



Associate editor: P. Molenaar

## Signalling pathways regulating galactosaminoglycan synthesis and structure in vascular smooth muscle: Implications for lipoprotein binding and atherosclerosis

Rizwana Afroz<sup>a</sup>, Yingnan Cao<sup>b</sup>, Muhamad Ashraf Rostam<sup>c</sup>, Hang Ta<sup>a,d</sup>, Suowen Xu<sup>e</sup>, Wenhua Zheng<sup>f</sup>, Narin Osman<sup>g</sup>, Danielle Kamato<sup>a</sup>, Peter J. Little<sup>a,b,g,\*</sup>

<sup>a</sup> School of Pharmacy, Pharmacy Australia Centre of Excellence, The University of Queensland, Woolloongabba, Queensland 4102, Australia

<sup>b</sup> Department of Pharmacy, Xinhua College of Sun Yat-sen University, Tianhe District, Guangzhou 510520, China

<sup>c</sup> Kulliyah of Allied Health Sciences, International Islamic University Malaysia, Kuantan, Pahang 25200, Malaysia

<sup>d</sup> Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Brisbane, Queensland 4072, Australia

<sup>e</sup> Aab Cardiovascular Research Institute, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA

<sup>f</sup> Faculty of Health Sciences, University of Macau, Taipa, Macau, China

<sup>g</sup> School of Health and Biomedical Sciences, RMIT University, Bundoora, Victoria 3083, Australia



## ARTICLE INFO

Available online 15 February 2018

## Keywords:

Atherosclerosis  
Proteoglycans  
Galactosaminoglycans  
Signalling pathways

## ABSTRACT

Atherosclerosis commences with the trapping of low density lipoproteins (LDLs) in blood vessels by modified proteoglycans (PGs) with hyperelongated glycosaminoglycan (GAG) chains. GAG chain synthesis and growth factor mediated hyperelongation regulates the composition and size of PGs in a manner that would cause low density lipoprotein (LDLs) retention in vessel wall. Galactosaminoglycans are a class of GAGs, commonly observed on PGs. Multiple enzymes are involved in galactosaminoglycan biosynthesis. Galactosaminoglycan synthesis is regulated by various signalling pathways which are amenable to pharmacological manipulation to treat atherosclerosis. Receptor mediated signalling pathways including protein tyrosine kinase receptors (PTKRs), serine/threonine kinase receptors (S/TKRs) and G-protein coupled receptors (GPCRs) pathways regulate galactosaminoglycan synthesizing enzyme expression. Increased expression of these enzymes modify galactosaminoglycan chain structure by making them hyperelongated. This review focuses on the signalling pathways regulating the expression of genes involved in galactosaminoglycan synthesis and modification. Furthermore, there are multiple other processes for inhibiting the interactions between LDL and galactosaminoglycans such as peptide mimetics of ApoB100 and anti-galactosaminoglycan antibodies and the therapeutic potential of these strategies is also addressed.

© 2018 Elsevier Inc. All rights reserved.

## Contents

1. Introduction . . . . .	89
2. Galactosaminoglycan structure contributes to LDL binding . . . . .	89
3. Enzymes involved in galactosaminoglycan biosynthesis and modification . . . . .	90
4. Signalling pathways regulating galactosaminoglycan synthesis and structure . . . . .	91

**Abbreviations:** Ang-II, angiotensin-II; CDKs, cyclin-dependent kinases; ChGn, chondroitin N-acetylgalactosaminyltransferase; ChPF, chondroitin polymerizing factor; ChSy, chondroitin synthase; CS, chondroitin sulfate; CVDs, cardiovascular diseases; C4S, chondroitin-4-sulfate; C6S, chondroitin-6-sulfate; C4ST, chondroitin 4-O-sulfotransferase; C6ST, chondroitin 6-O-sulfotransferase; DS, dermatan sulfate; DSE, dermatan sulfate epimerase; D4ST, dermatan 4-O-sulfotransferase; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; Erk1/2, extracellular signal-regulated kinase 1/2; ET-1, endothelin-1; GAGs, glycosaminoglycans; Gal, galactose; GalT,  $\beta$ -1,3-galactosyl transferase; GalNAc, N-acetyl-D-galactosamine; GalNAcT, N-acetylgalactosyl transferase; GalNAc4S-6ST, N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase; GlcA, glucuronic acid; GlcNAc, N-acetyl-D-glucosamine; GlcAT,  $\beta$ -1,3-glucuronosyl transferase; GPCR, G-protein coupled receptors; HMGCoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; IdoA, iduronic acid; IP<sub>3</sub>, inositol 1,4,5 triphosphate; LDLs, low density lipoproteins; mAb, monoclonal antibody; MMPs, matrix metalloproteinases; PCSK9, proprotein convertase subtilisin/kexin type 9; PDGF, platelet derived growth factor; PDGFR, platelet derived growth factor receptor; PGs, proteoglycans; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; PTKRs, protein tyrosine kinase receptors; Ser, serine; Sos, son of sevenless; S/TKRs, serine/threonine kinase receptors; TGF- $\beta$ , transforming growth factor- $\beta$ ; TGFBR, transforming growth factor- $\beta$  receptor; Thr, threonine; Tyr, tyrosine; UST, uronosyl 2-O-sulfotransferase; VSMCs, vascular smooth muscle cells; Xyl, xylose; XT, xylosyltransferase.

\* Corresponding author at: School of Pharmacy, The University of Queensland, Pharmacy Australia Centre of Excellence, 20 Cornwall Street, Woolloongabba QLD 4102, Australia.

E-mail addresses: [r.afroz@uq.edu.au](mailto:r.afroz@uq.edu.au) (R. Afroz), [ashrafrostam@ium.edu.my](mailto:ashrafrostam@ium.edu.my) (M.A. Rostam), [h.ta@uq.edu.au](mailto:h.ta@uq.edu.au) (H. Ta), [Suowen\\_Xu@URMC.Rochester.edu](mailto:Suowen_Xu@URMC.Rochester.edu) (S. Xu), [wenhuaxzheng@umac.mo](mailto:wenhuaxzheng@umac.mo) (W. Zheng), [narin.osman@rmit.edu.au](mailto:narin.osman@rmit.edu.au) (N. Osman), [d.kamato@uq.edu.au](mailto:d.kamato@uq.edu.au) (D. Kamato), [p.little@uq.edu.au](mailto:p.little@uq.edu.au) (P.J. Little).

