Glycoconj J (2014) 31:461–467
DOI 10.1007/s10719-014-9551-9

Roles of glycosaminoglycans and glycanmimetics in tumor progression and metastasis

Basappa · Kanchugarakoppal S. Rangappa · Kazuyuki Sugahara

Published online: 17 September 2014

Abstract Various tumor cells exhibit structural alterations in the sulfated modifications to glycosaminoglycans (GAGs). The altered expression of chondroitin sulfate (CS) and heparan sulfate (HS) on the surfaces of tumor cells is known to play a key role in malignant transformation and tumor metastasis. The receptor molecule for the CS chains containing E-disaccharide units (CS-E) expressed on Lewis lung carcinoma (LLC) cells was recently revealed to be Receptor for Advanced Glycation End-products (RAGE). RAGE is also involved in the development of various pathological conditions including aging, infection, pulmonary fibrosis, diabetes, and Alzheimer's disease, by binding to a wide range of ligands. RAGE binds strongly not only to CS-E, but also to HS-expressing LLC cells. Recombinant RAGE bound CS-E and HS with high affinity. Furthermore, in a mouse model, the colonization of the lungs by LLC cells was inhibited by intravenously injected CS-E, an anti-CS-E antibody, or an anti-RAGE antibody. These findings demonstrated that RAGE is at least one of the critical receptors for CS and HS chains expressed on the tumor cell surface and is involved in experimental lung metastasis, and also that CS/HS and RAGE are potential molecular targets for the treatment of pulmonary metastasis. We, hence, reviewed these findings and also several chemically synthesized small GAGmimetics that exhibit potent anti-metastatic and/or anti-tumor activities.

Keywords Heparan sulfate · Chondroitin sulfate · Glycosaminoglycans · Tumor metastasis · Tumor progression · RAGE

Abbreviations

CS	Chondroitin sulfate
DS	Dermatan sulfate
HS	Heparan sulfate
VEGF	Vascular endothelial growth factor
FGF2	Fibroblast growth factor-2
GAG	Glycosaminoglycan

Introduction

Cancer is the leading cause of deaths. Since the number of cancer-related deaths is increasing, there is an urgent need for clinical markers that can be used to determine the risk of developing tumors, and also serve as tools for initial diagnoses as well as monitoring tumor progression and the effects of medication. Glycans regulate tumor proliferation, invasion, hematogenous metastasis, and angiogenesis; therefore, a clearer understanding of these roles will set the fundamental stage for developing pharmaceutical agents that target these molecules. Such novel agents may be used alone or in combination with other strategies for treatment of cancers such as surgery and/or chemoradiation [1]. It is estimated that over 50 % of all human proteins are glycosylated [2]. Glycosylation has been detected on cell surfaces and in extracellular matrices that create the initial point of contact in cellular interactions [3]. Therefore, the effects of disease states

Basappa
Laboratory of Chemical Biology, Department of Chemistry, Bangalore University, Palace Road, Bangalore 560001, India
e-mail: salundibasappa@yahoo.com

K. S. Rangappa
Department of Chemistry, University of Mysore, Manasagangotri, Mysore 560001, India
e-mail: rangappaks@yahoo.com

K. Sugahara (✉)
Research Group of Proteoglycan Signaling and Therapeutics, Hokkaido University Graduate School of Life Science, Frontier Research Center for Post-Genomic Science and Technology, West-11, North-21, Kita-ku, Sapporo, Hokkaido 001-0021, Japan
e-mail: k-sugar@sci.hokudai.ac.jp

on the biosynthesis of glycans may be clearer than disease-associated changes to proteins. It is now well established that altered glycosylation varies more significantly among tumor cells than among normal cells [4–6]. The recognition of glycans as mediators of important biological processes has stimulated growing interest in glycobiology research [3].

Mechanism underlying glycosaminoglycan-mediated tumor metastasis

Previous studies from our and other laboratories demonstrated that various tumor cells exhibited structural alterations in the sulfated modifications to glycosaminoglycans (GAGs), including chondroitin sulfate (CS) and dermatan sulfate (DS), expressed on tumor cell surfaces during malignant progression [7,8]. The altered expression of CS and heparan sulfate (HS) on the surfaces of tumor cells has been shown to play a key role in malignant transformation and tumor metastasis [9,10]. A Lewis lung carcinoma (LLC)-derived tumor cell line with high metastatic potential had a higher proportion of E-disaccharide units, GlcUA-GalNAc (4,6-*O*-disulfate), in the CS chains than low metastatic LLC cells, in which GlcUA and GalNAc represent D-glucuronic acid and *N*-acetyl-D-galactosamine, respectively. Metastasis was inhibited by the pre-administration of CS-E rich in E-disaccharide units derived from squid cartilage or by pre-incubation with the phage display single chain antibody GD3G7 [9], which is specific to CS-E [11,12] and stains human pancreatic and ovarian cancer tissues [12,13]. Similar results were also obtained with a mouse osteosarcoma cell line metastatic to the liver [14] and a human B16 melanoma cell line metastatic to the lungs [8]. However, the molecular mechanism underlying this inhibition has yet to be investigated.

