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Correlation of C4ST-1 and ChGn-2 expression with chondroitin sulfate chain elongation in atherosclerosis

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ABSTRACT

Subendothelial retention of lipoproteins by proteoglycans (PGs) is the initiating event in atherosclerosis. The elongation of chondroitin sulfate (CS) chains is associated with increased low-density lipoprotein (LDL) binding and progression of atherosclerosis. Recently, it has been shown that 2 Golgi enzymes, chondroitin 4-*O*-sulfotransferase-1 (C4ST-1) and chondroitin *N*-acetylgalactosaminyltransferase-2 (ChGn-2), play a critical role in CS chain elongation. However, the roles of *C4ST-1* and *ChGn-2* during the progression of atherosclerosis are not known. The aim of this study was to analyze the expression of *C4ST-1* and *ChGn-2* in atherosclerotic lesions *in vivo* and determine whether their expression correlated with CS chain elongation.

Low-density lipoprotein receptor knockout (LDLr KO) mice were fed a western diet for 2, 4, and 8 weeks to stimulate development of atherosclerosis. The binding of LDL and CS PG in this mouse model was confirmed by chondroitinase ABC (ChABC) digestion and apolipoprotein B (apo B) staining. Gel filtration analysis revealed that the CS chains began to elongate as early as 2 weeks after beginning a western diet and continued as the atherosclerosis progressed. Furthermore, quantitative real-time polymerase chain reaction (qRT-PCR) showed that the mRNA levels of *C4ST-1* and *ChGn-2* increased after 8 weeks of this diet. In contrast, the mRNA levels of their homologs, *C4ST-2* and *ChGn-1*, were unchanged. In addition, immunohistochemical analysis demonstrated that the expression of *C4ST-1* and *ChGn-2* appeared to have similar site-specific patterns and coincided with biglycan expression at the aortic root.

Our results suggested that *C4ST-1* and *ChGn-2* may be involved in the elongation of CS chains in the arterial wall during the progression of atherosclerosis. Therefore, modulating their expression and activity might be a novel therapeutic strategy for atherosclerosis.

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1. Introduction

The "response to retention" hypothesis of atherosclerosis [1,2] proposes that the key initiating step in atherogenesis is subendothelial retention of atherogenic lipoproteins, such as low-density lipoprotein (LDL), by extracellular matrix (ECM) molecules, particularly chondroitin sulfate (CS)/dermatan sulfate (DS) proteoglycans (PGs). Lipoproteins bind to CS/DS glycosaminoglycan (GAG) chains on PGs [3–5]. Biglycan is a common type of CS/DS PG that is colocalized with apolipoprotein (Apo) B in early and advanced human atherosclerotic coronary arteries [6]. Many in vitro studies suggest that CS chains on PGs are essential for PGs to bind lipoproteins. In addition, several other atherogenic factors, such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and thrombin, stimulate the elongation of CS chains, which increases LDL binding [7–9]. Thus, CS chain elongation may be a therapeutic target for the prevention of atherosclerosis [10,11].

Several glycosyltransferases and sulfotransferases are involved in the biosynthesis of CS chains [12]. However, the precise mechanism of the elongation of CS chains is not known. Recently, Izumikawa et al. demonstrated that chondroitin 4-*O*-sulphotransferase-1 (*C4ST-1*) and chondroitin *N*-acetylgalactosaminyltransferase-2 (*ChGn-2*) regulate the CS chain length and amount of CS of PGs in vitro [13]. They also showed that chondroitin polymerizing factor (ChPF) exhibits polymerization activity only when it co-expressed with any of these chondroitin synthase (ChSy) enzymes, *ChSy-1*, *ChSy-2*, or *ChSy-3* [14–16]. However, the expression and role of *C4ST-1* and *ChGn-2* enzymes in atherosclerosis development *in vivo* have not been studied yet.

Here, we analyzed the expression of *C4ST-1* and *ChGn-2* during the progression of atherosclerosis *in vivo* and determined whether their expression correlated with CS chain elongation.

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2. Materials and methods

2.1. Animals

Low-density lipoprotein receptor knockout (LDLr KO) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). All animal protocols were approved by the Animal Facility of Kobe Pharmaceutical University, Kobe, Japan. The LDLr KO mice were fed standard CRF-1 mouse chow (Charles River Laboratories International, Inc.) until 10–12 week of age. Subsequently, they were switched to F2HFD1 mouse chow with 1.25% cholesterol to simulate a western diet (Oriental Yeast Co., Ltd., Japan) for 0, 2, 4, and 8 weeks.

2.2. Tissue collection

Fresh frozen aortas from LDLr KO mice that were fed a western diet for 0, 2, 4, or 8 weeks were used to analyze mRNA, disaccharide composition, and CS chain length. The heart (containing the aortic sinus) was either frozen in Tissue-Tek OCT (Sakura Finetek USA, Inc.) for cryosectioning or processed for paraffin sectioning.

2.3. Quantitative atherosclerosis analysis

Quantification of the atherosclerosis was performed as described previously [8,17].