5. Potential of the response to retention hypothesis for providing a therapeutic agent for the prevention or treatment of atherosclerosis . . . . .	93
6. Conclusions . . . . .	95
Acknowledgments . . . . .	95
Conflict of interest . . . . .	95
References . . . . .	95

## 1. Introduction

Cardiovascular diseases (CVDs) are a heterogeneous group of conditions afflicting heart and blood vessels which share common underlying pathological mechanisms (Aghamohammadzadeh, Ormandy, and Heagerty, 2015). CVDs are the largest single cause of death globally and more people die annually from CVDs than from any other cause (Mendis, Puska, and Norrving, 2011; WHO, 2017). Atherosclerosis is a progressive disease characterized by lipid accumulation in the large arteries and it constitutes the single most important contributor to this enormous burden of disease and it is accelerated in the presence of diabetes (Libby, 2002; Nigro, Osman, Dart, and Little, 2006; Yang et al., 2010). Atherosclerosis commences with the pre-inflammatory stage occurring as accumulation of lipids in the blood vessel wall followed by an inflammatory stage which is manifest ultimately as the formation of atherosclerotic plaques (Libby, 2002; Ross, 1999; Virmani et al., 2005). Plaques may be stable or unstable with plaque rupture, causing the acute clinical syndrome of a life threatening myocardial infarction or stroke (Davies, 1996; Falk, 1989). The major pathological mechanisms currently under investigation as causes of atherosclerosis are the retention of low density lipoproteins (LDLs) (Skalen et al., 2002), endothelial dysfunction (Davignon and Ganz, 2004), oxidative stress (Witztum, 1994), inflammation (Libby, 2002; Ross, 1999) and immune system defects (Fernández-Ruiz, 2016; Little, Chait, and Bobik, 2011). In human coronary arteries, atherosclerosis commences with the retention of LDLs by galactosaminoglycans on proteoglycans (PGs), specifically biglycan and this is followed by the immune response and the inflammatory cascade (Nakashima, Fujii, Sumiyoshi, Wight, and Sueishi, 2007). Utilization of genetically modified apolipoprotein knockout mice demonstrates the primary role of LDLs and PGs in atherogenesis (Skalen et al., 2002). Boren et al. (Kijani, Vázquez, Levin, Borén, and Fogelstrand, 2017) confirmed that, vascular LDL retention is almost exclusively dependent on electrostatic interactions between galactosaminoglycans and LDLs. In a murine model of atherosclerosis, the increased expression of galactosaminoglycan synthesizing genes is associated with lipid accumulation in the vessel wall (Anggraeni et al., 2011).

In early atherogenesis, negatively charged sulfate and carboxyl groups of galactosaminoglycan chains on PGs in the extracellular matrix of the arterial intima bind and trap LDLs via electrostatic interactions with positively charged aminoacyl residues in the apoB moiety of an LDL particle (Borén and Williams, 2016; Ohira and Iso, 2013). LDL particles normally flux into and out of the arterial wall, most LDL particles that enter the arterial wall leave without contributing to the development of the atherosclerotic lesion (Borén and Williams, 2016; Schwenke and Carew, 1989; Tran-Lundmark et al., 2008). LDL particles become trapped in the vessel wall because of the changes in the properties of both the LDLs and the extracellular matrix, particularly galactosaminoglycan chains on PGs (Haberland, Olch, and Fogelman, 1984; Little, Osman, and O'Brien, 2008; Nakashima et al., 2007; Tabas, 1999).

Multiple signalling pathways are involved in regulating galactosaminoglycan synthesizing enzyme gene expression, which direct structural changes in galactosaminoglycan chains on PGs and increase their binding affinity to LDLs in the vessel wall. These signalling pathways represent potential targets for therapeutic intervention of atherosclerosis and thus are the subject of the current review.

## 2. Galactosaminoglycan structure contributes to LDL binding

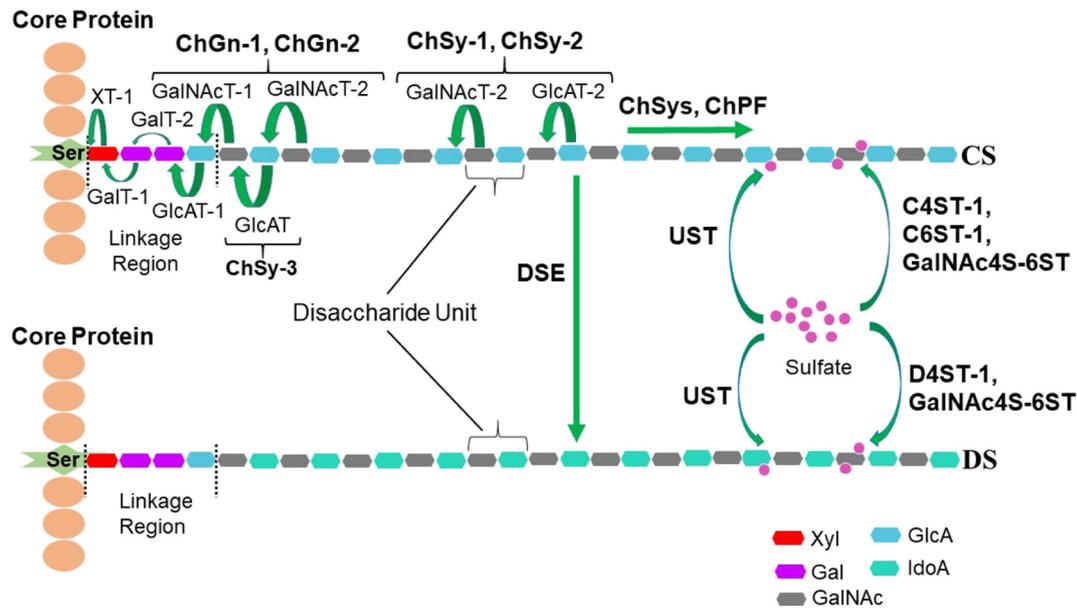
### 2.1. Structural features of galactosaminoglycans

The major glycosaminoglycans (GAGs) in vertebrates include glucosaminoglycans (heparan sulfate (HS), keratan sulfate (KS), heparin (Hep)) and galactosaminoglycans (chondroitin sulfate (CS) and dermatan sulfate (DS)) (Kjellén and Lindahl, 1991). The GAG chains are composed of alternating units of an amino sugar, either *N*-acetyl- $\beta$ -glucosamine (GlcNAc) or *N*-acetyl- $\beta$ -galactosamine (GalNAc) and a hexauronic acid either glucuronic acid (GlcA) or iduronic acid (IdoA). The presence of either type of amino sugar differentiates between glucosaminoglycans and galactosaminoglycans. GlcNAc or GalNAc is found to be present in disaccharide units of glucosaminoglycans and galactosaminoglycans, respectively. Although the polysaccharide backbones are simple, linear chains of repeating disaccharide units, Hep/HS and CS/DS acquire a considerable degree of structural variability by extensive modifications involving sulfation and uronic acid epimerization (Kjellén and Lindahl, 1991; Yamada, Sugahara, and Özbek, 2011).