The receptor molecule for CS chains containing E-disaccharides expressed on LLC cells was revealed to be Receptor for Advanced Glycation End-products (RAGE) [15], which is a member of the immunoglobulin superfamily predominantly expressed in the lung. RAGE is known to be involved in the development of various diseases and pathological conditions including aging, infection, pulmonary fibrosis, diabetes, and Alzheimer's disease, by binding to various ligands (Fig. 1). RAGE bound strongly not only to E-disaccharides, but also to HS-expressing LLC cells. Recombinant RAGE bound CS-E and HS with high affinity [15]. Furthermore, the colonization of the lungs by LLC cells was inhibited by an anti-RAGE antibody administered via intravenous injections [15]. These findings demonstrated that RAGE is at least one of the critical receptors for CS and HS chains expressed on the tumor cell surface and is involved in experimental lung metastasis, and also that CS/HS and RAGE are potential molecular targets in the treatment of pulmonary metastasis (Fig. 2). CS-E-derived decasaccharide sequences (Table 1) that may be involved in binding to RAGE have also been proposed [9,11,16].

GAGmimetic small compounds

In our recent studies, a novel heterocyclic compound that mimics such glycans was designed and synthesized, and exhibited the ability to bind several growth factors/cytokines. Preclinical data also indicated its potential anti-cancer effects, as described below.

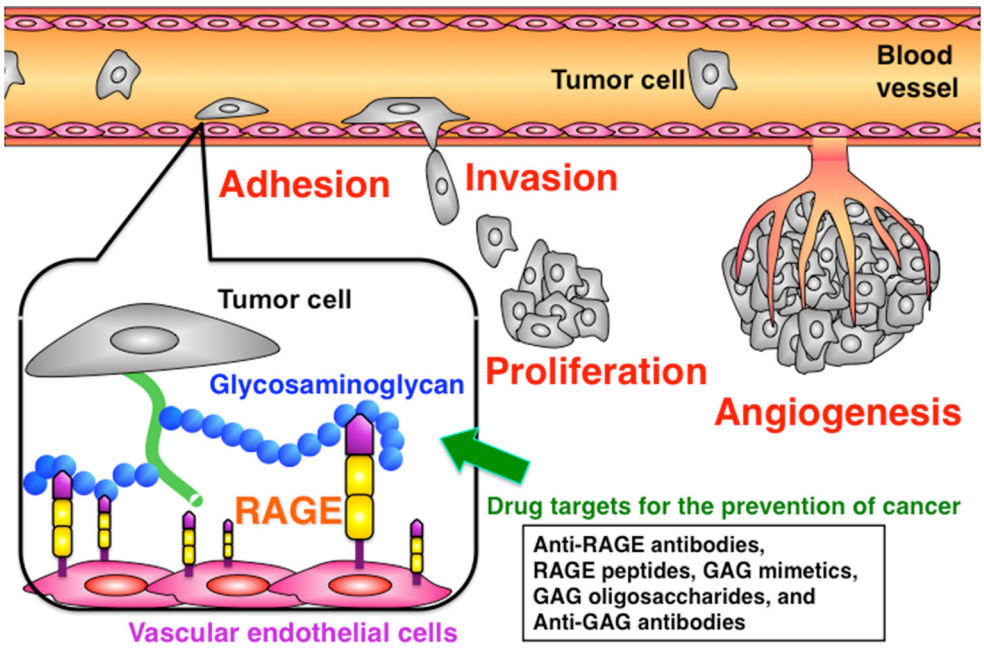

Fig. 1 Schematic presentation of pulmonary metastasis involving GAGs on tumor cells and RAGE in mouse lungs. Tumor cells proliferate at the primary site, are released therefrom, and migrate/invade into blood vessels. Tumor cells circulating in the bloodstream adhere to vascular endothelial cells in the lung through the capture of GAGs by RAGE (inset). Subsequently, the tumor cells exhibit invasion, proliferation and angiogenesis at the secondary sites. Hence, GAG, their fragments and GAGmimetics with RAGE-binding capacity or RAGEmimetics are potential targets for drug discovery. (taken from ref. [8] and modified)

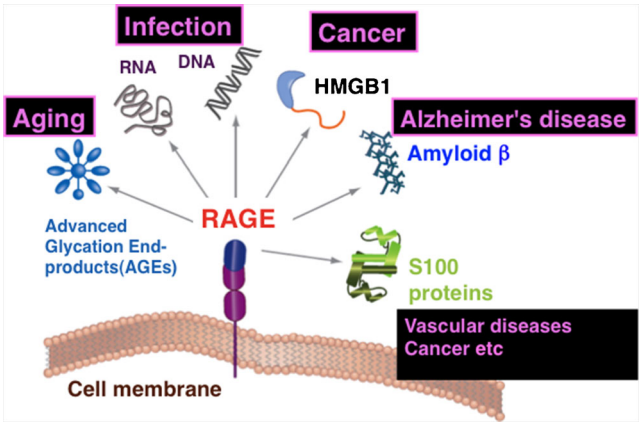

Fig. 2 Ligands of Receptor for Advanced Glycation End-products (RAGE) associated various pathological conditions. RAGE binds a variety of substances and is involved in pathological conditions of a variety of diseases. Figure 3 of ref. [Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ (2010) HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol 28:367–388] was modified

