Receptors & Clinical Investigation 2015; 2: e867. doi: 10.14800/rci.867; © 2015 by Yu-Xin Xu
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RESEARCH HIGHLIGHT

The perlecan LDL-binding receptor needs sugar: Implication of perlecan core protein interaction with LDL for atherosclerosis

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Received: June 13, 2015
Published online: July 29, 2015

Cardiovascular disease (CVD) is the deadliest disease in the US and finding cures for CVD has been a pressing and challenging problem in biomedical science. Atherosclerosis is a pathological condition caused by an elevated plasma LDL cholesterol level and its deposition in the arterial wall. Although cholesterol-lowering therapies are currently effective in reducing circulating LDL cholesterol level, clinical trials have shown limited impact on reducing CVD risk. Thus, a supplemental treatment is urgently needed. Subendothelial LDL retention in the arterial wall is an early step in the atherogenic process, and finding the regulators that can be targeted to block this retention offers an alternative approach for early prevention. This review first presents the current understanding about the mechanism of the atherosclerosis development, and then focus on perlecan and its role in atherosclerosis. Perlecan is a major arterial proteoglycan consisting of a core protein and three heparan sulfate (HS) side chains. Many studies have linked perlecan to atherosclerosis because its HS side chains interact with LDL. Recently, we reported that the perlecan core protein also interacts with LDL via its LDL receptor (LDLR)-like domain II. Critical to the interaction is the sialic acid modification on the domain. In this review, the recent findings and the potential role of the arterial sialic acid in the early subendothelial LDL retention are discussed.

To cite this article: Yu-Xin Xu. The perlecan LDL-binding receptor needs sugar: Implication of perlecan core protein interaction with LDL for atherosclerosis. Receptor Clin Invest 2015; 2: e867. doi: 10.14800/rci.867.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the US. Currently, more than 8 million people suffer from various types of CVD, accounting for one out of every three deaths [1]. The primary risk factor intimately linked to the disease is atherosclerosis, which is a pathological condition that arises from fibrous plaque build-up inside the artery wall. The accumulation of the plaque causes the stenosis or narrowing of the blood vessels and induces tissue ischemia. At later stages, plaque rupture and thrombosis can result in serious complications such as heart attack or stroke [2-4].

The development of atherosclerosis is complex and involves many biological processes. Tremendous efforts have been taken to investigate the development of atherosclerosis and many hypotheses have been proposed including the response-to-retention hypothesis [5]. The hypothesis illustrates a scenario in which the earliest atherogenic events occur and progress as described below. The prominent and indisputable risk factor associated with atherosclerosis is the elevated plasma LDL cholesterol level [6, 7]. High plasma LDL levels are sufficient to induce atherosclerosis [8] and the LDL particles infiltrate the endothelium through endocytosis although the efficiency of infiltration is much dependent on lipoprotein density and size [9-11]. The apolipoprotein (Apo) B-containing lipoproteins are

then trapped by the extracellular matrix of the arterial wall, mainly the proteoglycans. The prolonged retention of the lipoproteins leads them to be modified, such as oxidation and aggregate formation, which subsequently induce an inflammatory response: monocytes, macrophages, T cells, as well as other immune cells migrate into the retention site [3, 12, 13]. Monocytes then differentiate into macrophages, which take in the retained native LDL or modified LDL and become the cholesterol-enriched foam cells. The underlying smooth muscle cells (SMCs) are activated to proliferate and migrate to nascent lesion sites. The modified LDL stimulates SMCs and macrophages to express more proteoglycans, which contain longer glycosaminoglycans (GAGs) chains and have greater affinity for LDL [14, 15]. Both the native LDL and oxidized LDL enhance macrophages to produce more proteoglycans and increase the uptake of LDL. At the later stage, foam cell death results in debris and deposition of cholesterol crystals, which are covered by fibrous cap synthesized by the SMCs [13, 16]. The plaque eventually thickens the arterial intima and leads to its erosion or rupture, and production of thrombotic factors.