Galactosaminoglycan chains on PGs are complex, long, unbranched polysaccharides with important roles in cell growth, differentiation, morphogenesis, cell migration and microbial infection (Yamada et al., 2011). Galactosaminoglycan chains are covalently attached to the core protein to form PGs, which are ubiquitously distributed in the extracellular matrix (Kjellén and Lindahl, 1991). Particularly, galactosaminoglycan chains are attached to a serine (Ser) residue on the core protein through the common linkage region tetrasaccharide, which is made up of xylose-galactose-galactose-glucuronic acid (Xyl-Gal-Gal-GlcA) (Fig. 1). The galactosaminoglycan chains are generated principally by similar modifications to glucosaminoglycans with an initial polymerization product of the disaccharide unit GlcA/IdoA-GalNAc. The structural diversity of galactosaminoglycans is less pronounced than heparin related species as the GalNAc residues remain acetylated (Kjellén and Lindahl, 1991). The range of normal galactosaminoglycan chain lengths vary widely and extends from 20 kDa to approximately 60 kDa corresponding to approximately 40 to 120 disaccharides (Little, Ballinger, Burch, and Osman, 2008). Epimerization at the carbon-5 (C-5) position of GlcA in precursor chondroitin backbone results in the formation of repeating disaccharide unit GalNC-IdoA or dermatan backbone (Malmström and Fransson, 1975). Both CS and DS galactosaminoglycans are fully developed through sulfation by the action of sulfotransferases. Galactosaminoglycans are mainly sulfated at C-4 and C-6 positions of their GalNAc moieties. C-2 and C-3 positions of GlcA and IdoA are other positions available for sulfation of galactosaminoglycan chains and therefore various types of di and trisulfated disaccharide units may form (Karamanos, Syrokou, Vanky, Nurminen, and Hjerpe, 1994).

### 2.2. Galactosaminoglycan structure and LDL binding

There are three major structural properties of galactosaminoglycans that are regulated by signalling pathways and can influence their binding to apolipoproteins on LDLs. These include modification in the length of the chains (number of disaccharide units), the extent and pattern of sulfation and the isoform of the uronic acid moiety on the galactosaminoglycan chain (Ballinger, Nigro, Frontanilla, Dart, and Little, 2004). Treatment of VSMCs with multiple vascular agonists



**Fig. 1.** Schematic representation of the role of enzymes in galactosaminoglycan biosynthesis and modification: Galactosaminoglycan linkage region is made up of xylose-galactose-galactose-glucuronic acid (Xyl-Gal-Gal-GlcA). The xylosyltransferase-1 (XT-1) enzyme transfers a Xyl to serine (Ser) residue of PG core protein. Two Gal residues are added by the action of two enzymes,  $\beta$ -1,3-galactosyl transferase-1 (GalT-1) and GalT-2. The  $\beta$ -1,3-glucuronosyl transferase-1 (GlcAT-1) adds a GlcA residues to complete the linkage region. Six chondroitin synthase enzymes including chondroitin synthase-1 (ChSy-1), ChSy-2, ChSy-3, chondroitin polymerizing factor (ChPF), chondroitin *N*-acetylgalactosaminyltransferase-1 (ChGn-1) and ChGn-2 catalyse subsequent addition of repeating disaccharide unit (GalNAc-GlcA) in CS chain. The modification of this precursor chondroitin backbone is occurring by sulfation with the action of sulfotransferase enzymes including uronosyl 2-O-sulfotransferase (UST), chondroitin 4-O-sulfotransferase (C4ST), chondroitin 6-O-sulfotransferase (C6ST). Dermatan sulfate-glucuronyl C5-epimerase (DSE) enzyme catalyse epimerization at fifth carbon (C-5) position of GlcA in precursor chondroitin backbone to synthesize dermatan backbone. The dermatan backbone is then matured and modified by action of another set of sulfotransferases including UST and dermatan 4-O-sulfotransferase (D4ST). *N*-acetylgalactosamine 4-sulfate 6-O-sulfotransferase (GalNAc4S-6ST) transfers sulfate to C-6 position of *N*-acetylgalactosamine 4-sulfate (GalNAc(4SO<sub>4</sub>)) in both growing CS and DS chain.

stimulates cells to produce elongated galactosaminoglycan chains which is associated with an increased binding to LDL (Figueroa and Vijayagopal, 2002; Getachew et al., 2010). In aortic smooth muscle cells, oxidized LDL causes biglycan galactosaminoglycan chain elongation with increased LDL binding (Chang, Potter-Perigo, Tsoi, Chait, and Wight, 2000). Comparative studies with CS and DS PGs indicate that DS PGs are more efficient at LDL binding. Oversulfation is considered as the prime feature involved in increased LDL binding to DS molecules (Sambandam, Baker, Christner, and Ekborg, 1991). Both the degree of sulfation and the position of the sulfate groups on the galactosaminoglycans, determines their binding ability to LDLs (Ballinger et al., 2004; Sambandam et al., 1991). In early atherosclerosis, the ratio of deltadi-mono-6-sulfate/deltadi-mono-4-sulfate ( $\Delta$ di-mono6S/ $\Delta$ di-mono4S) in galactosaminoglycan chains is increased which reflects the similar increase in the ratio of CS/DS, since CS is mainly composed of C-6 sulfated disaccharides, whereas DS is mainly composed of C-4 sulfated units (Theocharis, Theocharis, De Luca, Hjerpe, and Karamanos, 2002). These changes may facilitate the LDL accumulation in the aortic wall. Similar pattern of increases in C-6 sulfation (6S)/4S of galactosaminoglycan chains in the aorta of rats fed a high cholesterol diet have also been reported (Oberkersch et al., 2014). Enzymatic epimerization of the carboxylic acid group on the C-5 position of the GlcA generates the isomer IdoA, by definition, the galactosaminoglycan chain changes from CS to DS. These two molecules show different levels of structural flexibility and rigidity that determine LDL binding. GlcA exists in a single well defined chair conformation, on the other hand IdoA residues exist in multiple isoenergetic conformers. IdoA containing galactosaminoglycan chains bind more avidly to LDL than GlcA containing galactosaminoglycans (Gigli, Ghiselli, Torri, Naggi, and Rizzo, 1993). Although the overall charge densities of the molecules are similar, IdoA containing galactosaminoglycans exhibit local concentrations of charge for ionic binding of galactosaminoglycan to the apolipoprotein on LDLs (Iverius, 1972).

Animal models of atherosclerosis have provided strong evidence to support the role of galactosaminoglycan in lipid binding and

atherosclerotic lesion development (Delgado-Roche et al., 2013, 2015; Sarduy et al., 2017). Mice immunized with chimeric mouse/human antibodies against galactosaminoglycans and fed a high-fat, high cholesterol diet showed a 40% reduction in aortic lesion area due to inhibition of LDL-CS binding and reduction of oxidative stress (Brito et al., 2012).