HS (Fig. 3a) on cell surfaces modulates signal transduction into tumor cells by interacting with various growth factors such as fibroblast growth factor-2 (FGF2), vascular endothelial growth factor (VEGF), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) [17–19]. HS on cell surfaces and in extracellular matrices plays a major role in tumor metastasis, and acts as a storage shed for various proteins. Heparanase, a family member of the endo-β-D-glucuronidases promotes tumor cell invasion by degrading HS in the extracellular matrix. Heparanase also promotes cell proliferation, metastasis, and angiogenesis by releasing growth factors such as FGF2 and VEGF from HS [20]. Several studies have shown that HSmimetics act as anti-tumor agents. For example, the anti-tumor and anti-heparanase activities of the non-sugar-based HSmimetic compound KI-105 (2-[3-nitro-4-(phenylthio)benzoyl]benzoic acid) have been reported previously [21].

Table 1 Structure of the decasaccharide sequences isolated from commercial CS-E, which was purified from the squid (Superspecial grade of Seikagaku Corp., Tokyo)

Disaccharide sequences detected in isolated decasachharide fractions from CS-E of the squid
E-E-E-E-E*
E-E-E-A-A
E-E-E-E-A
E-E-E-E-C

*A-, C-, and E-units represent GlcUA-GalNAc (4-*O*-sulfate) GlcUA-GalNAc (6-*O*-sulfate), and GlcUA-GalNAc (4,6-*O*-disulfate), respectively

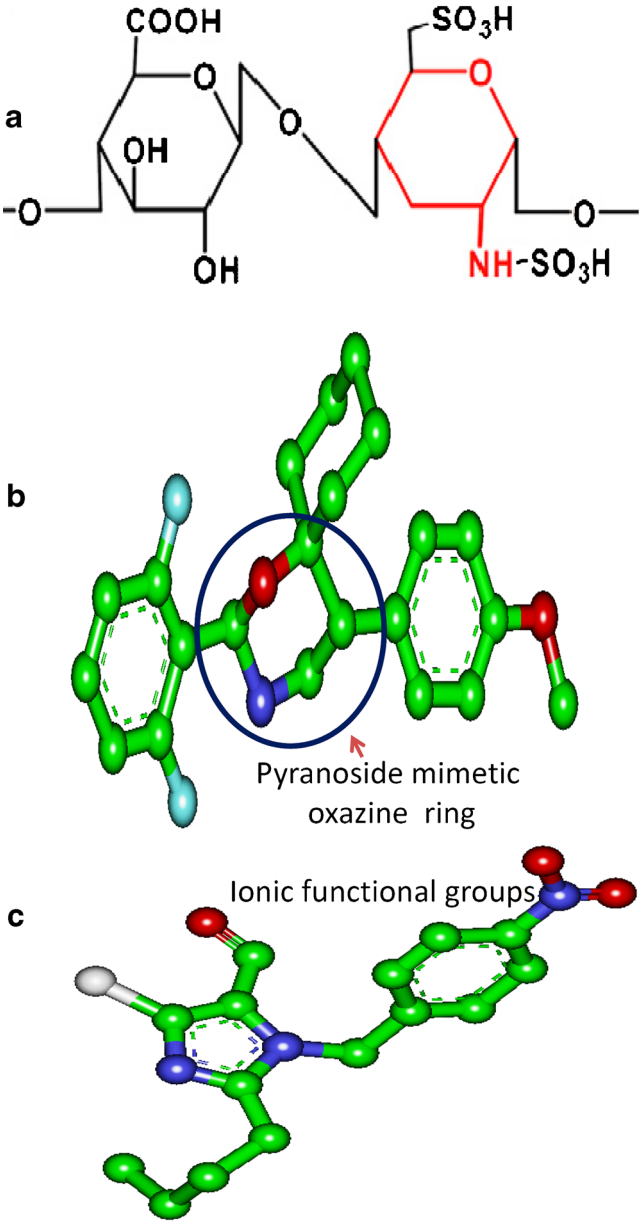

Fig. 3 **a** Structure of the representative repeating disaccharide unit, which is characteristic of major disaccharide units found in HS chains; **b** An oxazine sugarmimetic DMBO (2-(2,6-difluorophenyl)-4a,5,6,7,8,8a-hexahydro-4a-(4-methoxyphenyl)-4H-benzo[e][1,3]oxazine), and **c** Imidazole aldehyde sugarmimetic NIC (2-butyl-5-chloro-3-(4-nitro-benzyl)-3H-imidazole-4-carbaldehyde). In the search for effective therapeutic agents for cancers, which target multiple pathways, we designed and synthesized a novel six-member oxazine compound DMBO **b**, a class of sugarmimetic, in which the ring carbon has been replaced by a nitrogen atom. The oxazine nucleus of the DMBO appears to mimic the pyranoside ring structure, which is the monosaccharide backbone of the sugar residues in HS. NIC, a hybrid of HSmimetic core structure of KI-105, was prepared by replacing the benzene group by imidazole, since the chemistry of imidazole occupies an extremely important niche within the family of 5-membered heterocyclic compounds [23]. NIC is considered to mimic the HS non-structurally. We have reported that NIC, directly binds to the heparin-binding domain of VEGF, as detected by high throughput surface plasmon resonance (SPR) analysis, as well as by *in silico* binding analysis, and showed promising antitumor activity in experimental model of liver matastasis [23]