The arterial proteoglycans are the key for the ApoB-containing lipoprotein retention in the arterial wall

Proteoglycans are the major components of the extracellular matrix lining around the arterial wall [17, 18]. Typically, proteoglycans contain a core protein and one or multiple covalently linked GAGs, which are linear polysaccharide building from repeating disaccharide units [19]. Most of GAGs carry negatively charged sulfates and carboxyl groups. The matrix proteoglycans possess huge structural diversity that enables them to bind with a variety of ligands including growth factors. Many studies have demonstrated that LDL/VLDL particles bind with heparin, dermatan sulfate (DS), chondroitin sulfate (CS) and HS at the low ionic strength buffer at the physiological pH [20-27]. ApoB-100 is rich in positively charged amino acids such as arginine and lysine, and it contains 8 clusters of positively charged amino acid segments [28]. The binding is based on the ionic interaction of the positively charged amino acid segments of ApoB-100 with the negatively charged groups of GAGs. It was shown that the segment (residues 3359-3369) located at the C-terminus of ApoB-100 is responsible for binding arterial proteoglycans [29, 30]. The N-terminal region of ApoB-100 also contains the proteoglycan-binding site that is normally covered by the C-terminal region of ApoB-100. The binding site explains why ApoB-48 in the absence of the C-terminal binding site is equally atherogenic by interacting with the proteoglycans [17, 31-34].

Substantial evidence supports the role of arterial proteoglycans in mediating the ApoB-containing lipoprotein retention in the arterial wall [13, 17]. It has been observed that rapid increase of blood LDL by injecting human LDL resulted in the LDL retention in the intima of arteries within 2 hours in rabbits [35]. The focally accumulated LDL particles were markedly increased in the lesion-prone sites of the arterial wall, which were entangled with the arterial extracellular matrix [8, 35]. The major components of the matrix responsible for the LDL retention are the acidic proteoglycans. For example, chondroitin sulfate proteoglycan strongly interacts with LDL [24] and this interaction induces structural changes in the LDL particles [36]. Monocytes/macrophages subsequently ingest the lipoprotein particles eventually leading to the cholesterol ester accumulation and foam cell formation. The interaction between the arterial proteoglycans and ApoB-containing lipoprotein was confirmed by genetic analysis in mice. Mice carrying the mutant ApoB-100, defective in proteoglycan binding, developed much less atherosclerosis than the mice with the wildtype ApoB-100 [37]. Taken together, these studies support the role of arterial proteoglycans in the early LDL retention during the initiation of atherogenesis.

The early LDL retention and the subsequent biological response frequently take place at the lesion-prone sites of the arterial intima, which are accompanied with diffuse intimal thickening (DIT) [13]. DIT is rich in the extracellular matrix, which constitutes of up to 60% of the intima content and is composed of abundant proteoglycans [38]. DIT is not equally distributed in all arteries. DIT can be detected in the coronary arteries and aortas at the very early stage of life, even from 36 weeks of gestation [39]. The content of DIT in coronary and aorta and its proteoglycan expression increase with age. Consistent with these, DIT was demonstrated to be strongly associated with the distribution of atherosclerosis. It is also well known that the arterial LDL retention and oxidation precedes the fatty streak formation and monocyte recruitment into the arterial wall [40]. The example that provides a sequential scenario for all the events came from a pathological analysis of serial sections of the coronary arteries with no or early atherosclerosis [41]. The study revealed that the ApoB-containing lipid deposition in the normal DIT results in the fatty streak formation. The increasing lipid accumulation stimulates the infiltration of macrophages towards to the lipid deposition site, the accumulation of the lipids by the macrophages and conversion into foam cells. These events eventually lead to the advanced plaque formation. The pathological study suggests that, in the DIT without lipid deposition, proteoglycans are highly expressed in the areas that are susceptible to lipid deposition. As the lesion progresses, a correlation was observed between the distribution of lipid deposition and proteoglycan expression from the early and advanced stages.