### 3. Enzymes involved in galactosaminoglycan biosynthesis and modification

Biosynthesis of galactosaminoglycan chains on PGs involves the concerted action of multiple enzymes (Fig. 1). The initiation of the linkage region requires the xylosyltransferase-1 (XT-1) enzyme, to transfer a Xyl to Ser residue of PG core protein (Götting, Kuhn, and Kleesiek, 2007). Subsequently, the combined action of three enzymes  $\beta$ -1,3-galactosyl transferase-1 (GalT-1), GalT-2 and  $\beta$ -1,3-glucuronosyl transferase-1 (GlcAT-1) add two Gal and one GlcA residues to form the linkage region (Uyama, Kitagawa, and Sugahara, 2007). This linkage region connects the galactosaminoglycan chain to the core protein. Polymerization of repeating disaccharide units (GalNAc-GlcA) in CS chains occurs by the action of six chondroitin synthase enzymes including chondroitin synthase-1 (ChSy-1), ChSy-2, ChSy-3, chondroitin polymerizing factor (ChPF), chondroitin *N*-acetylgalactosaminyltransferase-1 (ChGn-1) and ChGn-2 (Izumikawa et al., 2008; Izumikawa, Uyama, Okuura, Sugahara, and Kitagawa, 2007). The precursor CS backbone is matured and modified by sulfation with the action of a number of enzymes including uronosyl 2-O-sulfotransferase (UST), chondroitin 4-O-sulfotransferase (C4ST), chondroitin 6-O-sulfotransferase (C6ST) (Uyama et al., 2007). CS backbone is modified to the DS with the epimerization action of the DS epimerase (DSE) enzyme at C-5 position of GlcA in CS backbone. DS backbone is fully matured by sulfation with the action of a different set of sulfotransferases, UST and dermatan 4-O-sulfotransferase (D4ST) (Evers, Xia, Kang, Schachner, and Baenziger, 2001). An enzyme *N*-acetylgalactosamine 4-sulfate 6-O-sulfotransferase (GalNAc4S-6ST) transfers sulfate to the C-6 position of *N*-acetylgalactosamine 4-sulfate (GalNAc(4SO<sub>4</sub>)) in both growing CS

and DS chains (Ohtake, Ito, Fukuta, and Habuchi, 2001). All these sulfotransferases (regardless CS and/or DS) transfer sulfate groups to the different corresponding positions of GlcA and IdoA (Malmström, Bartolini, Thelin, Pacheco, and Maccarana, 2012; Uyama et al., 2007). ChSy-1 and ChSy-2 are bifunctional glycosyltransferases that exhibit GlcAT-2 and N-acetylgalactosyl transferase-2 (GalNAcT-2) activity (Izumikawa et al., 2007, 2008). ChSy-3 exhibits only GlcAT activity (Izumikawa et al., 2008). Whereas ChSy-1 and ChSy-2 are responsible for CS chain elongation, ChGn-1 and ChGn-2 catalyse both the chain initiation and elongation (Sato et al., 2003). ChGn-1 and ChGn-2 enzymes exhibit GalNAcT-1 and GalNAcT-2 activity (Gotoh et al., 2002; Sato et al., 2003). Chondroitin polymerization can be achieved by the combined action of enzyme complexes composed of any of two ChSys and ChPF (Izumikawa et al., 2007, 2008). This enzyme complexes with chondroitin polymerase activity and interacts with C4ST-1 to regulate the length of CS chains (Izumikawa, Okuura, Koike, Sakoda, and Kitagawa, 2011). ChGn-1 regulates the chain numbers and level of CS and co-operates with C4ST-2 to increase the number of CS moieties (Izumikawa, Koike, and Kitagawa, 2012). Therefore, biosynthesis and chain length of galactosaminoglycans are not only regulated by chondroitin synthase family members but also CS sulfotransferases. Although an array of enzymes is involved in galactosaminoglycan chain elongation, ChGn-2 and C4ST-1 play a critical role in the regulation of galactosaminoglycan chain length in vitro (Izumikawa et al., 2011). An increase in the CS/DS synthesis was found in atherosclerotic mouse model study accompanied by an increased expression of these two enzymes (Anggraeni et al., 2011). Thus the critical role of ChGn-2 and C4ST-1 in galactosaminoglycan chain elongation regulation was demonstrated both in vivo and in vitro.

#### 4. Signalling pathways regulating galactosaminoglycan synthesis and structure

##### 4.1. Protein tyrosine kinase receptor (PTKR) pathways

The PTKRs are transmembrane glycoproteins that are activated by the binding of their ligands (Hubbard and Till, 2000). PTKRs are involved in activation of numerous signalling pathways leading to cell proliferation, differentiation, migration or metabolic changes (Schlessinger, 2000). The PTKR family includes insulin receptors and numerous growth factor receptors such as platelet derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor, vascular endothelial growth factor and nerve growth factor (Hubbard and Till, 2000). Growth factors such as PDGF and EGF are involved in the hyperelongation of galactosaminoglycan chains by altering gene expression utilizing classical PTKR signaling (Getachew et al., 2010; Yang et al., 2009).

PDGF via its cognate receptor stimulates the elongation of galactosaminoglycan chains on PGs (Getachew et al., 2010; Schonherr, Jarvelainen, Kinsella, Sandell, and Wight, 1993). In human VSMCs PDGF stimulates autophosphorylation and activation of the PDGFR $\alpha$  and PDGFR $\beta$  in their kinase activation domain tyrosine<sup>849</sup> (Tyr<sup>849</sup>) and Tyr<sup>857</sup>, respectively (Getachew et al., 2010). Imatinib, a small molecule tyrosine kinase inhibitor blocks PDGFR $\beta$  by inhibiting autophosphorylation on Tyr<sup>857</sup>, thus inhibiting galactosaminoglycan hyperelongation and LDL retention (Ballinger et al., 2010). Tyrosine kinase inhibitor, K11502 blocks Tyr<sup>857</sup> autophosphorylation on PDGFR $\beta$ , and is more potent than imatinib in inhibiting galactosaminoglycan elongation (Getachew, Ballinger, Burch, Little, and Osman, 2010). In contrast, genistein, a tyrosine kinase inhibitor does not block PDGFR autophosphorylation. Genistein inhibits PG core protein synthesis but has no effect on galactosaminoglycan chain elongation in VSMCs (Little et al., 2012). These studies demonstrated that PDGFR $\beta$  autophosphorylation in Tyr<sup>857</sup> is crucial to PDGF stimulated galactosaminoglycan chain elongation. PDGF also stimulates phosphorylation in Tyr<sup>751</sup> and Tyr<sup>1021</sup> on PDGFR, these are associated with downstream signalling

via phosphoinositide 3-kinase (PI3K) and phospholipase C (PLC). Although neither of these sites mediate galactosaminoglycan elongation however both sites are associated with increased PG core protein synthesis (Getachew et al., 2010).

