Chinese Journal of Cancer

Mini Review

Serglycin in human cancers

Xin-Jian Li[1] and Chao-Nan Qian[1,2]

Abstract

Serglycin belongs to a family of small proteoglycans with Ser-Gly dipeptide repeats, and it is modified with different types of glycosaminoglycan side chains. Intracellular serglycin affects the retention and secretion of proteases, chemokines, or other cytokines by physically binding to these factors in secretory granules. Extracellular serglycin has been found to be released by several types of human cancer cells, and it is able to promote the metastasis of nasopharyngeal carcinoma cells. Serglycin can bind to CD44, which is another glycoprotein located in cellular membrane. Serglycin's function of promoting cancer cell metastasis depends on glycosylation of its core protein, which can be achieved by autocrine as well as paracrine secretion mechanisms. Further investigations are warranted to elucidate serglycin signaling mechanisms with the goal of targeting them to prevent cancer cell metastasis.

Key words Serglycin, cancer, metastasis, proteoglycan

Serglycin is a proteoglycan that has its core peptide coded by the gene *SRGN* in humans. It belongs to a family of small proteoglycans with serine-glycine dipeptide repeats and is modified with various glycosaminoglycan side chains. Serglycin is also known as a secretory granule proteoglycan core protein or hematopoietic proteoglycan core protein[1]. Rat *Srgn* gene was originally cloned and sequenced from the rat yolk sac carcinoma cell line L2 in 1985; human serglycin was isolated from platelets in 1986[2,3]. The amino acid sequence of human serglycin is closely homologous to those of the mouse and rat[4].

Human serglycin consists of a core protein decorated with glycosaminoglycan chains, for example, chondroitin-4-sulfate (CS-4), CS-6, CS-E, CS-B, or heparin[5]. The core protein of human serglycin has 158 amino acid residues that form three functional domains. A signal peptide domain, encoded by exon 1, is composed of amino acid residues 1–27; the N-terminal domain, encoded by exon 2, is composed of amino acid residues 28–76; and the glycosylation domain, encoded by exon 3, is composed of amino acid residues 77–158[6], as shown in Figure 1.

Previous studies have shown that there are two forms of serglycin in human cells, intracellular and extracellular[7]. Intracellular serglycin is a key mediator of granulopoiesis in mast cells, cytolytic T lymphocytes (CTLs), and neutrophils[8-11], and it is involved in the retention or secretion of proteases, histamine, cytokines, and chemokines in the storage granules of mast cells or canine kidney cells[12-15]. Extracellular serglycin released from the storage granules of mast cells and CTLs (or from monocytes, macrophages, and endothelial cells that constitutively secrete serglycin) has been shown to interact with CD44 in hematopoietic cells, suggesting that serglycin may take on an important role in cell-cell interactions[16-18]. Secretion of serglycin is involved in the release of tissue-type plasminogen activator from endothelial cells, tumor necrosis factor-α from macrophages, and matrix metalloproteinase-9 (MMP-9) from monocytes[19-21]. Although serglycin-knockout mice have been generated by Abrink *et al.*[22], the biological functions of serglycin have not been studied in detail.

In this article, we focus on the biological roles of serglycin in tumor metastasis as well as some related physiological characteristics of this interesting molecule.

Expression of Serglycin in Hematopoietic Cells

Both serglycin mRNA and protein have been detected in human hematopoietic cancer cell lines. Niemann *et al.*[23] found that serglycin is distinctively

Authors' Affiliations: [1]State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060, P. R. China. [2]Laboratory of Cancer and Developmental Cell Biology, Van Andel Research Institute, Grand Rapids, MI 49503, USA.