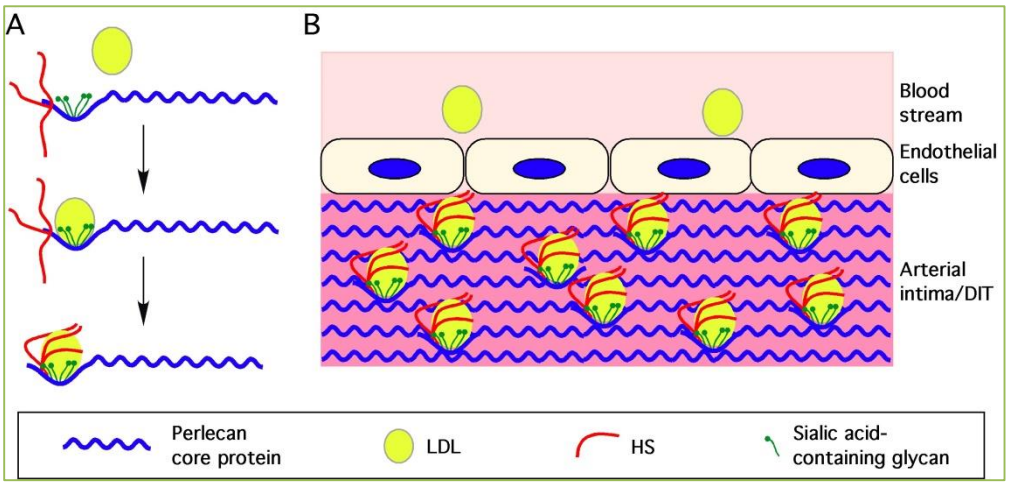

Figure 1. Schematic model for perlecan in LDL binding and LDL retention in the arterial wall. **A**. Coordinated interaction of LDL particles with perlecan domain II and I. LDL presumably first binds with the LDLR-like domain II and the binding is strengthened by the nearby three negatively charged HS side chains from domain I. **B**. The binding of LDL with perlecan facilitates its retention in the arterial wall, which initiates the atherogenic process.

Perlecan is an important factor for the ApoB-containing lipoprotein retention in the arterial wall

Perlecan is the major proteoglycan of the arterial wall that is normally synthesized by endothelial cells, and deposited into the subendothelial extracellular matrix [42]. However, during the lesion development, perlecan is largely synthesized by SMCs and macrophages [14, 43]. Perlecan is highly conserved across species and essential for growth. Deletion of perlecan gene in mice is either embryonic lethal or causes severe developmental defects [44]. Perlecan consists of a core protein with molecular weight ~450 kD and 3 HS side chains with approximate 70–100 kD. The core protein has five domains (I-V) with diverse structural features. The amino-terminal domain I contains attachment sites for the HS side chains [45]. Perlecan domain II has five repeat units, four of them are cysteine-rich and perfectly homologous to the ligand-binding portion of LDLR [46, 47]. It is important to note that the second repeat is different and does not contain cysteine but is rich in serine/threonine [48].

Several studies have linked perlecan to atherosclerosis and high levels of its expression are observed in the lesions of atherosclerosis-prone LDLR- and ApoE-deficient mice [49]. Perlecan expression correlates lesion progression. In advanced lesions with necrotic lipid cores, perlecan is remarkably increased [43, 49]. Mice with heterozygous deletion of perlecan exhibited a modest reduction in perlecan in the arterial wall and the deletion resulted in less atherosclerosis in young ApoE-deficient mice [43]. The role of perlecan in the development of atherosclerosis is based on the ionic interaction of HS and LDL as described above [17, 27]. Perlecan's HS is known in LDL binding activity and promotes vascular permeability and increases LDL retention. Depletion of endogenous perlecan HS in ApoE-null mice significantly reduced atherosclerosis [27].

Domain II of the perlecan core protein is an LDLR-like binding domain that is functional in LDL binding

Previously, no direct evidence supports a role of the perlecan core protein in LDL binding. Perlecan may represent an exceptional case for the binding because it has unique domain II that is highly homologous to the LDL-binding portion of LDLR [46]. This observation provided a rationale to test the potential of this domain to interact with LDL.