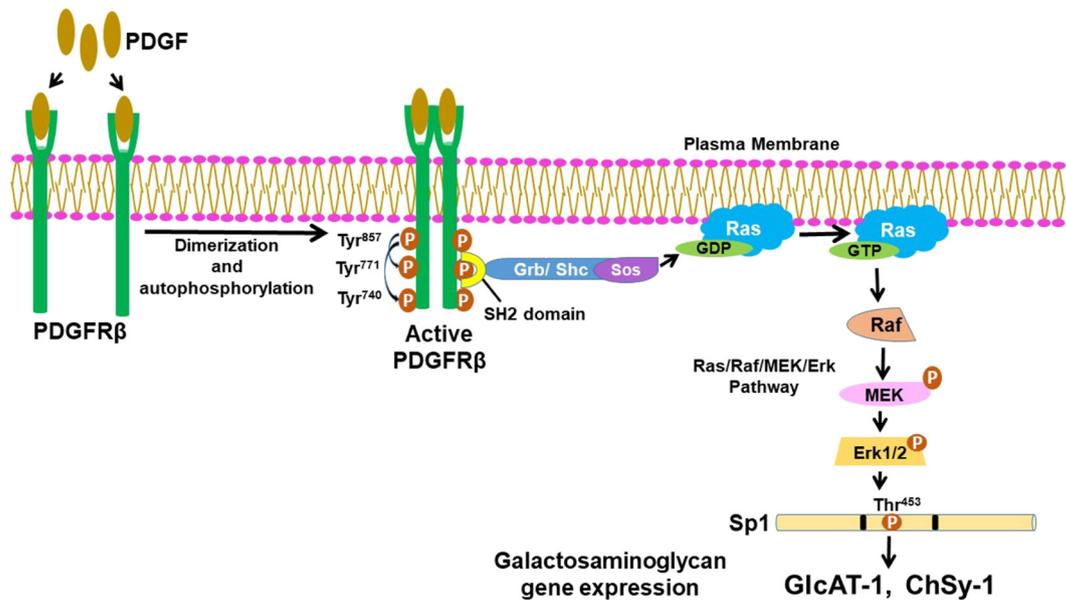
Phosphorylation of PDGFR $\beta$  Tyr<sup>857</sup> initiates extensive autophosphorylation of 11 Tyr residues within the cytoplasmic region of the receptor. These sites serve as tyrosine kinase docking sites for a number of adaptor molecules containing SH2 domains such as Grb and Shc. For instance, Grb and Shc can bind to Tyr<sup>740</sup> and Tyr<sup>771</sup>, respectively on PDGFR $\beta$ . These molecules form complexes with son of sevenless (Sos), a guanine nucleotide exchange factor to activate Ras (Heldin and Westermark, 1999). Activated Ras sequentially activates extracellular signal-regulated kinase 1/2 (Erk1/2) through the Ras/Raf/MEK/Erk pathway. Erk1/2 is associated with galactosaminoglycan elongation in VSMCs (Getachew et al., 2010). Transcription factor Sp1 is phosphorylated by Erk1/2 on threonine<sup>453</sup> (Thr<sup>453</sup>) (Tan and Khachigian, 2009). PDGF treatment in VSMCs, produced peak of Thr<sup>453</sup> Sp1 phosphorylation which is blocked in the presence of Erk inhibitors. This suggests the possible role of the Sp1 in galactosaminoglycan chain elongation (Getachew et al., 2010). Barre et al. (Barré et al., 2006) demonstrated that Sp1 phosphorylated and activated by Erk1/2 is a transactivator of GlcAT-1 gene promoter leading to increased expression of the GlcAT-1 enzyme in HeLa cells. In a recent study, ChSy-1 expression was found to be induced by Erk1/2 activated Sp1 in rat nucleus pulposus cells (Hu et al., 2015). Together these results indicate an involvement of Sp1 in PDGF stimulated expression of CS enzymes and subsequent hyperelongation of CS chains (Fig. 2).

PDGF leads to modifications in the sulfation pattern of CS chains. An increase in the the chondroitin-6-sulfate (C6S) to chondroitin-4-sulfate (C4S) ratio was found in PDGF treated monkey arterial smooth muscle cells. PDGF utilizes protein kinase C (PKC) signaling leading to the activation Ras/Raf/MEK/Erk pathway to mediate the changes in sulfation pattern of CS chains, but PKC activity alone is insufficient to generate this response (Cardoso et al., 2010). The serine/threonine kinase, Akt is found to be phosphorylated in response to PDGF stimulation. Akt phosphorylation is associated with PG core protein synthesis but it has no effect on PTKR stimulated galactosaminoglycan elongation or the expression of C4ST-1 gene (Osman et al., 2014). Another PTKR, EGF receptor (EGFR) stimulates the mRNA expression of galactosaminoglycan synthesizing gene ChSy-1 and C4ST-1 via downstream signaling intermediate Erk1/2, which is inhibited by EGFR antagonists AG1478 in VSMCs (Kamoto et al., 2016). PTKR mediated signaling pathways in response to their agonist stimulation are directly involved in inducing expression of galactosaminoglycan synthesizing genes leading to the synthesis of hyperelongated galactosaminoglycan chains. These hyperelongated galactosaminoglycan chains render the vessel wall “more sticky” to trap more LDLs and initiates the pre-inflammatory stage of atherosclerosis.

##### 4.2. Serine/threonine kinase receptors (S/TKRs) pathways

A family of transmembrane receptors that contain intracellular serine/threonine kinase domain is known as S/TKRs and ligands of these receptors include TGF- $\beta$  superfamily members such as TGF- $\beta$ , activin, nodal and others (ten Dijke et al., 1994). TGF- $\beta$  is a pleiotropic growth factor associated with the development of all stages of atherosclerosis. TGF- $\beta$  family members consist of dimeric ligands and usually remain in a latent form bound to their polypeptide. When released from their polypeptide, these ligands become active and bind to dimers of membrane TGF- $\beta$  receptors (TGFBRs). Following ligand binding, TGFBR1 dimers combine with TGFBR2 dimers to form heterotetrameric receptor complexes. Phosphorylation of TGFBR1 by TGFBR2 leads to the formation of active heterotetrameric receptor complexes (Massague, Seoane, and Wotton, 2005).

TGF- $\beta$ 1 has a very specific action on galactosaminoglycan elongation and has no effects on the C6S to C4S ratio (Schonherr et al., 1993).



**Fig. 2.** Platelet derived growth factor (PDGF) mediated signalling pathway leading to galactosaminoglycan genes expression: PDGF binding to the PDGF receptor  $\beta$  (PDGFR $\beta$ ) induce receptor dimerization and autophosphorylation on Tyr<sup>857</sup>. Tyr<sup>857</sup> Phosphorylation initiates autophosphorylation of downstream tyrosine residues within the cytoplasmic region of the receptor. Adaptor molecules such as Grb and Shc contain SH2 domain that can bind these tyrosine kinase docking sites on PDGFR. These adaptor molecules form complex with son of sevenless (Sos), a guanine nucleotide exchange factor. Sos mediate the exchange for GDP bound to inactive Ras to GTP and activates Ras. Subsequent activation of Raf leading to phosphorylation and activation of mitogen activated protein kinase (MAPK) Erk kinase (MEK). Activated MEK sequentially phosphorylates and activates extracellular signal-regulated kinase 1/2 (Erk1/2). Sp1 transcription factor is phosphorylated on threonine<sup>453</sup> (Thr<sup>453</sup>) by Erk1/2 and induces expression of galactosaminoglycan synthesizing enzymes GlcAT-1 and ChSy-1.