The small oxazine compound, DMBO (2-(2,6-difluorophenyl) -4a,5,6,7,8,8a-hexahydro-4a-(4-methoxyphenyl) -4H-benzo[e][1,3]oxazine), as an anti-tumor agent

DMBO (Fig. 3b) mimics the pyranosidic ring structure of HS. DMBO also possesses the ability to bind various growth factors/cytokines such as VEGF, HB-EGF, and TNF-α, and also synergistically exhibits anti-tumor activity with heparin (Fig. 4a and b) [22]. This appears to have been the first study on a synthetic oxazine compound that can mimic the functional groups and pyranosidic ring structure of HS by interacting directly with growth factors/cytokines with high affinity. Thus, it has been proposed that DMBO may bind VEGF and effectively block the ability of VEGF to activate its receptor and subsequent signaling. Furthermore, the *in vivo* anti-metastatic mechanism of DMBO on LM8G7 cells has been attributed to its ability to interrupt heparanase-associated pathological events, which is the most important and comparable to other anti-metastatic sugarmimetic agents (Fig. 4c).

Anti-tumor activity of the novel HSmimetic NIC (2-butyl-5-chloro-3-(4-nitro-benzyl) -3H-imidazole-4-carbaldehyde): a VEGF-binding small molecule

We compared the structure of NIC (originally reported as Compound 8) [23] (Fig. 3c) with HS, both of which have common functional ionic groups such as sulfate, nitro, and carbaldehyde, which can be located at similar positions in the disaccharide structural units of HS. Molecular docking studies revealed that NIC binds the heparin-binding domain of VEGF through strong hydrogen bonding with Lys-30 and Gln-20 amino acid residues, which is consistent with the prediction that NIC inhibited the binding of VEGF to immobilized heparin. *In vitro* studies showed that the compound NIC inhibited the VEGF-induced proliferation, migration, and tube formation of mouse vascular endothelial cells, and also the invasion of a murine osteosarcoma cell line (LM8G7) [23], which secretes high levels of VEGF. *In vivo*, these effects significantly reduced the tumor burden in an experimental

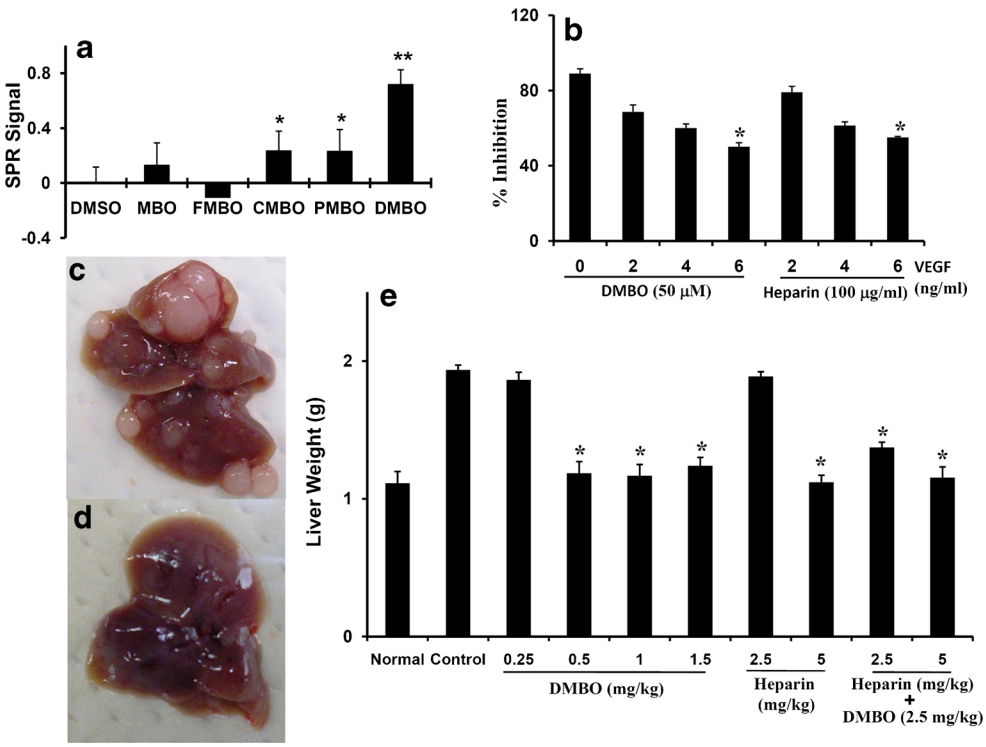

Fig. 4 Anti-tumor activity of DMBO. **a** Interaction between immobilized DMBO and soluble VEGF is shown by SPR analysis with maximum signal strength as compared with DMBO's analogs. **b** Effects of DMBO and heparin on the proliferation LM8G7 cells is presented. LM8G7 cells were incubated with the indicated concentrations of DMBO or heparin in the presence of various concentrations of VEGF (2–6 ng/mL) and its inhibition of the binding of VEGF was measured. **c**–**e** Effects of DMBO against the metastatic potential of mouse osteosarcoma cells are presented. LM8G7 cells were intravenously injected with DMBO into C3H/HeN mice, with the indicated doses on days 3, 5 and 10. After 4 weeks, the number of liver nodules in the mice was counted macroscopically, and the liver weight was measured in the control and the tested mice injected with DMBO, heparin or both. **c** and **d**) Representative livers from mice injected with LM8G7 cells treated with DMEM (**c**, negative control) and DMBO (0.5 mg/kg body weight) **d** are shown. **e** The average liver weight of the control and the DMBO-treated mice. The possible synergistic effects of DMBO and heparin were investigated. Data represent mean values±S.D. for three independent experiments and each experiment was conducted with six mice per group. **P* <0.05 versus control. Mann–Whitney *U* test