2.4. Immunohistochemistry

Sections (4 μm) were obtained from 4% paraformaldehyde-fixed, paraffin-embedded tissue. Immunostaining was performed with the following antibodies: goat polyclonal anti-biglycan antibody (1:50; Abcam, USA), goat anti-apolipoprotein B (1:100; Rockland Immunochemicals, Inc., Gilbertsville, PA), goat polyclonal anti-C4ST-1 (1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), rabbit polyclonal anti-CSGalNact-2 (1:50; Abgent), fluorescein isothiocyanate (FITC) conjugated mouse monoclonal anti-α-smooth muscle actin (1:500; Sigma, St. Louis, MO, USA), anti-mouse Mac-3 (1:250; BD Biosciences Pharmingen, San Jose, CA), and mouse monoclonal anti-proteoglycan ΔDi-4S (1:200, Seikagaku Corp.). Appropriate secondary antibodies were used. The antibody binding was visualized with 3,3′-diaminobenzidine (DAB) from DAKO.

2.5. Chondroitinase ABC digestion

Sections (4 μm) were obtained from 4% paraformaldehyde-fixed, paraffin-embedded tissue. Section was permeabilized with 0.2% Triton X-100/phosphate buffered saline (PBS) for 15 min at room temperature, and then incubated with chondroitinase buffer (50 mmol/L Tris–HCl (pH 7.5), 0.2 mol/L sodium chloride) for 15 min at room temperature. Section was digested with 5 mIU chondroitinase ABC (ChABC) (Seikagaku Corp.), which selectively removes CS and DS chains from PGs [18,19] for 1 h at 37 °C.

2.6. Real-time polymerase chain reaction

Total RNA was extracted from aorta tissue by using Trizol reagent (Invitrogen, Paisley, UK). The relative mRNA expression levels of *C4ST-1*, *C4ST-2*, *ChGn-1*, *ChGn-2*, *ChSy-1*, *ChPF*, and biglycan were determined by using quantitative real-time polymerase chain reaction (qRT-PCR) with the One Step SYBR Prime Script RT PCR kit II (TaKaRa Biotechnology Co., Ltd.), with glyceraldehyde-3-phosphate dehydrogenase (G3PDH) as a reference. The primers for each gene were designed from publicly available mouse mRNA sequences (Table 1).

Table 1
Primers for quantitative real-time polymerase chain reaction.

Target gene	Sequence
C4ST-1mus	
Forward	ACC TCG TGG GCA AGT ATG AG
Reverse	TCT GGA AGA ACT CCG TGG TC
C4ST-2mus	
Forward	ATC AGC ATC ACC AGC AAC A
Reverse	TGT GGC CTG GAG AGA GAC
ChGn-1mus	
Forward	TAA ACA GCC CTG TGG AGA G
Reverse	GTC GAA ATA GGA CAA GTC GC
ChGn-2mus	
Forward	TTA ATA TCA TTG TGC CAC TTG CG
Reverse	TAG AAT AGA CTT GAC TTT AGA TAG TCC TT
ChSy-1mus	
Forward	ACC ACA CAT TGG CAA GT
Reverse	TGT ACC CTT TCT TGT TCT GTT CA
ChPFmus	
Forward	CAC GTA CCA GGA GAT TCA AGA
Reverse	GAA GTA GTC CCA GCG CA
m-Biglycan	
Forward	CCT GGA GAA CAG TGG CTT TGA
Reverse	GGC CTC TGA GAT GCG CAG
G3PDHmus	
Forward	CAT CTG AGG GCC CAC TG
Reverse	GAG GCC ATG TAG GCC ATG A

2.7. Isolation and characterization of glycosaminoglycans

Dried homogenized aortas were prepared as described previously [20,21].

2.8. Disaccharide composition analysis

Purified glycosaminoglycans (GAGs) were digested with ChABC. Subsequently, the GAGs were labeled with 2-aminobenzamide (2-AB), and then identified and quantified by high performance liquid chromatography (HPLC), as described previously [22].

2.9. Glycosaminoglycan chain length analysis

Purified GAGs were subjected to reductive β-elimination using $NaBH_4$/NaOH, and then analyzed by gel filtration chromatography analysis on a Superdex 200 column (10 × 300 mm) eluted with 0.2 M ammonium bicarbonate at a flow rate of 0.4 mL/min. Fractions were collected at 3 min intervals, digested with ChABC, labeled with 2-AB, and then analyzed with HPLC, as described previously [23].

2.10. Statistical analysis

Statistically significant differences between means were determined by using one-way analysis of variance followed by Fisher's protected least significant difference (PLSD) test with equal or unequal variances. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Quantitative analysis of atherosclerosis in the aorta and aortic root

The extent of atherosclerotic lesions in the aortic root of mice is shown in Fig. 1E–H. The mice that were fed a western diet for 4 or 8 weeks had significantly more plaque areas than those that were fed the same diet for 0 or 2 weeks (Fig. 1I). In the mice that were