We recently demonstrated that the perlecan core protein indeed interacts with LDL via its LDLR-like domain II [48]. We found that the secreted domain II is heavily modified with *O*-linked glycans and the interaction is dependent on the glycosylation. Of the five repeat units of domain II, the second repeat unit diverges from the canonical LDLR ligand-binding module [47]. Interestingly, most of the glycosylation sites are from this unit. Most of the glycans are capped with negatively charged (α2-3) linked sialic acids [50] and are critical for this interaction. Consistent with the findings from LDLR- or ApoE-deficient mice [43, 49], we determined that perlecan is also overexpressed in the lesions of human atherosclerotic arteries and co-localized with the elevated (α2-3) linked sialic acid modifications, indicating a strong link of sialylation with atherosclerosis [48].

Our study on the perlecan LDLR-like domain revealed some unique features of its interaction with LDL as

compared with other members of LDLR or LDLR-related protein (LRP) families. The prototype LDLR contains 7 ligand-binding modules [47], whereas LRP1 possesses 31 such modules [51, 52]. Extensive structural and functional studies of LDLR showed that at least five repeat modules (repeats 3-7) are required for the ligand binding [53, 54]. Perlecan domain II only has four homologous repeat units, and they are interrupted by the second unit that is highly deviated as described above [46]. Therefore, this structure would make the domain inactive in LDL binding. Indeed, domain II without the glycosylation does not interact with LDL. The glycosylation-dependent interaction suggests that the glycans may change the properties of the domain II structure. As we demonstrated recently [48], the important sugar moieties for the interaction are the sialic acids. For a typical LDLR binding module, there are many acidic residues located between the fourth and sixth cysteines [53, 54], which are critical for high affinity binding of the positively charged domain of ApoB-100. It is likely that the negatively charged sialic acids may substitute the acidic residues to make the second repeat functional in the coordinated binding activity of domain II with LDL. We showed that there is an additive effect between perlecan domain II and the HS of domain I on binding with LDL. The coordinated binding would enhance the subendothelial LDL retention. The perlecan core protein's binding with LDL and the coordinated binding activity between domain II and the HS of domain I are illustrated in Figure 1.

Do the arterial sialic acids play an independent role in the subendothelial LDL retention?

The sialic acid-mediated perlecan domain II interaction with LDL raises the question that the sugar may play an independent role in the early subendothelial LDL retention. Sialic acids are the common sugar at the terminal position of various types of glycans. Numerous cell surface and extracellular glycoproteins are modified with sialic acid-containing glycans. Because of their terminal positions, sialic acids are frequently used as receptors for pathogens or participate in specific recognition events [19]. For example, the human influenza virus recognizes the α(2-6)-linked sialic acid, whereas the avian influenza virus binds to α(2-3)-linked sialic acid [55]. Sialic acids have about 50 structural variants and each of them can be attached to the subterminal galactose via various linkages [50]. All these features generate a great number of structural diversity that enables sialic acid to interact with different partners.

Sialic acids are a family of nine-carbon sugar units. Because the carboxylate group at the 1-carbon position is typically ionized at physiological pH, sialic acids are normally negatively charged and hydrophilic [19]. Therefore, similar to the electrostatic interaction of GAG of proteoglycans, the arterial sialic acids should be sufficient to interact with LDL independent of perlecan domain II. And the sialic acids and proteoglycans could play a synergistic role in the early LDL retention. However, the information about this is quite limited. Several studies related to sialic acids show that their content on LDL is associated with atherosclerosis [56, 57]. It was proposed that the sialic acids on LDL may play a role for the LDL particles to infiltrate through the luminal endothelial cells [58]. But the association was not observed in other similar studies [59, 60]. It should be mentioned that the infiltration of LDL across the endothelial cells is not a rate-limiting step in the subendothelial LDL retention [61]. Relevant to the arterial sialic acids, a report by Cuniberti *et al* [62] suggests that ablation of the arterial sialic acid content induced neointimal thickening, presumably due to SMC proliferation. But the key questions remain: What are the content and types of sialic acid-containing glycans in the arterial wall? Do they interact with LDL? And what is the significance of the interaction in the development of atherosclerosis? The answers to these questions await future studies.