model of liver metastasis [23]. Collectively, these findings indicate that NIC prevents the growth of tumors by directly influencing tumor cell proliferation and inhibiting VEGF-mediated endothelial cell migration and angiogenesis. In conclusion, the GAGmimetic NIC may improve the vasculature and microenvironment in tumors by inhibiting the binding of VEGF to its receptor.

Analysis of the energy-minimized structures of HexUA (2-*O*-sulfate)-GlcNAc (6-*O*-sulfate), in which HexUA and GlcNAc stand for hexuronic acid and *N*-acetyl-D-glucosamine, respectively, and NIC revealed that anionic groups, such as sulfate, carboxylic acid, and carbaldehyde as well as nitro functional groups, are located in similar positions and directions. Because of this structural similarity (by functional groups) to HS, NIC may be able to bind to $VEGF_{165}$ at the heparin-binding site. A surface plasmon resonance assay revealed that NIC interacted strongly with VEGF, and weakly with FGF-2. Molecular docking analysis of the GAGmimetic NIC to the heparin-binding site of VEGF using the Ligand Docker (CDOCKER) of Discovery Studio (DS) program revealed that NIC bound to the heparin-binding domain of VEGF (Fig. 5a) with high affinity. Visual analysis of the docked imidazole and benzene moiety of NIC showed that it interacted with the key amino acid residues Val-19, Phe-18, His-15, Lys-16, and Pro-9 of the heparin-binding domain of VEGF. In addition, NIC bound through hydrogen bonding to Lys-30 and Gln-20 residues at the heparin-binding pocket of VEGF (Fig. 5b). A similar finding has been reported previously for the compound KI-105, which binds to the HS-binding site of heparanase [21]. These findings indicated that

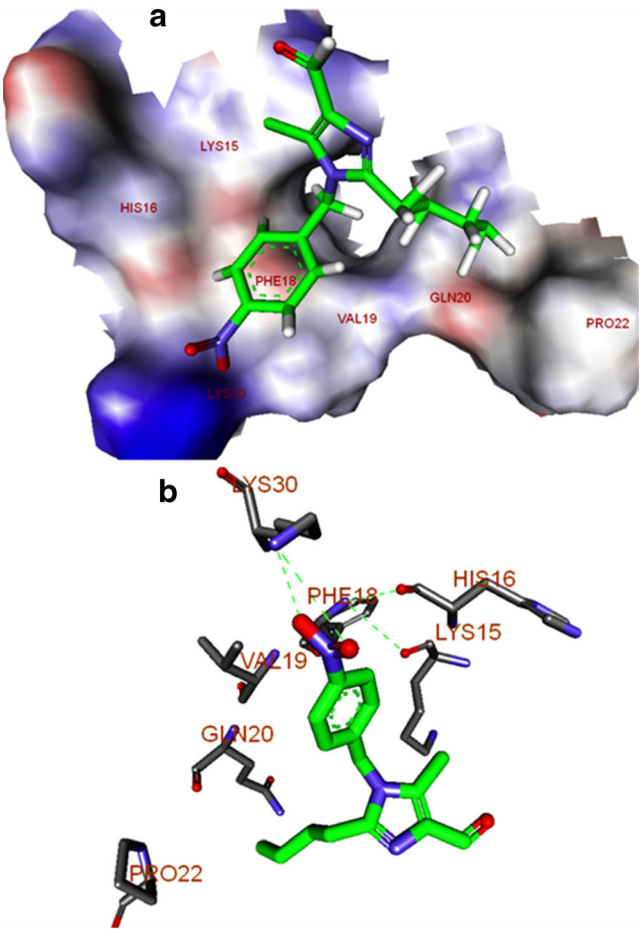

Fig. 5 Molecular basis for the interaction between Compound NIC and heparin-binding domain of $VEGF_{165}$. **a** Binding mode of NIC with heparin-binding site of $VEGF_{165}$. The amino acid residues of heparin-binding site of $VEGF_{165}$ are shown in stick models. **b** Interactions of NIC within the heparin-binding pocket of $VEGF_{165}$ with putative hydrogen bonds shown as green dotted lines. NIC is shown in green color. The bonding between NIC and the heparin-binding domain of $VEGF_{165}$ are shown in yellow color. All the hydrogen atoms are not shown. The picture is rendered in Discovery Studio, version 2.5 (right panel) (taken from ref. [23])