Transcription factor Smad2 is an immediate downstream target of TGF $\beta$ 1 in the TGF- $\beta$  stimulated signalling pathway. Smad2 consists of an amino-terminal or “Mad-homology 1” (MH1) domain, a linker region and a carboxy-terminal or MH2 domain (Massague et al., 2005). The canonical TGF- $\beta$  signalling pathway via the TGF $\beta$ 1 involves the phosphorylation of the Smad2 carboxy-terminal, which forms a complex with Smad4 to translocate to the nucleus (Burch, Zheng, and Little, 2011; Derynck, Zhang, and Feng, 1998; Massague et al., 2005). In addition to carboxy terminal phosphorylation, the Smad2 linker region can undergo phosphorylation on Thr<sup>220</sup>, Ser<sup>245</sup>, Ser<sup>250</sup>, Ser<sup>255</sup> residues (Kamato et al., 2014; Rostam et al., 2016). Smad linker region phosphorylation can occur by multiple serine/threonine kinases such as MAP kinases (Erk and p38), PI3K, cyclin-dependent kinases (CDKs), Rho associated protein kinase (ROCK) and glycogen synthase kinase 3 (GSK3) (Burch et al., 2010; Kamato et al., 2013; Matsuzaki, 2013; Rezaei, Kamato, Ansari, Osman, and Little, 2012). Highly specific signalling pathways from TGF- $\beta$  to Smad2 linker region phosphorylation exist in human VSMCs (Rostam et al., 2016). Serine/threonine kinases Erk, p38, PI3K and CDK mediate the phosphorylation of the Ser<sup>245</sup>/Ser<sup>250</sup>/Ser<sup>255</sup> residues whereas only CDK is found to phosphorylate the Thr<sup>220</sup> residue in Smad2 linker region. Phosphorylation of three Ser residues (Ser<sup>245</sup>, Ser<sup>250</sup> and Ser<sup>255</sup>) in Smad2 linker region induces expression of two galactosaminoglycan synthesizing enzymes ChSy-1 and C4ST-1, however phosphorylation of Thr<sup>220</sup> is associated with induction of XT-1, the enzyme involved in the initiation of the galactosaminoglycan linkage region (Rostam et al., 2016) (Fig. 3). Phosphorylation of any of the three Ser residues on Smad2 linker region is involved in galactosaminoglycan chain elongation. In contrast, Thr<sup>220</sup> phosphorylation on Smad2 linker region can induce galactosaminoglycan chain initiation. XT-1 expression is up-regulated by TGF $\beta$ 1 in cardiac fibroblasts with a corresponding increased synthesis of galactosaminoglycans in cardiac tissues. A specific knock-down of the XT-1 mRNA with siRNA resulted in a decreased XT-1 activity and lower galactosaminoglycan synthesis in vivo (Prante et al., 2007).

S/TKR signalling pathways play a crucial role in galactosaminoglycan synthesizing enzyme expression via Smad2 linker region phosphorylation. TGF- $\beta$  stimulated overexpression of these genes involved in both

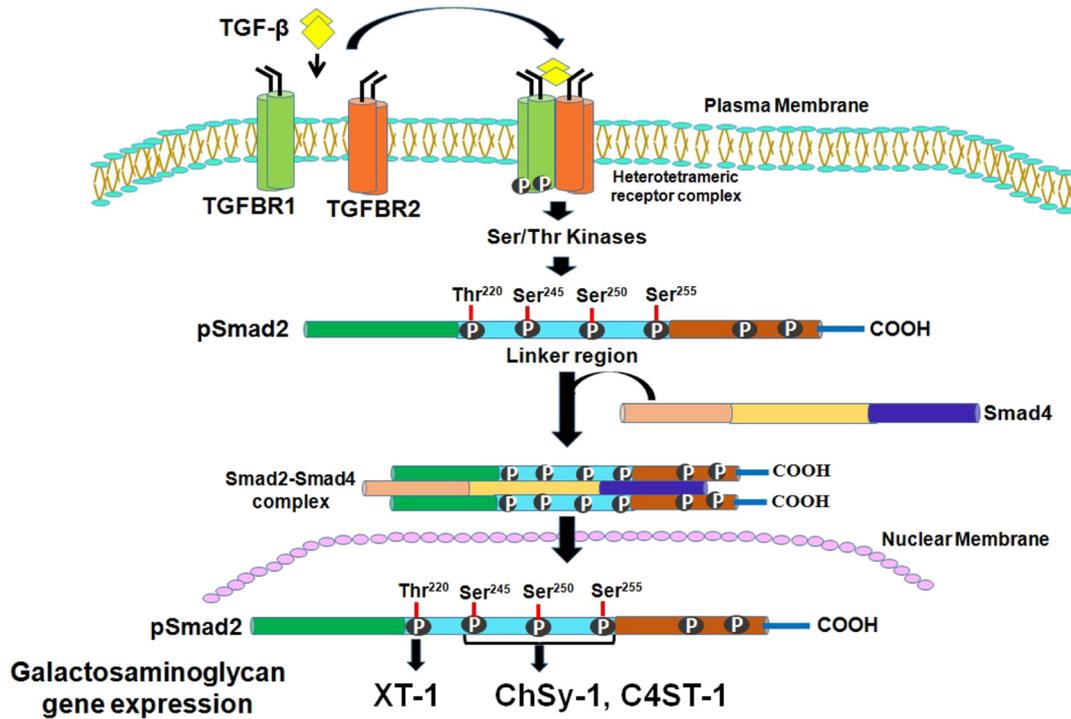
galactosaminoglycan chain initiation and elongation render the vessel wall more susceptible to LDL binding.

#### 4.3. G-protein coupled receptors (GPCRs) pathways

Seven transmembrane GPCRs are the most prolific and polyfunctional group of cell surface receptors in mammalian biology and are heavily implicated in physiology and pathology. These receptors are coupled to G proteins with  $\alpha$ ,  $\beta$  and  $\gamma$  subunits which act as effectors of GPCR signalling (Pierce, Premont, and Lefkowitz, 2002). Downstream mediators of GPCR signaling include inositol 1,4,5 triphosphate (IP<sub>3</sub>) leading to the increased cytosolic calcium and activation of protein kinase C (PKC) (Gilman, 1987).