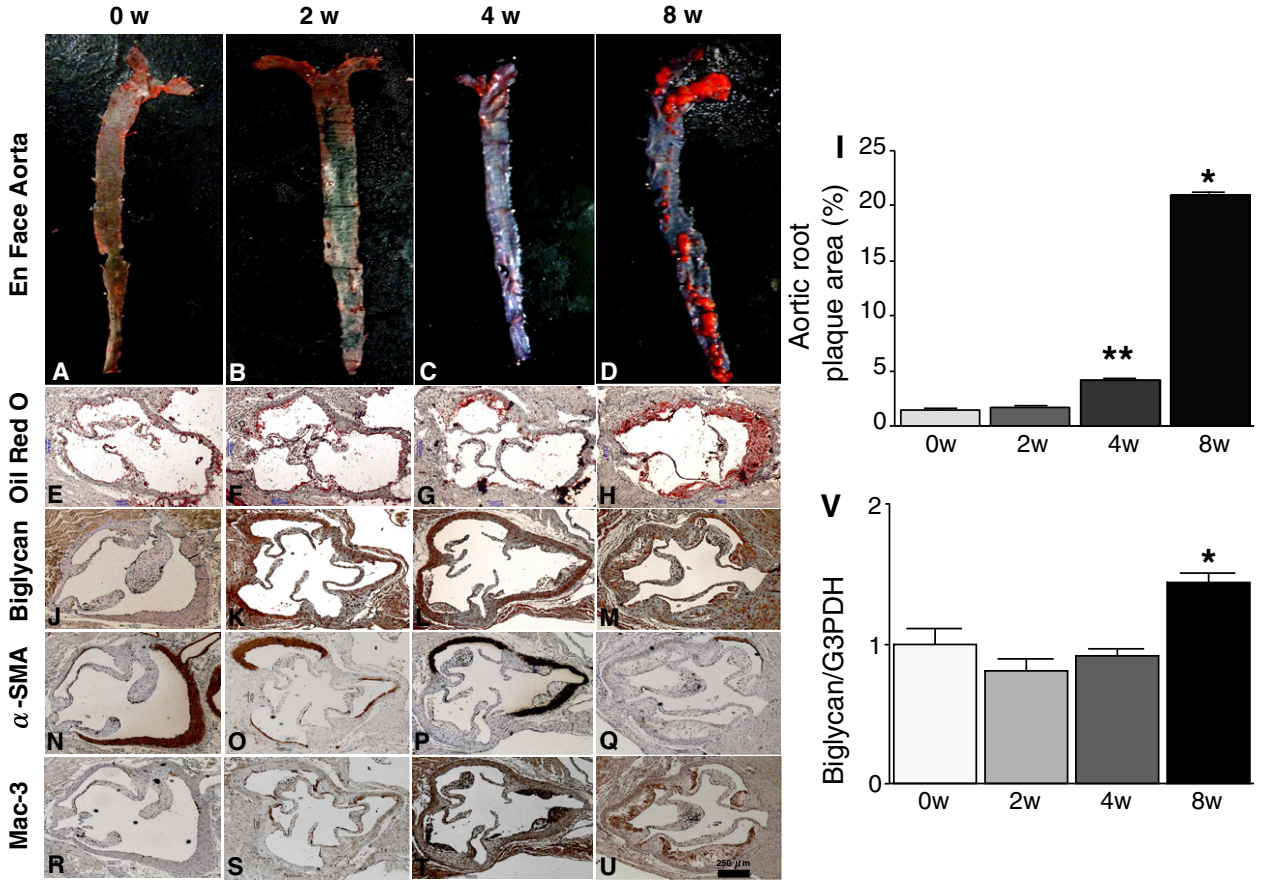

Fig. 1. (A–I) Quantification of atherosclerotic plaque areas. (A–D) Lipid deposition in the aorta as shown by Oil Red O staining on the en face aorta of low-density lipoprotein receptor (LDLr) knockout (KO) mice after consuming a western diet for 0, 2, 4, or 8 weeks. (E–H) Oil Red O staining of the aortic sinus of LDLr KO mice after consuming a western diet for 0, 2, 4, and 8 weeks. (I) Plaque areas at the aortic root were determined by using Image J software. Data are expressed as mean (SE) ($n = 6$). $^{*}p < 0.0001$ versus 0, 2, and 4 weeks of consuming a western diet. $^{**}p < 0.005$ versus 0, 2, and 8 weeks of consuming a western diet. (J–M) Biglycan expression during progression of atherosclerosis. Representative images of immunostained biglycan (J–M), α-smooth muscle actin (α-SMA) (N–Q), and Mac-3 (R–U) in the aortic sinus of LDLr KO mice after consuming a western diet for 0, 2, 4, or 8 weeks, respectively. (V) mRNA expression of biglycan in the aorta of LDLr KO mice after consuming a western diet for 0, 2, 4, or 8 weeks.

fed a western diet for 0, 2, or 4 weeks, the samples could not be further analyzed by en face aorta analysis because there were very few lesions (Fig. 1A–D).

3.2. Biglycan expression during atherosclerosis progression

Immunohistochemistry showed that biglycan expressed at the aortic root at the beginning of the experimental period and increased as atherosclerosis progressed (Fig. 1J–M). Biglycan immunostaining also was expressed in the neointima and media. In addition, the accumulation of biglycan immunostaining coincided with the expression of α-SMA (Fig. 1N–Q) and Mac-3 immunostaining. After 8 weeks of consuming a western diet, the mRNA expression level of biglycan in mice were significantly higher than those that had consumed the same diet for 0, 2, or 4 weeks (Fig. 1V).