Corresponding Authors: Chao-Nan Qian, [1]State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060, P. R. China; [2]Laboratory of Cancer and Developmental Cell Biology, Van Andel Research Institute, 333 Bostwick Ave. N.E., Grand Rapids, MI 49503, USA. Email: qianchn@sysucc.org.cn.

doi: 10.5732/cjc.011.10314

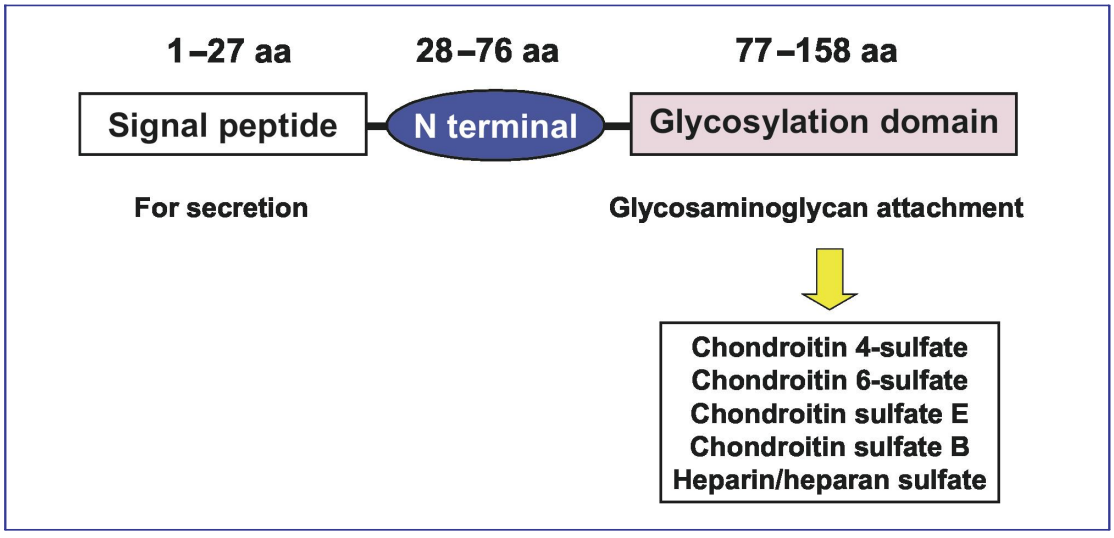

Figure 1. The protein structure of serglycin.

expressed in acute myeloid leukemia (AML) relative to lymphoblastic leukemia. Serglycin is also a selective marker distinguishing AML from Philadelphia chromosome-negative chronic myeloproliferative disorders [23]. In multiple myeloma cells, serglycin is a secreted protein decorated predominantly with CS-4, suggesting that serglycin has an effect on inhibition of bone mineralization[24]. Moreover, serglycin demonstrates a role in protecting myeloma cells from complement attacks induced by antibody immunotherapy, therefore promoting the survival of malignant myeloma cells [25]. Up-regulation of serglycin expression is found in drug-resistant tumor cell lines of hematopoietic origin, indicating that serglycin may be involved in the drug resistance of human cancer cells [26]. In a variety of hematopoietic cells, including myelomonocytes, macrophages, and lymphoma, myeloma, mastochytoma, or thymoma cells, serglycin has been shown to interact with cell surface protein CD44 if serglycin has attached CS-4 or CS-6 moieties, but not heparin or heparan sulfate[16].

Serglycin Promotes Metastasis of Nasopharyngeal Carcinoma Cells

Serglycin is highly expressed in high-metastasis nasopharyngeal carcinoma (NPC) cells[27]. In primary NPC tissues, a higher level of serglycin serves as an independent prognostic indicator for disease-free survival and distant metastasis-free survival of patients. *In vitro* and *in vivo* studies have proven that serglycin can promote motility, invasion, and metastasis of NPC cells via induction of a mesenchymal molecule vimentin [27]. This important function of serglycin depends on full glycosylation of the core protein [27]. In NPC cells, intracellular serglycin has a molecular weight of about 130 kDa, but the secreted form is about 300 kDa. Overexpression of serglycin by NPC cells dramatically increases only the amount of secreted serglycin but does not alter the intracellular serglycin level, suggesting that its metastasis-promoting function is mainly determined by the secreted form[27].