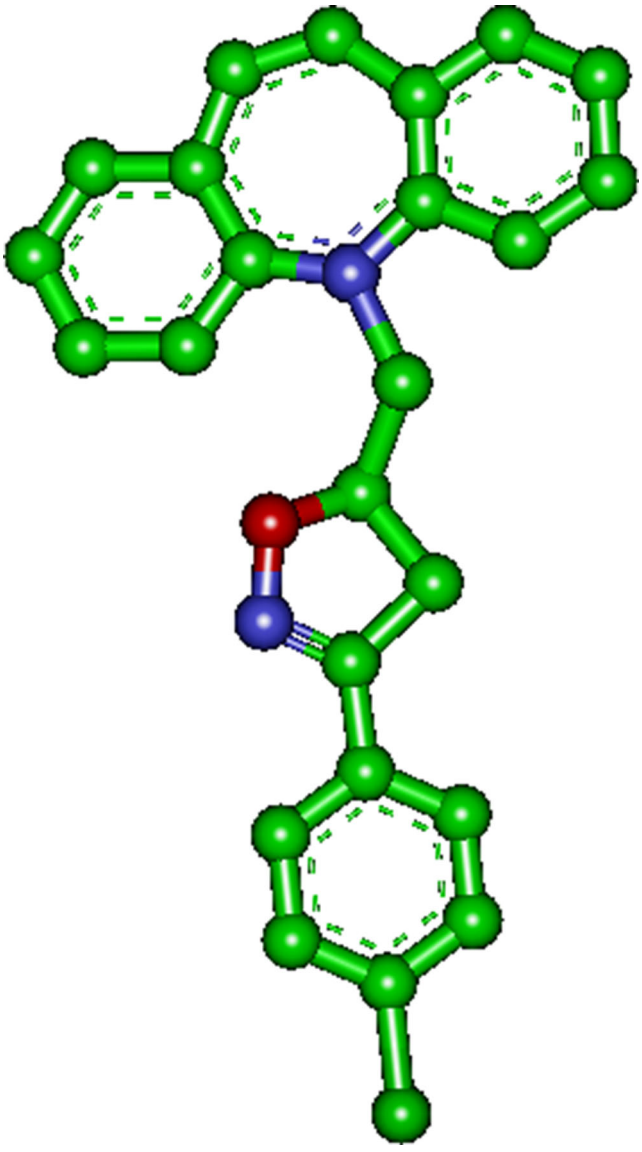

Fig. 6 The ball-and stick model of the most active anti-cancer compound, CDD ((5-[−3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl-methyl]-5H-dibenzo [b,f] azepine), which belongs to the isoxazoline tethered to dibenzoazepine (DB) was given

NIC initially competes or binds to the heparin-binding domain of VEGF, thereby decreasing the binding of VEGF to immobilized heparin.

Anti-cancer activities of novel dibenzo [b,f] azepine tethered isoxazoline derivatives

Dibenzoazepine (DB) derivatives are important and valuable compounds in medicinal chemistry. The replacement of the ribose ring with an isoxazolidine nucleus has also emerged as an important class of dideoxynucleoside analogues [24]. These analogues mimic natural nucleosides, undergo phosphorylation by cellular kinases, are metabolized by enzymes, and are then inserted in the DNA growing chain, in which they are finally modified as chain terminators [25]. Therefore, we synthesized a DB-fused hybrid structure containing isoxazolines. The effects of CDD (Fig. 6) (originally reported as Compound 4 g) (5-[−3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl-methyl]-5H-dibenzo [b,f] azepine) on the invasion of highly metastatic murine osteosarcoma (LM8G7) cells were studied using Matrigel™-coated porous membranes [26]. LM8G7 cells, which expres high amounts of CS, were found to be highly invasive. Among the tested molecules, compound CDD was found to more strongly inhibit the invasion of LM8G7 cells than other structurally related compounds. The compound CDD completely inhibited the invasion of MDA-MB-231 cells at 10 μM (Fig. 7). In addition to its anti-invasion properties the CDD also dose-dependently inhibited the migration of LM8G7 and human ovarian cancer cells (OVSAHO) dose-dependently. The compound CDD inhibited the proliferation of LM8G7, OVSAHO, human breast cancer cells (MCF-7), and human melphalan-resistant multiple myeloma (RPMI8226-LR5) cells in a similar manner to cisplatin and suramin.

Conclusions

Cell-surface/extracellular-matrix HS glycosaminoglycans are complex polysaccharides ubiquitously found in the animal kingdom, and regulate several aspects of cancer biology, including tumorigenesis, tumor progression, and metastasis. An insight into the above findings will reveal that glycans provide an opportunity to better understand the mimicking efficacy of novel heterocyclic compounds towards GAG components on cell surface surfaces such as CS/HS/DS chains, and new