3.3. Disaccharide composition and glycosaminoglycan chain length analysis of proteoglycans

The total amount of CS and DS in the aortas of LDLr KO mice that were fed a western diet increased as their atherosclerosis progressed. Approximately 73% of the CS disaccharides were comprised of ΔHexA-GalNAc (4S). However, this proportion was not significantly different in any group (Table 2).

Gel filtration analysis revealed that CS chain from the aorta of LDLr KO mice with developing atherosclerotic lesions, began to elongate as early as 2 weeks after the mice began consuming a western diet and increased in length as the atherosclerosis progressed (Fig. 2A–D).

3.4. Characterization of biglycan binding to low-density lipoprotein

At the beginning of the experimental period, apo B and biglycan immunostaining coincided with tiny fatty streak lesions and near sub-endothelial regions where foam cells had not yet appeared (Supplementary Fig. 1A–H). These data showed that both biglycan accumulation and lipoprotein deposition occur during the initial stage of atherosclerosis.

To characterize the binding of apo B to CS chains, the aortic roots with developing lesions were immunostained for apo B in the absence or presence of ChABC (Fig. 3A and B, respectively). The digested aortic sinus was strongly immunopositive for anti-proteoglycan ΔDi-4S, which represents the remaining four sulfated CS and DS "stubs" after extensive ChABC digestion (Fig. 3C). In contrast, apo B immunostaining in sections that were digested with ChABC showed almost no positive staining, which indicated that apo B directly binds PGs through CS chains.

3.5. Enzymes involved in the elongation of GAG chains

The mRNA expression level of *C4ST-1* and *ChGn-2* increased after the mice consumed a western diet for 8 weeks (Fig. 4A and B). However, the expression levels of their homologs, *C4ST-2* and

Table 2
Disaccharide composition of chondroitin sulfate (CS)/dermatan sulfate (DS) in the aorta of low-density lipoprotein receptor knockout mice after consumption of a western diet.

Disaccharide	Composition (pmol/mg) (mol%)[a]			
	0 weeks[b]	2 weeks[b]	4 weeks[b]	8 weeks[b]
ΔHexA-GalNAc	188 ± 53 (6)	244 ± 11 (6)	288 ± 1 (6)	951 ± 6 (15)
ΔHexA-GalNAc (6S)	696 ± 9 (20)	781 ± 84 (19)	887 ± 10 (17)	695 ± 23 (11)
ΔHexA-GalNAc (4S)	2411 ± 205 (72)	2946 ± 481 (73)	3737 ± 53 (75)	4519 ± 20 (72)
ΔHexA(2S)-GalNAc(6S)	64 ± 1 (2)	80 ± 10 (2)	92 ± 5 (2)	100 ± 8 (2)
Total CS	3361 ± 269	4052 ± 587	5006 ± 39	6266 ± 4
Total DS	138 ± 15	174 ± 15	229 ± 2	263 ± 9
Sulfation degree	0.94 ± 0.01	0.93 ± 0.03	0.94 ± 0.01	0.84 ± 0.02

[a] The value represented as pmol of disaccharide per mg of dried homogenated aorta.
[b] The values are the means ± SE of three determinations.

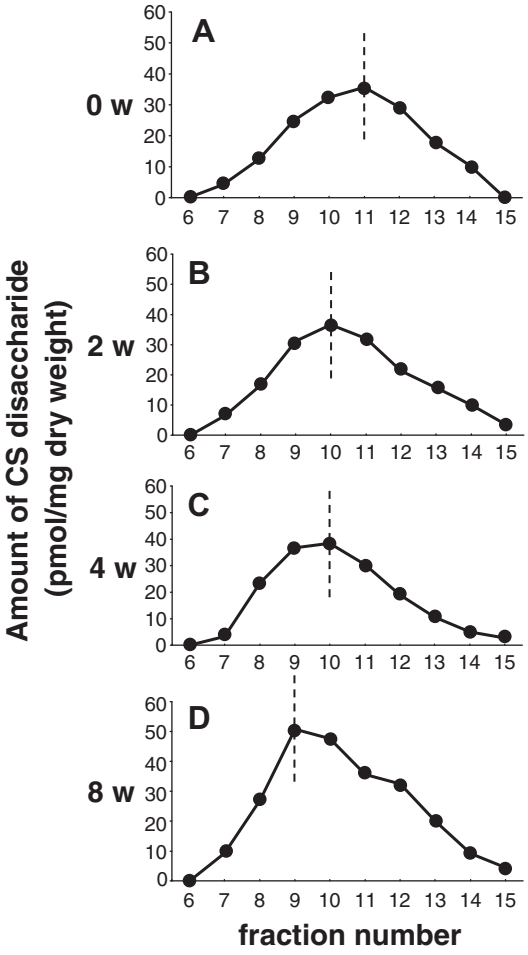

Fig. 2. Glycosaminoglycan chain length analysis of aortas in low-density lipoprotein receptor knockout mice. (A–D) Analysis of digested GAGs fractions. Samples were obtained from the aorta of LDLr KO mice after consuming a western diet for 0 weeks (A) 2 weeks (B), 4 weeks (C), or 8 weeks (D).