Serglycin Secretory Mechanism in Human Cells

Sequence analyses of the serglycin core protein revealed that amino acid residues 1–27 form the signal peptide[5]. In some hematopoietic cells, including monocytes or macrophages, myeloma cells, and human endothelial cells, serglycin is constitutively secreted [19,20,24,25,28,29]. In other hematopoietic cells, including neutrophils and mast cells, serglycin is stored in secretory granules and secreted after cell activation[9,13]. Cytokines, chemokines, and proteases interact with serglycin during secretion from secretory granules and can still be in complex with serglycin after its release from cells [12,30-34]. In the extracellular matrix (ECM), serglycin is involved in the activation of MMPs, which have important roles in inflammation, wound repair, cellular invasion, and other fundamental processes[21,35,36]. Secretion of tumor necrosis factor from macrophages has been reported to be regulated by serglycin[20]. In addition, serglycin from CTLs or natural killer (NK) cells can form macro-complexes with granzyme B and perforin to induce the apoptosis of target cells [37-40]. The apoptosis-promoting effect of serglycin was found in mast cells, and this effect was associated with the release of serglycin and serglycin-dependent proteases into the cytosol[41]. Studies

of serglycin released from human cancer cells should provide insight into new signaling pathways through which serglycin is involved in tumor progression.

Diversity of Glycan Chains on Serglycin

The glycosaminoglycan chains attached to serglycin vary among different cell types. In hematopoietic cells—including platelets, CTLs, NK cells, and mucosal mast cells (other than mast cells in connective tissues or the peritoneal cavity)—chondroitin sulfate is the major glycan, and CS-4 is the dominant form in most of these cell types[42]. Heparin is the glycan attachment to serglycin in peritoneal or connective tissue mast cells[15].

The localization of serglycin is determined by the types of glycan attached to the serglycin protein. In Madin-Darby canine kidney (MDCK) cells, chondroitin sulfate serglycin is generally secreted into the apical medium and heparin serglycin is mainly transported to basolateral membrane of the cells[43-45]. In human endothelial cells, serglycin is a major proteoglycan that carries mainly chondroitin sulfate chains and some heparin chains. A major amount of serglycin is secreted into apical medium and a small amount co-localizes with growth-related oncogene α (GROα /CXCL1) in vesicles[46]. In acinar pancreatic cells, serglycin without attached glycans is not able to be sorted into secretory granules[47]. Glycan modification of serglycin is a key factor determining its localization and biological roles in mammalian cells. Although serglycin's function in promoting metastasis depends on full glycosylation[27], glycan modification of serglycin is poorly understood in human cancer cells.

Serglycin Signaling for Clinical Applications

As a secreted glycoprotein, serglycin could be an ideal serum marker for predicting and monitoring cancer progression. Further, serglycin's role in promoting metastasis depends on interaction between the secreted protein and cancer cells, making that interaction an ideal drug target. Serglycin promotes NPC cell metastasis via autocrine and paracrine signaling[27], but how secreted serglycin triggers the cascade leading to cellular motility remains unknown. It is therefore crucial to identify the membrane-binding protein(s) of serglycin that help promote migration, invasion, and metastasis.

Serglycin binds to CD44 on hematopoietic cells[16]. CD44 itself is a glycosylated protein found at cell surface, and it is involved in cell-cell interactions, cell adhesion, and cellular migration[48,49]. CD44 is a cancer stem cell marker for a variety of cancer types, including carcinomas of the pancreas, colon, ovary, breast, and liver[50-54]. Some aggressive behaviors of cancer cells, e.g., apoptosis resistance, epithelial-mesenchymal transition, and metastasis, have been linked to CD44[49]. It is therefore important to clarify whether serglycin signaling depends on binding to CD44 at the cell surface.

Summary

Serglycin is widely expressed in various hematopoietic cells and highly metastatic NPC cells, and it is an important molecule in regulating NPC metastasis. Although great potential for clinical applications can be predicted, the exact molecular mechanism of serglycin signaling remains unclear. Further explorations in identifying serglycin-binding proteins and its signaling cascade in promoting cellular motility are needed.

Acknowledgements

This work was supported by a grant from the State Key Program of National Natural Science Foundation of China (No. 81030043). We thank David Nadziejka, Grand Rapids, Michigan, for critical reading of the manuscript.