Vasoactive GPCR agonists endothelin-1 (ET-1) (Little, Burch, Getachew, Al-aryahi, and Osman, 2010), angiotensin-II (Ang-II) (Figuroa and Vijayagopal, 2002) and thrombin (Burch et al., 2010) stimulate PG synthesis in VSMCs. ET-1 stimulates PG synthesis with longer galactosaminoglycan chains and increased C6S to C4S ratio (Ballinger, Ivey, Osman, Thomas, and Little, 2009). Ang-II induces an increase in PG synthesis in human thoracic aorta smooth muscle cells, which is due to galactosaminoglycan chain hyperelongation and increased sulfation (Figuroa and Vijayagopal, 2002). Previous studies targeted the involvement of classical GPCR signaling pathways in PG synthesis and galactosaminoglycan chain elongation (Burch et al., 2010; Survase, Ivey, Nigro, Osman, and Little, 2005). IP<sub>3</sub> and cytosolic calcium ions do not effect PG synthesis in VSMCs (Burch et al., 2010; Survase et al., 2005). Although, PKC can play an important role in PG synthesis and galactosaminoglycan chain elongation (Cardoso et al., 2010). GPCR agonists do not stimulate PG synthesis and galactosaminoglycan chain elongation via classical GPCR mediated signalling pathways.

GPCRs can transactivate both PTKRs (Daub, Weiss, Wallasch, and Ullrich, 1996) and S/TKRs (Burch et al., 2010; Kamato et al., 2017; Kamato, Burch, Osman, Zheng, and Little, 2013). Indeed, the recognition of the ability of GPCRs to transactivate PTKRs and S/TKRs was discovered in the context of galactosaminoglycan chain elongation (Kamato et al., 2016). Thrombin can stimulate PG synthesis in VSMCs



**Fig. 3.** Transforming growth factor- $\beta$  (TGF- $\beta$ ) mediated signalling pathway leading to galactosaminoglycan genes expression: Ligand binding induces TGF- $\beta$  receptor 1 (TGFBR1) and TGFBR2 dimers to form heterotetrameric complex followed by the activation of TGFBR1. TGFBR1 can induce the activation of multiple Ser/Thr kinases. Individual Smad2 linker region sites can be specifically phosphorylated by these serine/threonine kinases. Phosphorylation of three Ser residues on Ser<sup>245</sup>, Ser<sup>250</sup> and Ser<sup>255</sup> leading to induce the mRNA expression of two crucial galactosaminoglycan synthesizing enzyme ChSy-1 and C4ST-1. Thr<sup>220</sup> phosphorylation is associated with expression of galactosaminoglycan chain initiating enzyme XT-1. These indicates that phosphorylation on one of the three Ser residues play key role in galactosaminoglycan chain elongation. In contrast, threonine<sup>220</sup> (Thr<sup>220</sup>) phosphorylation plays critical role in initiation of galactosaminoglycan chain in TGF- $\beta$  stimulated galactosaminoglycan synthesis.

which utilizes both PTKRs and S/TKRs transactivation pathways (Burch, Getachew, Osman, Febbraio, and Little, 2013). Thrombin elicits dual transactivation-dependent signaling pathways to stimulate ChSy-1 and C4ST-1 mRNA expression (Kamato et al., 2016) (Fig. 4). Thrombin stimulation of galactosaminoglycan synthesizing enzyme expression occurs entirely via transactivation-dependent pathways and conversely does not involve transactivation-independent pathways (Kamato et al., 2015). The transactivation of PTKR involves matrix metalloproteinases (MMPs) and the phosphorylation of Erk. In contrast, the S/TKR mediated response involves phosphorylation of Ser residues on Smad2 carboxy terminal and does not involve MMPs. Chung et al. (Chung, Ramachandran, Hollenberg, and Muruve, 2013) showed similar results with TGFBR1 transactivation by proteinase-activated receptor-2 (PAR-2), except transactivation of TGFBR1 was reported to be dependent on MMPs. Smad2 linker region phosphorylation via thrombin stimulation is inhibited by the MMP inhibitor, GM6001 (Kamato et al., 2016). MMPs are involved in GPCR transactivation of EGFR but not TGFBR1. Therefore, thrombin mediated Smad2 linker region phosphorylation occurs possibly via GPCR transactivation of PTKR not GPCR transactivation of S/TKRs. Smad2 linker region phosphorylation is of considerable interest because identifying a common target molecule downstream to distinct receptor mediated signaling pathways for the regulation of PG synthesis and galactosaminoglycan hyperelongation would be a potential therapeutic target for the prevention of atherosclerosis.

### 5. Potential of the response to retention hypothesis for providing a therapeutic agent for the prevention or treatment of atherosclerosis

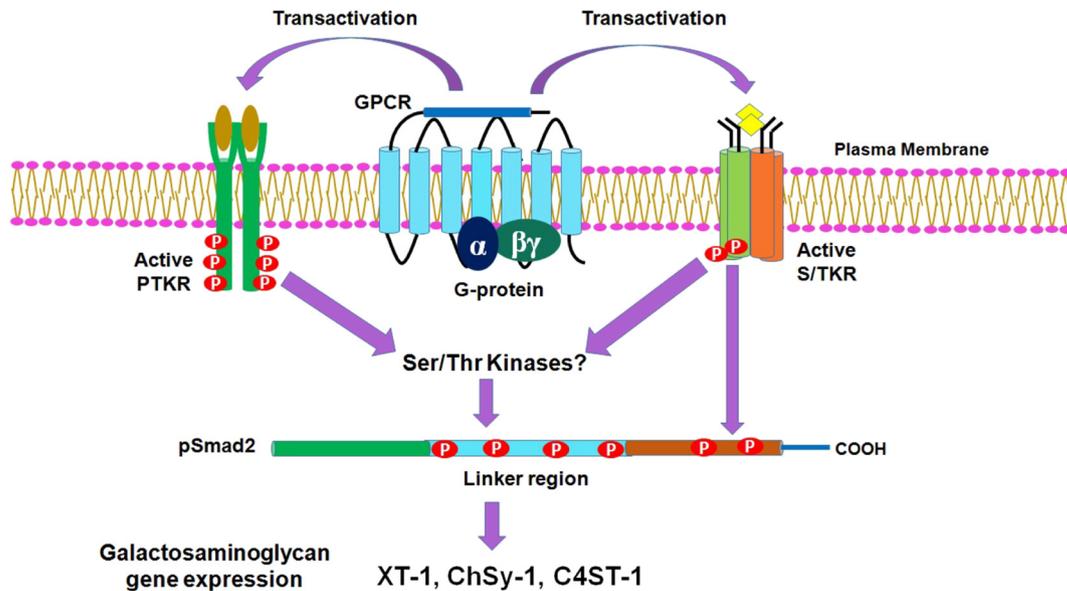
The response to retention hypothesis proposes that atherosclerosis commences with the entry, binding and trapping of atherogenic LDLs by modified PGs in the vessel wall. So what are the potential therapeutic modalities to exploiting this hypothesis therapeutically?

#### 5.1. Lowering plasma LDL cholesterol

LDL cholesterol lowering is the current gold standard and recommended treatment for preventing atherosclerosis. Simply lowering plasma cholesterol should reduce the cholesterol flux into the vessel wall. Statins were consistently successful in multiple clinical trials and have been very successful in reducing CVD risk and events in real world therapeutic use for over two decades. There are multiple statins available and their actions and outcomes have been extensively reviewed (Collins et al., 2016; Ehrenstein, Jury, and Mauri, 2005; Taylor et al., 2013).