ChGn-1, did not change significantly during the progression of atherosclerosis (Supplementary Fig. 2A and B). In addition biglycan (Fig. 4C–F), *C4ST-1* (Fig. 4G–J), and *ChGn-2* (Fig. 4K–N) immunostainings were expressed at the beginning of the experimental period and increased as the atherosclerosis progressed. Furthermore, the expressions of *C4ST-1* (Fig. 4G–J) and *ChGn-2* (Fig. 4K–N) immunostainings coincided with that of biglycan (Fig. 4C–F) immunostaining at the aortic root in the neointima and media of initial lesions as well as with α-SMA (Fig. 4O–R) and Mac-3 (Fig. 4S–V) immunostaining. Together, these results suggested that *C4ST-1* and *ChGn-2* have similar site-specific expression patterns.

We also observed that the mRNA expression levels of *ChSy-1* and *ChPF* increased significantly after the mice had consumed a western diet for 8 weeks (Supplementary Fig. 2C and D).

4. Discussion

Although PGs are present in the normal arterial wall, they vary in their core proteins, sulfation pattern, and GAG chain length. These structural differences change dynamically during the progression of atherosclerosis [24–28]. Hyperelongated biglycan may be the key factor for the development and progression of atherosclerosis because they enhance the LDL binding affinity [1,2,7,29]. As a result, CS chain elongation may be a therapeutic target for the prevention of atherosclerosis [10,11]. The increase in chain length may be due to increases in the amount or activity of the enzymes that synthesize the CS chain. However, the molecular mechanism of CS elongation *in vivo* is not clear.

Our results demonstrated that the synthesis of longer CS chains during the development of atherosclerosis is accompanied by increased expression of *C4ST-1* and *ChGn-2*. We noticed that mRNA expression of *C4ST-1* and *ChGn-2* was not up-regulated as early as the CS chain elongation. This discrepancy may have arisen from the sensitivity of the detection methods and sample preparation, i.e. quantitative RT-PCR using mRNA prepared from whole aorta vs. gel filtration and HPLC analysis with purified GAGs. Furthermore, we showed that these enzymes colocalized with biglycan and apo B, which were already present at the initial stage of atherosclerosis. In contrast, their homologs, *C4ST-2* (Supplementary Fig. 2A) [30] and *ChGn-1* (Supplementary Fig. 2B) [31], did not appear to be involved in CS chain elongation since their expression levels did not change significantly during the progression of atherosclerosis. Our results are consistent with those of Izumikawa et al. [13], who demonstrated that *C4ST-1* and *ChGn-2* regulated the chain length and amount of CS in vitro, and suggested these 2 enzymes may be involved in the elongation of CS chains in the arterial wall during the progression of atherosclerosis.

The retention of atherogenic lipoprotein by PGs is the key initiating event in atherosclerosis, because it is already presented in the diffuse intimal thickening (DIT) of human arteries before atherosclerosis develops [32]. The retention of atherogenic lipoproteins by vascular PGs can be enhanced by modification of the atherogenic lipoprotein and the vascular PGs to produce a more atherogenic profile. For example, LDL retention increases its time within the arterial wall and, therefore, its susceptibility to oxidation. Oxidized LDL stimulates vascular smooth muscle cells to produce PGs with elongated chains that enhance the binding affinity of LDL [33–38]. Thus, the entrapment of atherogenic lipoproteins in the arterial wall by elongated CS chains on PGs fuels a vicious cycle of atherosclerosis [6], which may be centered on *C4ST-1* and *ChGn-2*.

The binding of atherogenic lipoproteins to arterial wall PGs is mediated by ionic interactions between the positively charged residues of apo B and negatively charged CS of PGs [4,39,40]. Since

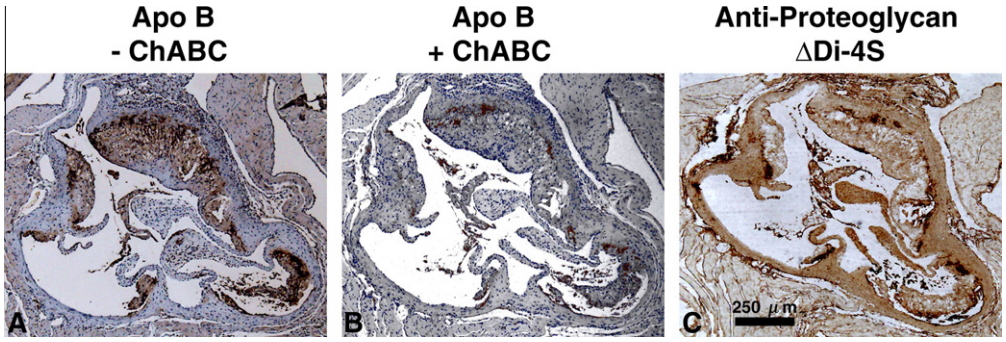

Fig. 3. Characterization of proteoglycan binding to apolipoprotein B. Representative images of the aortic sinus of LDLr KO mice that consumed a western diet for 8 weeks. Samples were immunostained for apo B in the absence (A) or presence of ChABC (B). (C) Immunostained for anti-proteoglycan ΔDi-4S.