Received: 2011-08-01; accepted: 2011-08-04.

References

[1] Schick BP. Regulatio n of expression of megakaryocyte and platelet proteoglycans [J]. Stem Cells, 1996,14 Suppl 1:220–231.
[2] Bourdon MA, Oldberg A, Pierschbacher M, et al. Molecular cloning and sequence analysis of a chondroitin sulfate proteoglycan cDNA [J]. Proc Natl Acad Sci U S A, 1985,82(5):1321–1325.
[3] Okayama M, Oguri K, Fujiwara Y, et al. Purification and characterization of human platelet proteoglycan [J]. Biochem J, 1986,233(1):73–81.
[4] Avraham S, Stevens RL, Nicodemus CF, et al. Molecular cloning of a cDNA that encodes the peptide core of a mouse mast cell secretory granule proteoglycan and comparison with the analogous rat and human cdna [J]. Proc Natl Acad Sci U S A, 1989,86(10):3763–3767.
[5] Kolset SO, Tveit H. Serglycin—structure and biology [J]. Cell Mol Life Sci, 2008,65(7–8):1073–1085.
[6] Nicodemus CF, Avraham S, Austen KF, et al. Characterization of the human gene that encodes the peptide core of secretory granule proteoglycans in promyelocytic leukemia HL-60 cells