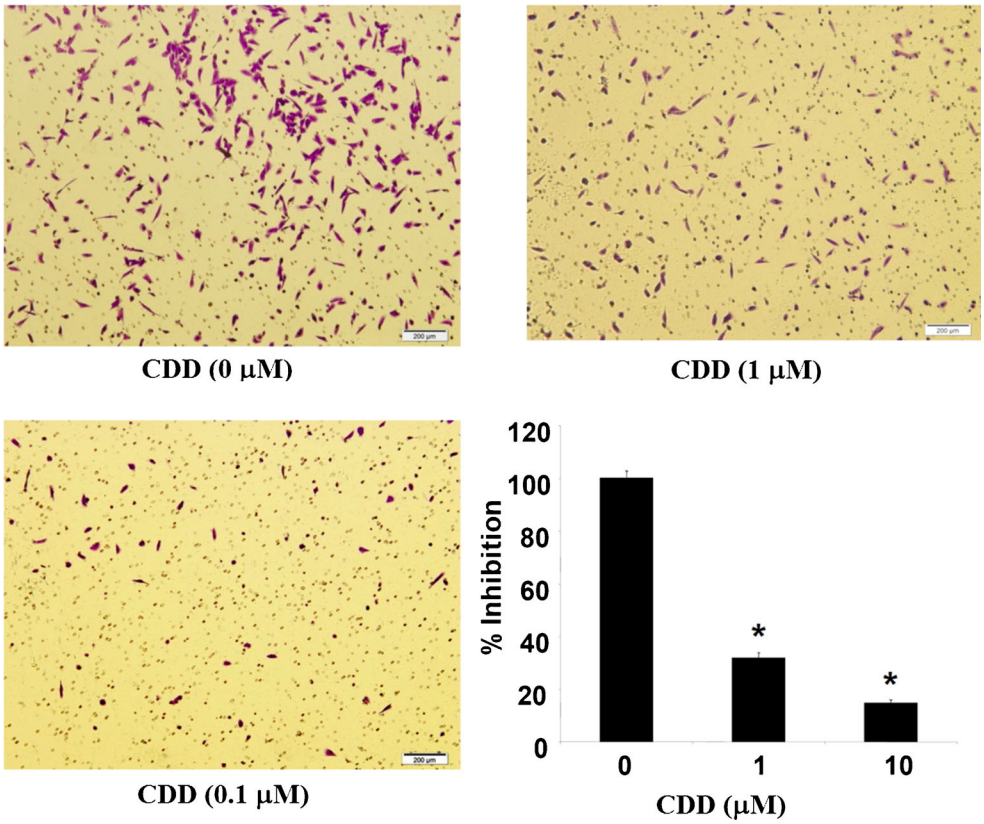

Fig. 7 Compound CDD completely inhibited the invasion of MDA-MB-231 cells. Cells were seeded in Matrigel™-coated Boyden chambers and were incubated for 24 h with DMEM (untreated) or a medium containing 1 μM or 10 μM of CDD; the photographs show the cell on the lower surface of the filter (invaded) stained with the Diff-Quick solution. The % inhibition of the invasion of MDA-MB-231 by CDD is presented. The data represent the mean value±S.D. for three independent experiments. *P <0.05 versus control, Student's t-test

therapeutic approaches using GAGmimetics will be in demand for not only cancers, but also other GAG-related diseases.

Acknowledgments This work was supported in part by Grants-in-Aid for Scientific Research (B) 23390016 20390019 (to K. S.) and a Grant-in-Aid for Challenging Exploratory Research 25670018 (to K. S.) from the Japan Society for the Promotion of Science (JSPS). This research was supported in part by the University Grants Commission (41-257-2012-SR), Vision Group Science and Technology, Department of Science and Technology (No. SR/FT/LS-142/2012) to Basappa. Basappa also thanks Karnataka University, INDIA for the PAVATE fellowship.