This review summarizes the current information about the mechanism of atherosclerosis with an emphasis on the role of perlecan in atherosclerosis. Our study provided some novel insight of the perlecan core protein's interaction with LDL via its LDLR-like domain II. The dependence of the interaction on the sialic acid-containing glycans suggests that the arterial sialic acids may play an independent role in the early subendothelial LDL retention. Thus, perlecan and sialic acid-containing glycans may be the important targets that can be used to block the retention, though further studies are needed to confirm the hypothesis.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgements

The author would like to thank Dr. Sekar Kathiresan for support to this project, and thank Drs. Matthew Layne and Li Liu for critical reading of this manuscript. The project was supported by SDG grant 11SDG7670007 from the American Heart Association.

References

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, *et al*. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. Circulation 2014; 129:e28-e292.

2. Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell 2001; 104:503-516.

3. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell 2011; 145:341-355.

4. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011; 473:317-325.

5. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 1995; 15:551-561.

6. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, *et al*. Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009; 302:1993-2000.

7. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J, 3rd. Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med 1961; 55:33-50.

8. Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. Arteriosclerosis 1989; 9:908-918.

9. Khalil MF, Wagner WD, Goldberg IJ. Molecular interactions leading to lipoprotein retention and the initiation of atherosclerosis. Arterioscler Thromb Vasc Biol 2004; 24:2211-2218.

10. Proctor SD, Mamo JC. Intimal retention of cholesterol derived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. Arterioscler Thromb Vasc Biol 2003; 23:1595-1600.

11. Nordestgaard BG, Stender S, Kjeldsen K. Reduced atherogenesis in cholesterol-fed diabetic rabbits. Giant lipoproteins do not enter the arterial wall. Arteriosclerosis 1988; 8:421-428.

12. Hurt-Camejo E, Camejo G, Rosengren B, Lopez F, Ahlstrom C, Fager G, *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 1992; 12:569-583.

13. Tabas I, Williams KJ, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation 2007; 116:1832-1844.

14. Maor I, Hayek T, Hirsh M, Iancu TC, Aviram M. Macrophage-released proteoglycans enhance LDL aggregation: studies in aorta from apolipoprotein E-deficient mice. Atherosclerosis 2000; 150:91-101.

15. Chang MY, Olin KL, Tsoi C, Wight TN, Chait A. Human monocyte-derived macrophages secrete two forms of proteoglycan-macrophage colony-stimulating factor that differ in their ability to bind low density lipoproteins. J Biol Chem 1998; 273:15985-15992.

16. Dzau VJ, Braun-Dullaeus RC, Sedding DG. Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. Nat Med 2002; 8:1249-1256.

17. Camejo G, Hurt-Camejo E, Wiklund O, Bondjers G. Association of apo B lipoproteins with arterial proteoglycans: pathological significance and molecular basis. Atherosclerosis 1998; 139:205-222.

18. Tannock LR, King VL. Proteoglycan mediated lipoprotein retention: a mechanism of diabetic atherosclerosis. Rev Endocr Metab Disord 2008; 9:289-300.

19. Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J (1999) Essentials of Glycobiology (Cold Spring Harbor Press, Cold Spring Harbor, New York).

20. Bernfeld P, Nisselbaum J. Reaction of human betalipoglobulin with marcromolecular polysulfated esters. Fed Proc 1956; 15:220-227.

21. Iverius PH. The interaction between human plasma lipoproteins and connective tissue glycosaminoglycans. J Biol Chem 1972; 247:2607-2613.

22. Olsson U, Camejo G, Hurt-Camejo E, Elfsber K, Wiklund O, Bondjers G. Possible functional interactions of apolipoprotein B-100 segments that associate with cell proteoglycans and the ApoB/E receptor. Arterioscler Thromb Vasc Biol 1997; 17:149-155.

23. Olsson U, Ostergren-Lunden G, Moses J. Glycosaminoglycan-lipoprotein interaction. Glycoconj J 2001; 18:789-797.

24. Cardoso LE, Mourao PA. Glycosaminoglycan fractions from human arteries presenting diverse susceptibilities to atherosclerosis have different binding affinities to plasma LDL. Arterioscler Thromb 1994; 14:115-124.