and analysis of the translated product [J]. J Biol Chem, 1990,265(10):5889–5896.
[7] Kolset SO, Prydz K, Pejler G. Intracellular proteoglycans [J]. Biochem J, 2004,379(Pt 2):217–227.
[8] Niemann CU, Cowland JB, Klausen P, et al. Localization of serglycin in human neutrophil granulocytes and their precursors [J]. J Leukoc Biol, 2004,76(2):406–415.
[9] Niemann CU, Abrink M, Pejler G, et al. Neutrophil elastase depends on serglycin proteoglycan for localization in granules [J]. Blood, 2007,109(10):4478–4486.
[10] Whitaker-Menezes D, Schechter NM, Murphy GF. Serine proteinases are regionally segregated within mast cell granules [J]. Lab Invest, 1995,72(1):34–41.
[11] Grujic M, Braga T, Lukinius A, et al. Serglycin-deficient cytotoxic T lymphocytes display defective secretory granule maturation and granzyme B storage [J]. J Biol Chem, 2005,280(39):33411–33418.
[12] Henningsson F, Hergeth S, Cortelius R, et al. A role for serglycin proteoglycan in granular retention and processing of mast cell secretory granule components [J]. Febs J, 2006,273 (21):4901–4912.
[13] Braga T, Grujic M, Lukinius A, et al. Serglycin proteoglycan is required for secretory granule integrity in mucosal mast cells [J]. Biochem J, 2007,403(1):49–57.
[14] Zernichow L, Dalen KT, Prydz K, et al. Secretion of proteases in serglycin transfected Madin-Darby canine kidney cells [J]. Febs J, 2006,273(3):536–547.
[15] Humphries DE, Wong GW, Friend DS, et al. Heparin is essential for the storage of specific granule proteases in mast cells [J]. Nature, 1999,400(6746):769–772.
[16] Toyama-Sorimachi N, Kitamura F, Habuchi H, et al. Widespread expression of chondroitin sulfate-type serglycins with CD44 binding ability in hematopoietic cells [J]. J Biol Chem, 1997,272(42):26714–26719.
[17] Toyama-Sorimachi N, Miyasaka M. A sulfated proteoglycan as a novel ligand for CD44 [J]. J Dermatol, 1994,21(11):795–801.
[18] Toyama-Sorimachi N, Sorimachi H, Tobita Y, et al. A novel ligand for CD44 is serglycin, a hematopoietic cell lineage-specific proteoglycan. Possible involvement in lymphoid cell adherence and activation [J]. J Biol Chem, 1995,270 (13): 7437–7444.
[19] Schick BP, Gradowski JF, San Antonio JD. Synthesis, secretion, and subcellular localization of serglycin proteoglycan in human endothelial cells [J]. Blood, 2001,97(2):449–458.
[20] Zernichow L, Abrink M, Hallgren J, et al. Serglycin is the major secreted proteoglycan in macrophages and has a role in the regulation of macrophage tumor necrosis factor-alpha secretion in response to lipopolysaccharide [J]. J Biol Chem, 2006,281 (37):26792–26801.
[21] Winberg JO, Kolset SO, Berg E, et al. Macrophages secrete matrix metalloproteinase 9 covalently linked to the core protein of chondroitin sulphate proteoglycans [J]. J Mol Biol, 2000,304 (4):669–680.
[22] Abrink M, Grujic M, Pejler G. Serglycin is essential for maturation of mast cell secretory granule [J]. J Biol Chem, 2004,279(39):40897–40905.
[23] Niemann CU, Kjeldsen L, Ralfkiaer E, et al. Serglycin proteoglycan in hematologic malignancies: a marker of acute myeloid leukemia [J]. Leukemia, 2007,21(12):2406–2410.
[24] Theocharis AD, Seidel C, Borset M, et al. Serglycin constitutively secreted by myeloma plasma cells is a potent inhibitor of bone mineralization in vitro [J]. J Biol Chem, 2006,281(46):35116–35128.
[25] Skliris A, Happonen KE, Terpos E, et al. Serglycin inhibits the classical and lectin pathways of complement via its glycosaminoglycan chains: Implications for multiple myeloma [J]. Eur J Immunol, 2011,41(2):437–449.
[26] Beyer-Sehlmeyer G, Hiddemann W, Wormann B, et al. Suppressive subtractive hybridisation reveals differential expression of serglycin, sorcin, bone marrow proteoglycan and prostate-tumour-inducing gene I (PTI-1) in drug-resistant and sensitive tumour cell lines of haematopoetic origin [J]. Eur J Cancer, 1999,35(12):1735–1742.
[27] Li XJ, Ong CK, Cao Y, et al. Serglycin is a theranostic target in nasopharyngeal carcinoma that promotes metastasis [J]. Cancer Res, 2011,71(8):3162–3172.
[28] Kolset SO, Zernichow L. Serglycin and secretion in human monocytes [J]. Glycoconj J, 2008,25(4):305–311.
[29] Uhlin-Hansen L, Wik T, Kjellen L, et al. Proteoglycan metabolism in normal and inflammatory human macrophages [J]. Blood, 1993,82(9):2880–2889.
[30] Ringvall M, Ronnberg E, Wernersson S, et al. Serotonin and histamine storage in mast cell secretory granules is dependent on serglycin proteoglycan [J]. J Allergy Clin Immunol, 2008,121 (4):1020–1026.
[31] Kolset SO, Mann DM, Uhlin-Hansen L, et al. Serglycin-binding proteins in activated macrophages and platelets [J]. J Leukoc Biol, 1996,59(4):545–554.
[32] Matsumoto R, Sali A, Ghildyal N, et al. Packaging of proteases and proteoglycans in the granules of mast cells and other hematopoietic cells. A cluster of histidines on mouse mast cell protease 7 regulates its binding to heparin serglycin proteoglycans [J]. J Biol Chem, 1995,270(33):19524–19531.
[33] Serafin WE, Katz HR, Austen KF, et al. Complexes of heparin proteoglycans, chondroitin sulfate E proteoglycans, and [3H] diisopropyl fluorophosphate-binding proteins are exocytosed from activated mouse bone marrow-derived mast cells [J]. J Biol Chem, 1986,261(32):15017–15021.
[34] Pejler G, Abrink M, Wernersson S. Serglycin proteoglycan: regulating the storage and activities of hematopoietic proteases [J]. Biofactors, 2009,35(1):61–68.
[35] Lundequist A, Abrink M, Pejler G. Mast cell-dependent activation of pro matrix metalloprotease 2: a role for serglycin proteoglycan-dependent mast cell proteases [J]. J Biol Chem, 2006,387(10–11):1513–1519.
[36] Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior [J]. Annu Rev Cell Dev Biol, 2001,17:463–516.
[37] Metkar SS, Wang B, Aguilar-Santelises M, et al. Cytotoxic cell granule-mediated apoptosis: perforin delivers granzyme B-serglycin complexes into target cells without plasma membrane pore formation [J]. Immunity, 2002,16(3):417–428.
[38] Galvin JP, Spaeny-Dekking LH, Wang B, et al. Apoptosis induced by granzyme B-glycosaminoglycan complexes: implications for granule-mediated apoptosis in vivo [J]. J Immunol, 1999,162(9):5345–5350.
[39] Raja SM, Wang B, Dantuluri M, et al. Cytotoxic cell granule-mediated apoptosis. Characterization of the macromolecular complex of granzyme B with serglycin [J]. J Biol Chem, 2002,277(51):49523–49530.
[40] Veugelers K, Motyka B, Goping IS, et al. Granule-mediated killing by granzyme B and perforin requires a mannose 6-phosphate receptor and is augmented by cell surface heparan sulfate [J]. Mol Biol Cell, 2006,17(2):623–633.
[41] Melo FR, Waern I, Ronnberg E, et al. A role for serglycin proteoglycan in mast cell apoptosis induced by a secretory granule-mediated pathway [J]. J Biol Chem, 2011,286 (7): 5423–5433.
[42] Kolset SO, Gallagher JT. Proteoglycans in haemopoietic cells [J]. Biochim Biophys Acta, 1990,1032(2–3):191–211.
[43] Kolset SO, Vuong TT, Prydz K. Apical secretion of chondroitin sulphate in polarized madin-darby canine kidney (MDCK) cells [J]. J Cell Sci, 1999,112(Pt 11):1797–1801.
[44] Mertens G, Van der Schueren B, van den Berghe H, et al. Heparan sulfate expression in polarized epithelial cells: the apical sorting of glypican (GPI-anchored proteoglycan) is inversely related to its heparan sulfate content [J]. J Cell Biol,

