL5. The presence of mast cells was

shown to correlate with either favorable or poor prognosis depending on the tumor. Mast cells can exert pro-tumorigenic effects by secreting factors like VEGF, angiopoietin-1, CXCL1, and IL-8 that promote tumor angiogenesis, as well as growth factors such as PDGF, NGF, SCF, FGF2, and proteases that facilitate tumor cell growth and metastases (118). Mast cell proteases exert dual roles in the regulation of inflammatory process. Several pro-inflammatory chemokines and cytokines are substrates of mast cell proteases and their cleavage results in the activation or inactivation of inflammatory mediators (119). Chymase also indirectly influences ECM remodeling via its ability to activate various MMPs (120, 121). Both tryptase and chymase are directly involved in ECM degradation. Tryptase degrades collagen type IV present in basement membranes while chymase cleaves vitronectin and procollagen and both degrade fibronectin (119).

Endothelial cells synthesize and secrete chemokines, cytokines, and growth factors such as CXCL1 and respond to inflammatory stimuli enhancing their production in a serglycin-dependent manner (22). Cancer associated fibroblasts which are activated by cancer cells also overexpress inflammatory mediators as well as serglycin and a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), promoting cancer cell invasion (24).

Considering that single GAG chains and serglycin interact with growth factors, cytokines, and chemokines (7, 122), it is plausible that serglycin secreted by cancer and stromal cells is important for the protection of inflammatory mediators in ECM and the creation of chemotactic gradients. Furthermore, serglycin may be involved in the presentation of these molecules to high affinity receptors thus enhancing signaling events (Figure 3).

Platelets have long been believed to play a critical role in cancer metastasis through the enhancement of circulating tumor cells survival and adhesion to the endothelium in the circulation (123). Serglycin may be involved in tumor cell metastasis either via its proven role in activation of platelets or directly affecting the binding of tumor cells to platelets (Figure 3). The presence of serglycin in platelets is critical for packaging and secretion of selected α -granule proteins. The reduced secretion of dense granule contents results in impaired platelet activation and aggregation (7, 96). Tumor cells may bind to the surface of activated platelets via platelet receptors glycoprotein IIb/IIIa (integrin α IIb β 3) and P-selectin or by attachment to platelets microparticles. This most likely requires the activation of platelets, the fusion of α -granule membrane with the cell membrane, the exposure of activation-induced surface proteins and the secretion of chemokines, cytokines, and growth factors (7). Platelet serglycin may influence the release of these factors in the tumor microenvironment, which promote tumor cell growth and metastasis. Platelet-derived TGF- β as well as direct platelet-tumor cell contacts synergistically activate the TGF- β /Smad and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathways in cancer cells, resulting in their transition to an invasive mesenchymal-like phenotype and enhanced metastasis *in vivo* (124) (Figure 3). Another possibility is that released serglycin either by tumor cells or platelets may participate in bridging tumor cells with activated platelets or platelet microparticles as well as endothelial cells. It is proposed that serglycin is bound on the

surface of platelets (7), whereas serglycin has been identified on the surface of tumor cells (6, 16). Secreted serglycin substituted with CS chains may associate with tumor cell surface via CD44 (55) and the membrane of activated platelets and endothelial cells via P-selectin (116) and/or other unidentified receptors (Figure 3). The inhibition of platelets-tumor cell interaction has been targeted for treatment of metastasis. HP and other GAGs isolated from various sources prevent metastasis. Although the anti-metastatic effect of HP was initially believed to associate with its anticoagulant activity, later it was found that interfered with binding of activated platelets with ligands on tumor cells (125).

CONCLUSION

Serglycin is a dominant PG in immune cells with a major impact on their biology. Numerous studies in the past using mainly a valuable serglycin knockdown mouse model demonstrated important functional roles for serglycin in immune system processes and inflammation. Recent studies have revealed emerging roles for serglycin in tumorigenesis. Collectively, the expression of serglycin seems to benefit tumor cells in multiple ways. It may act as a modulator of immune system in tumor microenvironment and enrich tumor cells with resistance to various therapeutic agents. Serglycin augments the invasion and metastasis of tumor cells with yet unknown molecular mechanisms. Importantly, it serves as an ideal molecular partner for multiple molecular effectors, such as proteolytic enzymes, chemokines, cytokines, and growth factors regulating their biosynthesis and secretion or/and enhancing their activities by protecting and accompanying them to specific target sites.

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