25. Hollmann J, Schmidt A, von Bassewitz DB, Buddecke E. Relationship of sulfated glycosaminoglycans and cholesterol content in normal and arteriosclerotic human aorta. Arteriosclerosis 1989; 9:154-158.

26. Cardin AD, Randall CJ, Hirose N, Jackson RL. Physical-chemical interaction of heparin and human plasma low-density lipoproteins. Biochemistry 1987; 26:5513-5518.

27. Tran-Lundmark K, Tran PK, Paulsson-Berne G, Friden V, Soininen R, Tryggvason K, *et al*. Heparan sulfate in perlecan promotes mouse atherosclerosis: roles in lipid permeability, lipid retention, and smooth muscle cell proliferation. Circ Res 2008; 103:43-52.

28. Chen SH, Yang CY, Chen PF, Setzer D, Tanimura M, Li WH, *et al*. The complete cDNA and amino acid sequence of human apolipoprotein B-100. J Biol Chem 1986; 261:12918-12921.

29. Camejo G, Olofsson SO, Lopez F, Carlsson P, Bondjers G. Identification of Apo B-100 segments mediating the interaction of low density lipoproteins with arterial proteoglycans. Arteriosclerosis 1988; 8:368-377.

30. Hirose N, Blankenship DT, Krivanek MA, Jackson RL, Cardin AD. Isolation and characterization of four heparin-binding cyanogen bromide peptides of human plasma apolipoprotein B. Biochemistry 1987; 26:5505-5512.

31. Chatterton JE, Phillips ML, Curtiss LK, Milne R, Fruchart JC, Schumaker VN. Immunoelectron microscopy of low density lipoproteins yields a ribbon and bow model for the conformation of apolipoprotein B on the lipoprotein surface. J Lipid Res 1995; 36:2027-2037.

32. Segrest JP, Jones MK, Mishra VK, Pierotti V, Young SH, Boren J, *et al*. Apolipoprotein B-100: conservation of lipid-associating amphipathic secondary structural motifs in nine species of

vertebrates. J Lipid Res 1998; 39:85-102.