References

1. Fuster, M.M., Esko, J.D.: The sweet and sour of cancer: glycans as novel therapeutic targets. Nat Rev Cancer **5**, 526–542 (2005)
2. Apweiler, R., Hermjakob, H., Sharon, N.: On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. Biochim Biophys Acta **1473**, 4–8 (1999)
3. Ohtsubo, K., Marth, J.D.: Glycosylation in cellular mechanisms of health and disease. Cell **126**, 855–867 (2006)
4. Cazet, A., Julien, S., Bobowski, M., Krzewinski-Recchi, M.-A., Harduin-Lepers, A., S. Groux-Degroote, S., Delannoy, P.: Consequences of the expression of sialylated antigens in breast cancer. Carbohydr Res **345**, 1377–1383 (2010)
5. Rachagani, S., Torres, M.P., Moniaux, N., Batra, S.K.: Current status of mucins in the diagnosis and therapy of cancer. Biofactors **35**, 509–527 (2009)
6. Dube, D.H., Bertozzi, C.R.: Glycans in cancer and inflammation: Potential for therapeutics and diagnostics. Nat Rev Drug Discov **4**, 477–488 (2005)
7. Yamada, S., Sugahara, K.: Potential therapeutic application of chondroitin sulfate/dermatan sulfate. Curr Drug Discov Technol **5**, 289–301 (2008)
8. Mizumoto, S., Sugahara, K.: FEBS J **280**, 2462–2470 (2013)
9. Li, F., ten Dam, G.B., Murugan, S., Yamada, S., Hashiguchi, T., Mizumoto, S., Oguri, K., Okayama, M., van Kuppevelt, T.H., Sugahara, K.: Involvement of highly sulfated chondroitin sulfate in the metastasis of the Lewis lung carcinoma cells. J Biol Chem **283**, 34294–34304 (2008)
10. Xu, D., Young, J., Song, D., Esko, J.D.: Heparan sulfate is essential for high mobility group protein 1 (HMGB1) signaling by the receptor for advanced glycation end products (RAGE). J Biol Chem **286**, 41736–41744 (2011)
11. Purushothaman, A., Fukuda, J., Mizumoto, S., ten Dam, G.B., van Kuppevelt, T.H., Kitagawa, H., Mikami, T., Sugahara, K.: Functions of chondroitin sulfate/dermatan sulfate chains in the brain development: Critical roles of E and iE disaccharide units recognized by a single chain antibody GD3G7. J Biol Chem **282**, 19442–19452 (2007)
12. ten Dam, G.B., van de Westerlo, E.M.A., Purushothaman, A., Stan, R.V., Bulten, J., Sweep, F.C.G.J., Massuger, L.F., Sugahara, K., van Kuppevelt, T.H.: Antibody GD3G7 selected against embryonic glycosaminoglycans defines chondroitin sulfate-E domains highly up-regulated in ovarian cancer and involved in VEGF binding. Am J Pathol **171**, 1324–1333 (2007)
13. Sugahara, K.N., Hirata, T., Tanaka, T., Ogino, S., Takeda, M., Terasawa, H., Shimada, I., Tamura, J., ten Dam, G.B., van Kuppevelt, T.H., Miyasaka, M.: Chondroitin sulfate E fragments enhance CD44 cleavage and CD44-dependent motility in tumor cells. Cancer Res **68**, 7191–7199 (2008)
14. Basappa, Murugan, S., Sugahara, K.N., Lee, C.M., ten Dam, G.B., van Kuppevelt, T.H., Miyasaka, M., Yamada, S., Sugahara, K.: Involvement of chondroitin sulfate E in the liver tumor focal formation of murine osteosarcoma cells. Glycobiology **19**, 735–742 (2009)
15. Mizumoto, S., Takahashi, J., Sugahara, K.: Receptor for advanced glycation gnd products (RAGE) functions as a receptor for specific sulfated glycosaminoglycans, and anti-RAGE antibody or the sulfated glycosaminoglycans delivered *in vivo* inhibit pulmonary metastasis of tumor cells. J Biol Chem **287**, 18985–18994 (2012)
16. Deepa, S.S., Kalayanamitra, K., Ito, Y., Kongtaweelert, P., Fukui, S., Yamada, S., Mikami, T., Sugahara, K.: Novel sulfated octa- and decasaccharides from squid cartilage chondroitin sulfate-E: Sequencing and application for determination of the epitope structure of monoclonal antibody MO-225. Biochemistry **46**, 2453–2465 (2007)
17. Mundhenke, C., Meyer, K., Drew, S., Friedl, A.: Heparan sulfate proteoglycans as regulators of fibroblast growth factor-2 receptor binding in breast carcinomas. Am J Pathol **160**, 185–194 (2002)
18. Iozzo, R.V., San Antonio, J.D.: Heparan sulfate proteoglycans: heavy hitters in the angiogenesis arena. J Clin Invest **108**, 349–355 (2001)
19. Chu, C.L., Goerges, A.L., Nugent, M.A.: Identification of common and specific growth factor binding sites in heparan sulfate proteoglycans. Biochemistry **44**, 12203–12213 (2005)
20. Pisano, C., Vlodavsky, I., Ilan, N., Zunino, F.: The potential of heparanase as a therapeutic target in cancer. Biochem Pharmacol **89**, 12–19 (2014)
21. Ishida, K., Wierzba, M.K., Teruya, T., Simizu, S., Osada, H.: Novel heparan sulfate mimetic compounds as antitumor agents. Chem Biol **11**, 367–377 (2004)
22. Basappa, Murugan,S., Kavitha, V.C., Purushothaman, A., Nevin, G.K., Sugahara, K., Rangappa, S.K.: A small oxazine compound as an anti-tumor agent: A novel pyranoside mimetic that binds to VEGF, HB-EGF, and TNF-α. Cancer Lett **297**, 231–243 (2010)
23. Basappa, Sugahara, K., Thimmaiah, K.N., Bid, H.K., Houghton, P.J., Rangappa, K.S.: Anti-tumor activity of a novel HS-mimetic-vascular endothelial growth factor binding small molecule. PLoS ONE **7**(8), e39444 (2012). doi:10.1371/journal.pone.0039444
24. Gi, H.J., Xiang, Y., Schinazi, R.F., Zhao, K.: Synthesis of dihydroisoxazole nucleoside and nucleotide analogs. J Org Chem **62**, 88–92 (1997)
25. Wagner, C.R., Iyer, V.V., McIntee, E.J.: Pronucleotides: toward the *in vivo* delivery of antiviral and anticancer nucleotides. Med Res Rev **20**, 417–451 (2000)
26. Sadashiva, M.P., Basappa, N.S., Li, F., Manu, K.A., Sengottuvelan, M., Prasanna, D.S., Anilkumar, N.C., Sethi, G., Sugahara, K., Rangappa, K.S.: Anti-cancer activity of novel dibenzo [b, f] azepine tethered isoxazoline derivatives. BMC Chem Biol **12**, 5 (2012). doi: 10.1186/1472-6769-12-5