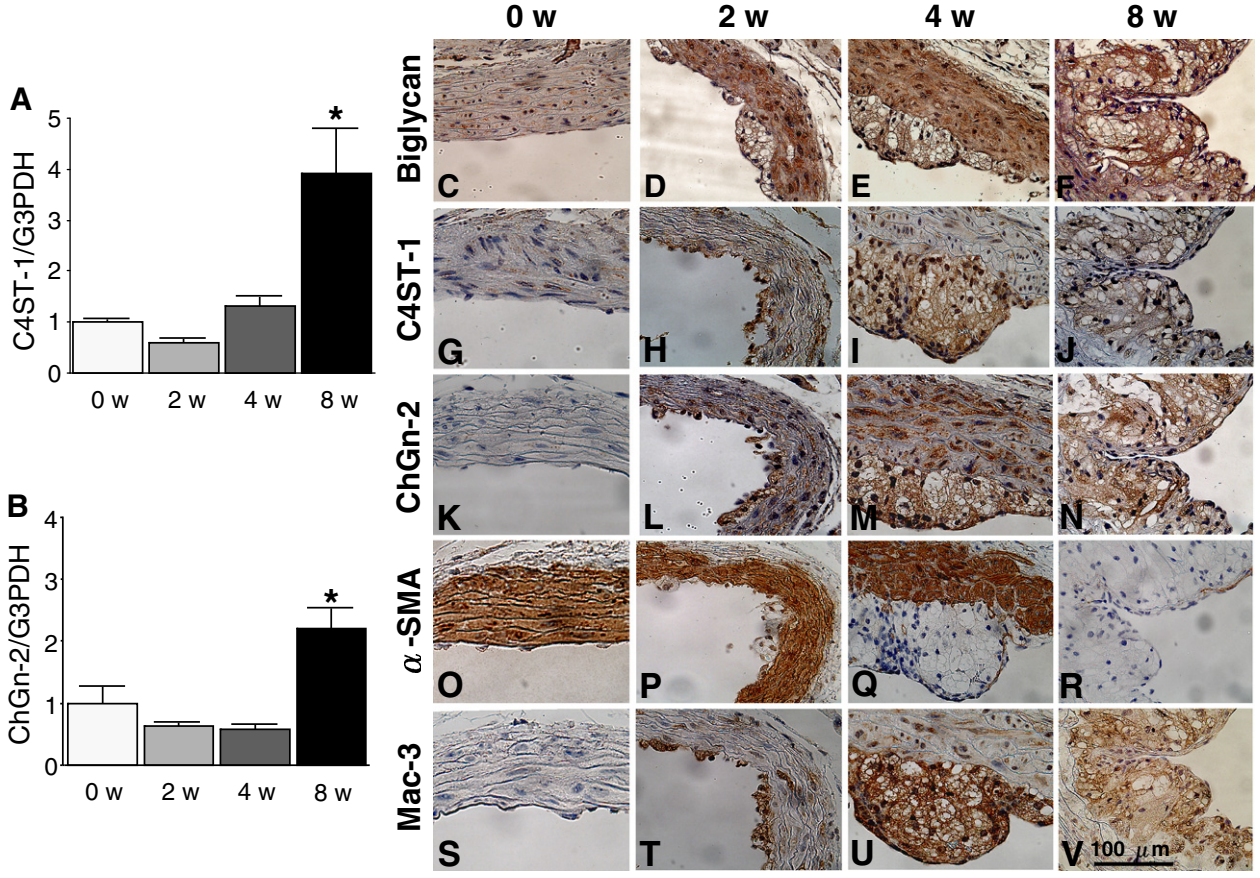

Fig. 4. mRNA expression of biglycan, *C4ST-1*, and *ChGn-2* in the aortic sinus of low-density lipoprotein receptor knockout mice. mRNA expression of *C4ST-1* (A) and *ChGn-2* (B) in the aorta of LDLr KO mice after consuming a western diet for 0, 2, 4, or 8 weeks ($n = 6$). (C–V) Representative images of immunostained biglycan (C–F), C4ST-1 (G–J), ChGn-2 (K–N), α-SMA (O–R), and Mac-3 (S–V) in the aortic sinus of LDLr KO mice after consuming a western diet for 0, 2, 4, or 8 weeks, respectively.

treatment of CS with ChABC eliminated almost all of the apo B immunoreactivity in atherosclerotic lesions (Fig. 3B), it is likely that apo B no longer bound PGs because the CS chains were not present. We hypothesize that the residual apo B could not be removed by ChABC digestion because they were bound to PGs indirectly, via intermediate molecules, such as lipoprotein lipase [4,41–43]. Nevertheless, our results suggested that the CS side chain is essential for apo B to bind CS/DS PGs, and therefore it has a critical role in the initiation and progression of atherosclerosis [27,44].

In conclusion, our results showed that *C4ST-1* and *ChGn-2* are involved in CS chain elongation during the development of atherosclerosis. However, we have not yet elucidated their mechanism of elongating CS chains. Currently, we are conducting *in vitro* and *in vivo* experiments with these enzymes by using siRNA-mediated knockdown and knockout mice, to determine their roles in the progression of atherosclerosis. Since *C4ST-1* and *ChGn-2* may be involved in the retention of atherogenic lipoproteins by mediating CS chain elongation, these enzymes may be a novel therapeutic targets to prevent the initiation and progression of atherosclerosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.01.096.