1996,132(3):487–497.
[45] Hafte TT, Fagereng GL, Prydz K, et al. Protein core-dependent glycosaminoglycan modification and glycosaminoglycan-dependent polarized sorting in epithelial madin-darby canine kidney cells [J]. Glycobiology, 2011,21(4):457–466.
[46] Meen AJ, Oynebraten I, Reine TM, et al. Serglycin is a major proteoglycan in polarized human endothelial cells and is implicated in the secretion of the chemokine GROalpha/CXCL1 [J]. J Biol Chem, 2011,286(4):2636–2647.
[47] Biederbick A, Licht A, Kleene R. Serglycin proteoglycan is sorted into zymogen granules of rat pancreatic acinar cells [J]. Eur J Cell Biol, 2003,82(1):19–29.
[48] Spring FA, Dalchau R, Daniels GL, et al. The Ina and Inb blood group antigens are located on a glycoprotein of 80,000 MW (the CDw44 glycoprotein) whose expression is influenced by the In(Lu) gene [J]. Immunology, 1988,64(1):37–43.
[49] Zoller M. CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? [J]. Nat Rev Cancer, 2011,11 (4):254–267.
[50] Li C, Lee CJ, Simeone DM. Identification of human pancreatic cancer stem cells [J]. Methods Mol Biol, 2009,568:161–173.
[51] Casagrande F, Cocco E, Bellone S, et al. Eradication of chemotherapy-resistant CD44 + human ovarian cancer stem cells in mice by intraperitoneal administration of clostridium perfringens enterotoxin [J]. Cancer, 2011 Jun 20. doi: 10.1002/cncr.26215. [Epub ahead of print]
[52] Liu LL, Fu D, Ma Y, et al. The power and the promise of liver cancer stem cell markers [J]. Stem Cells Dev, 2011 Jun 8. [Epub ahead of print]
[53] Ricardo S, Vieira AF, Gerhard R, et al. Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype [J]. J Clin Pathol, 2011 Jun 16. [Epub ahead of print]
[54] Su YJ, Lai HM, Chang YW, et al. Direct reprogramming of stem cell properties in colon cancer cells by CD44 [J]. EMBO J, 2011,30(15):3186–3199.