33. Boren J, Lee I, Zhu W, Arnold K, Taylor S, Innerarity TL. Identification of the low density lipoprotein receptor-binding site in apolipoprotein B100 and the modulation of its binding activity by the carboxyl terminus in familial defective apo-B100. J Clin Invest 1998; 101:1084-1093.
34. Hurt-Camejo E, Olsson U, Wiklund O, Bondjers G, Camejo G. Cellular consequences of the association of apoB lipoproteins with proteoglycans. Potential contribution to atherogenesis. Arterioscler Thromb Vasc Biol 1997; 17:1011-1017.
35. Nievelstein PF, Fogelman AM, Mottino G, Frank JS. Lipid accumulation in rabbit aortic intima 2 hours after bolus infusion of low density lipoprotein. A deep-etch and immunolocalization study of ultrarapidly frozen tissue. Arterioscler Thromb 1991; 11:1795-1805.
36. Camejo G, Rosengren B, Olson U, Lopez F, Olofson SO, Westerlund C, *et al*. Molecular basis of the association of arterial proteoglycans with low density lipoproteins: its effect on the structure of the lipoprotein particle. Eur Heart J 1990; 11 Suppl E:164-173.
37. Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL, *et al*. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature 2002; 417:750-754.
38. Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W, Jr., Richardson M, *et al*. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1992; 85:391-405.
39. Nakashima Y, Chen YX, Kinukawa N, Sueishi K. Distributions of diffuse intimal thickening in human arteries: preferential expression in atherosclerosis-prone arteries from an early age. Virchows Arch 2002; 441:279-288.
40. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, *et al*. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J Clin Invest 1997; 100:2680-2690.
41. Nakashima Y, Fujii H, Sumiyoshi S, Wight TN, Sueishi K. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. Arterioscler Thromb Vasc Biol 2007; 27:1159-1165.
42. Iozzo RV. Basement membrane proteoglycans: from cellar to ceiling. Nat Rev Mol Cell Biol 2005; 6:646-656.
43. Vikramadithyan RK, Kako Y, Chen G, Hu Y, Arikawa-Hirasawa E, Yamada Y, *et al*. Atherosclerosis in perlecan heterozygous mice. J Lipid Res 2004; 45:1806-1812.
44. Arikawa-Hirasawa E, Watanabe H, Takami H, Hassell JR, Yamada Y. Perlecan is essential for cartilage and cephalic development. Nat Genet 1999; 23:354-358.
45. Iozzo RV, Cohen IR, Grassel S, Murdoch AD. The biology of perlecan: the multifaceted heparan sulphate proteoglycan of basement membranes and pericellular matrices. Biochem J 1994; 302 (Pt 3):625-639.
46. Iozzo RV. Perlecan: a gem of a proteoglycan. Matrix Biol 1994; 14:203-208.
47. Goldstein JL, Brown MS, Anderson RG, Russell DW, Schneider WJ. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. Annu Rev Cell Biol 1985; 1:1-39.
48. Xu YX, Ashline D, Liu L, Tassa C, Shaw SY, Ravid K, *et al*. The glycosylation-dependent interaction of perlecan core protein with LDL: implications for atherosclerosis. J Lipid Res 2015; 56:266-276.
49. Kunjathoor VV, Chiu DS, O'Brien KD, LeBoeuf RC. Accumulation of biglycan and perlecan, but not versican, in lesions of murine models of atherosclerosis. Arterioscler Thromb Vasc Biol 2002; 22:462-468.
50. Varki A. Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins. Nature 2007; 446:1023-1029.
51. Nykjaer A, Willnow TE. The low-density lipoprotein receptor gene family: a cellular Swiss army knife? Trends Cell Biol 2002; 12:273-280.
52. Herz J, Strickland DK. LRP: a multifunctional scavenger and signaling receptor. J Clin Invest 2001; 108:779-784.
53. Russell DW, Brown MS, Goldstein JL. Different combinations of cysteine-rich repeats mediate binding of low density lipoprotein receptor to two different proteins. J Biol Chem 1989; 264:21682-21688.
54. Esser V, Limbird LE, Brown MS, Goldstein JL, Russell DW. Mutational analysis of the ligand binding domain of the low density lipoprotein receptor. J Biol Chem 1988; 263:13282-13290.
55. Nelli RK, Kuchipudi SV, White GA, Perez BB, Dunham SP, Chang KC. Comparative distribution of human and avian type sialic acid influenza receptors in the pig. BMC Vet Res 2010; 6:4.
56. Lindbohm N, Gylling H, Rajaratnam RA, Miettinen TA. Sialic acid content of low-density lipoprotein in women with coronary artery disease. J Lab Clin Med 2000; 136:110-115.
57. Ruelland A, Gallou G, Legras B, Paillard F, Cloarec L. LDL sialic acid content in patients with coronary artery disease. Clin Chim Acta 1993; 221:127-133.
58. Born GV, Palinski W. Unusually high concentrations of sialic acids on the surface of vascular endothelia. Br J Exp Pathol 1985; 66:543-549.
59. Sanchez-Quesada JL, Camacho M, Anton R, Benitez S, Vila L, Ordonez-Llanos J. Electronegative LDL of FH subjects: chemical characterization and induction of chemokine release from human endothelial cells. Atherosclerosis 2003; 166:261-270.
60. Chappey B, Beyssen B, Foos E, Ledru F, Guermonprez JL, Gaux JC, *et al*. Sialic acid content of LDL in coronary artery disease: no evidence of desialylation in subjects with coronary stenosis and increased levels in subjects with extensive atherosclerosis and acute myocardial infarction: relation between desialylation and in vitro peroxidation. Arterioscler Thromb Vasc Biol 1998; 18:876-883.
61. Schwenke DC, St Clair RW. Influx, efflux, and accumulation of LDL in normal arterial areas and atherosclerotic lesions of white Carneau pigeons with naturally occurring and cholesterol-aggravated aortic atherosclerosis. Arterioscler Thromb 1993; 13:1368-1381.
62. Cuniberti LA, Martinez V, Schachter J, Magarinos G, Meckert PC, Laguens RP, *et al*. Sialic acid as a protective barrier against neointima development. Atherosclerosis 2005; 181:225-231.