References

[1] K.J. Williams, I. Tabas, The response-to-retention hypothesis of early atherogenesis, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 551–561.
[2] I. Tabas, K.J. Williams, J. Boren, Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications, Circulation 116 (2007) 1832–1844.
[3] A. Chait, T.N. Wight, Interaction of native and modified low-density lipoproteins with extracellular matrix, Curr. Opin. Lipidol. 11 (2000) 457–463.
[4] C. GermÃ¡n, H.-C. Eva, W. Olov, et al., Association of apo B lipoproteins with arterial proteoglycans: pathological significance and molecular basis, Atherosclerosis 139 (1998) 205–222.
[5] K.J. Williams, Arterial wall chondroitin sulfate proteoglycans: diverse molecules with distinct roles in lipoprotein retention and atherogenesis, Curr. Opin. Lipidol. 12 (2001) 477–487.
[6] Y. Nakashima, T.N. Wight, K. Sueishi, Early atherosclerosis in humans: role of diffuse intimal thickening and extracellular matrix proteoglycans, Cardiovasc. Res. 79 (2008) 14–23.
[7] H. Dadlani, M.L. Ballinger, N. Osman, et al., Smad and p38 MAP kinase-mediated signaling of proteoglycan synthesis in vascular smooth muscle, J. Biol. Chem. 283 (2008) 7844–7852.
[8] R. Getachew, M.L. Ballinger, M.L. Burch, et al., PDGF {beta}-receptor kinase activity and ERK1/2 mediate glycosaminoglycan elongation on biglycan and increases binding to LDL, Endocrinology 151 (2010) 4356–4367.
[9] M.E. Ivey, P.J. Little, Thrombin regulates vascular smooth muscle cell proteoglycan synthesis via PAR-1 and multiple downstream signalling pathways, Thromb. Res. 123 (2008) 288–297.
[10] M.L. Ballinger, J. Nigro, K.V. Frontanilla, et al., Regulation of glycosaminoglycan structure and atherogenesis, Cell. Mol. Life Sci. 61 (2004) 1296–1306.
[11] P.J. Little, M.L. Ballinger, N. Osman, Vascular wall proteoglycan synthesis and structure as a target for the prevention of atherosclerosis, Vasc. Health Risk Manage. 3 (2007) 117–124.
[12] J.E. Silbert, G. Sugumaran, Biosynthesis of chondroitin/dermatan sulfate, IUBMB Life 54 (2002) 177–186.
[13] T. Izumikawa, Y. Okuura, T. Koike, H. Kitagawa, Chondrotin 4-*O*-sulfotransferase-1 regulates the chain length of chondroitin sulfate in cooperation with chondroitin *N*-acetylgalactosaminyltransferase-2, Biochem. J. 434 (2011) 321–331.
[14] T. Izumikawa, T. Koike, S. Shiozawa, et al., Identification of chondroitin sulfate glucuronyltransferase as chondroitin synthase-3 involved in chondroitin polymerization, J. Biol. Chem. 283 (2008) 11396–11406.
[15] H. Kitagawa, T. Izumikawa, T. Uyama, et al., Molecular cloning of a chondroitin polymerizing factor that cooperates with chondroitin synthase for chondroitin polymerization, J. Biol. Chem. 278 (2003) 23666–23671.
[16] T. Izumikawa, T. Uyama, Y. Okuura, K. Sugahara, H. Kitagawa, Involvement of chondroitin sulfate synthase-3 (chondroitin synthase-2) in chondroitin polymerization through its interaction with chondroitin synthase-1 or chondroitin-polymerizing factor, Biochem. J. 403 (2007) 545–552.
[17] A.S. Plump, C.J. Scott, J.L. Breslow, Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse, Proc. Natl. Acad. Sci. USA 91 (1994) 9607–9611.
[18] S. Nadanaka, M. Ishida, M. Ikegami, et al., Chondroitin 4-*O*-sulfotransferase-1 modulates Wnt-3a signaling through control of E disaccharide expression of chondroitin sulfate, J. Biol. Chem. 283 (2008) 27333–27343.
[19] T. Uyama, M. Ishida, T. Izumikawa, et al., Chondroitin 4-*O*-sulfotransferase-1 regulates E disaccharide expression of chondroitin sulfate required for herpes simplex virus infectivity, J. Biol. Chem. 281 (2006) 38668–38674.
[20] H. Kitagawa, A. Kinoshita, K. Sugahara, Microanalysis of glycosaminoglycan-derived disaccharides labeled with the fluorophore 2-aminoacridone by capillary electrophoresis and high-performance liquid chromatography, Anal. Biochem. 232 (1995) 114–121.
[21] T. Koike, T. Izumikawa, J.I. Tamura, et al., FAM20B is a kinase that phosphorylates xylose in the glycosaminoglycan protein linkage region, Biochem. J. 421 (2009) 157–162.
[22] J. Sorrell, F. Mahmoodian, I. Schafer, et al., Identification of monoclonal antibodies that recognize novel epitopes in native chondroitin/dermatan sulfate glycosaminoglycan chains: their use in mapping functionally distinct domains of human skin, J. Histochem. Cytochem. 38 (1990) 393–402.
[23] W.-C. Huang, W.-C. Kuo, J.-H. Cherng, et al., Chondroitinase ABC promotes axonal re-growth and behavior recovery in spinal cord injury, Biochem. Biophys. Res. Commun. 349 (2006) 963–968.
[24] Y. Nakashima, Y.-X. Chen, N. Kinukawa, et al., Distributions of diffuse intimal thickening in human arteries: preferential expression in atherosclerosis-prone arteries from an early age, Virchows Arch. 441 (2002) 279–288.
[25] S. Yla-Herttuala, T. Solakivi, J. Hirvonen, et al., Glycosaminoglycans and apolipoproteins B and A-I in human aortas. Chemical and immunological analysis of lesion-free aortas from children and adults, Arterioscler. Thromb. Vasc. Biol. 7 (1987) 333–340.
[26] K.D. O'Brien, K.L. Olin, C.E. Alpers, W. Chiu, M. Ferguson, K. Hudkins, T.N. Wight, A. Chait, Comparison of apolipoprotein and proteoglycan deposits in human coronary atherosclerotic plaques: colocalization of biglycan with apolipoproteins, Circulation 98 (1998) 519–527.
[27] T.N. Wight, M.J. Merrilees, Proteoglycans in atherosclerosis and restenosis: key roles for versican, Circ. Res. 94 (2004) 1158–1167.
[28] L. Tannock, V. King, Proteoglycan mediated lipoprotein retention: a mechanism of diabetic atherosclerosis, Rev. Endocr. Metab. Dis. 9 (2008) 289–300.
[29] E. Schonherr, H. Jarvelainen, M. Kinsella, et al., Platelet-derived growth factor and transforming growth factor-ft differentially affect the synthesis of biglycan and decorin by monkey arterial smooth muscle cells, Arterioscler. Thromb. Vasc. Biol. 1995 (1993) 1026–1036.
[30] N. Hiraoka, H. Nakagawa, E. Ong, et al., Molecular cloning and expression of two distinct human chondroitin 4-*O*-sulfotransferases that belong to the HNK-1 sulfotransferase gene family, J. Biol. Chem. 275 (2000) 20188–20196.
[31] T. Uyama, H. Kitagawa, J. Tanaka, J.-i. Tamura, T. Ogawa, K. Sugahara, Molecular cloning and expression of a second chondroitin *N*-acetylgalactosaminyltransferase involved in the initiation and elongation of chondroitin/dermatan sulfate, J. Biol. Chem. 278 (2003) 3072–3078.
[32] Y. Nakashima, H. Fujii, S. Sumiyoshi, et al., Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 1159–1165.
[33] M. Aviram, Modified forms of low density lipoprotein and atherosclerosis, Atherosclerosis 98 (1993) 1–9.
[34] H.F. Hoff, J.A. O'Neil, Oxidation of LDL: role in atherogenesis, Klin. Wochenschr. 69 (21–23) (1991) 1032–1038.
[35] E. Hurt-Camejo, G. Camejo, B. Rosengren, et al., Effect of arterial proteoglycans and glycosaminoglycans on low density lipoprotein oxidation and its uptake by human macrophages and arterial smooth muscle cells, Arterioscler. Thromb. Vasc. Biol. 12 (5) (1992) 569–583.
[36] M. Kaplan, M. Aviram, Retention of oxidized LDL by extracellular matrix proteoglycans leads to its uptake by macrophages: an alternative approach to study lipoproteins cellular uptake, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 386–393.
[37] M.Y. Chang, S. Potter-Perigo, C. Tsoi, et al., Oxidized low density lipoproteins regulate synthesis of monkey aortic smooth muscle cell proteoglycans that have enhanced native low density lipoprotein binding properties, J. Biol. Chem. 275 (2000) 4766–4773.
[38] K. Skalen, M. Gustafsson, E.K. Rydberg, et al., Subendothelial retention of atherogenic lipoproteins in early atherosclerosis, Nature 417 (2002) 750–754.
[39] C. Bancells, S.n. BenÃ-tez, M. Jauhiainen, et al., High binding affinity of electronegative LDL to human aortic proteoglycans depends on its aggregation level, J. Lipid Res. 50 (2009) 446–455.
[40] J.E. Figueroa, P. Vijayagopal, Angiotensin II stimulates synthesis of vascular smooth muscle cell proteoglycans with enhanced low density lipoprotein binding properties, Atherosclerosis 162 (2002) 261–268.
[41] M. Gustafsson, M. Levin, K. Skalen, et al., Retention of low-density lipoprotein in atherosclerotic lesions of the mouse: evidence for a role of lipoprotein lipase, Circ. Res. 101 (2007) 777–783.
[42] M.F. Khalil, W.D. Wagner, I.J. Goldberg, Molecular interactions leading to lipoprotein retention and the initiation of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 2211–2218.
[43] M.O. Pentikainen, R. Oksjoki, K. Oorni, et al., Lipoprotein lipase in the arterial wall: linking LDL to the arterial extracellular matrix and much more, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 211–217.
[44] K. Hashimura, K. Sudhir, J. Nigro, et al., Androgens stimulate human vascular smooth muscle cell proteoglycan biosynthesis and increase lipoprotein binding, Endocrinology 146 (2005) 2085–2090.