More recently proprotein convertase subtilisin kexin 9 (PCSK9) inhibitors have been developed – these agents bind to PCSK9 preventing its action in degrading LDL receptors leading to major upregulation of LDL receptors and an increased uptake of cholesterol and lowered plasma cholesterol (Yadav, Sharma, and Ferdinand, 2016). With statins expressing such high efficacy the rationale for PCSK9 inhibitors is less than clear. Both statins and PCSK9 inhibitors cause mild hyperglycaemia meaning an increase in diabetes depending upon the starting glycaemia status and susceptibility of the patient cohort (Schmidt et al., 2017; Shah and Goldfine, 2012).

The efficacy of statins in preventing CVD is such that some clinical guidelines (Lloyd-Jones, Goff, and Stone, 2014) even indicate the use of a statin without ongoing reference to the plasma cholesterol level. This is related to the demonstrated clinical efficacy but also at least in part the wide range of favourable, pleiotropic, cardiovascular protective actions which have been demonstrated for statins (Collins et al., 2016; Ford, Murray, McCowan, and Packard, 2016; Taylor et al., 2013). Importantly, in the current context, statins have no effects on PG synthesis in VSMCs (Little and Osman, unpublished observations) so such an action is not in the group of anti-inflammatory, anti-oxidation and other actions which add to the cholesterol lowering actions of statins and thus targeting PG-LDL interactions is a bona fide approach in combination with a statin.



**Fig. 4.** G-protein coupled receptor (GPCR) transactivation of PTKR and S/TKR leading to galactosaminoglycan gene expression: Thrombin mediated galactosaminoglycan chain elongation does not involve classical GPCR signalling rather it utilizes transactivation pathways for both PTKR and S/TKR to elicit response. GPCR transactivation of PTKR involve matrix metalloproteinases (MMPs), but S/TKR does not. Activated S/TKRs can directly phosphorylate transcription factor Smad2 on its carboxy terminal. S/TKRs also phosphorylates the linker region of Smad2 via serine/threonine kinases (Ser/Thr kinases). Combined speculation of different studies also provide an indication to the Smad2 linker region phosphorylation by PTKRs. Smad2 linker region phosphorylation can induce the expression of galactosaminoglycan synthesizing enzymes XT-1, ChSy-1 and C4ST-1.

## 5.2. Targeting the interaction between LDLs and PGs

### 5.2.1. Small peptide mimetics of ApoB100

One potential mechanism to block the interaction between amino acids on ApoB100 and sulfate groups on galactosaminoglycan chains is to saturate the galactosaminoglycan chain binding sites with small peptide mimetics and this has been successfully demonstrated in a mouse model (Skalen et al., 2002). The rationale underlying this approach can be demonstrated by mutating the residues on ApoB100. Thus, as the LDL and PG interaction involves clusters of amino acids in the apoB100 that bind to the negatively charged sulfate groups on the galactosaminoglycan chain, site A at residues 3148–3158 and site B residues 3359–3369 (Boren et al., 1998; Olsson et al., 1997). Site A in apoB100 becomes functional and cooperates with site B to increase the PG binding activity in phospholipase A2 modified LDL (Flood et al., 2004). Transgenic mice expressing mutated site B apoB100 at the PG binding site fed a hypercholesterolemic diet for 20 weeks displayed significant protection from atherosclerotic lesion development as compared to transgenic mice expressing wild-type apoB100 under the same conditions (Skalen et al., 2002).

### 5.2.2. Galactosaminoglycan directed antibodies

Administration of a chimeric mouse/human monoclonal antibody (mAb) to induce responses against galactosaminoglycans has been shown to be effective at preventing the progression of atherosclerotic lesions (Brito et al., 2012, 2017; Delgado-Roche et al., 2013; Sarduy et al., 2017; Soto et al., 2012). The anti-atherosclerotic antibody was based on the chimeric mouse/human immunoglobulin G1 (IgG1) variant of P3 mAb that recognises glycolylated gangliosides and sulfatides. The ChP3R99 antibody has an additional arginine at position 99 displaying a higher reactivity with antigens. The initial work to characterise ChP3R99 as an anti-atherogenic antibody shows that it did not have direct impact on the oxidized LDL or foam cell formation, however in an in vitro binding assay, ChP3R99 inhibited 70% of LDL binding to CS (Soto et al., 2012). Pre-treatment with the mAb in an atherosclerosis induced rabbit model completely inhibited lesion progression and resulted in lower macrophage infiltration (Soto et al., 2012). ChP3R99 was compared with ChP3S98, a mutant variant with lower reactivity

to the antigen in a murine model of atherosclerosis fed a hypercholesterolemic diet (Brito et al., 2012). Multiple administration of ChP3R99 at biweekly or weekly intervals prevented lesion formation in apoE<sup>-/-</sup> mice as compared to treatment with ChP3S98 which had no apparent reduction in aortic lesion formation (Brito et al., 2012). This can be explained with ChP3S98's lower capacity to generate antibody response against galactosaminoglycans. Pre and post immunization of ChP3R99 in apoE<sup>-/-</sup> induced galactosaminoglycan antibody production as compared to the low reactive mAb (Brito et al., 2012).

In 2017, two major studies in this area were published (Brito et al., 2017; Sarduy et al., 2017). Earlier work with the anti-galactosaminoglycan antibodies was in young and adolescent apoE<sup>-/-</sup> male mice (Brito et al., 2012). However as age and sex are major risk factors in the development of atherosclerosis, Vazquez et al. (Sarduy et al., 2017) assess the influence of these variable on the capacity of the ChP3R99 mAb to generate anti-galactosaminoglycan response. Immunization with ChP3R99 mAb reduced the mean aortic lesion area by 31% and 38% in male and female mice, respectively compared with their respective control-treated groups and to 31% in middle aged female mice with spontaneous lesions (Sarduy et al., 2017). Thus neither of the two major risk factors had an impact on the anti-galactosaminoglycan properties induced by immunization with this mAb. In the latter study the ChP3R99 mAb was used in established atherosclerosis in apoE<sup>-/-</sup> mice. Atherosclerosis was allowed to develop for 11 weeks with apoE<sup>-/-</sup> mice fed a high fat diet followed by subcutaneous injections of ChP3R99 mAb (Sarduy et al., 2017), atherosclerotic lesion progression was reduced by 88% as compared to the control treated group. Collectively these studies define the use of an anti-galactosaminoglycan antibody for the prevention of lesion development and as a treatment to halt lesion progression in advanced atherosclerosis (Brito et al., 2012, 2017; Delgado-Roche et al., 2013; Sarduy et al., 2017; Soto et al., 2012). Both of the above strategies are valid approaches but are not easily converted into a small chemical entity suitable for long term therapy in humans as is required for the treatment of a chronic disease such as atherosclerosis for the prevention of CVDs. Nevertheless, there are emerging pharmaceutical delivery techniques being developed, so a therapeutic agent is possible and as above such a drug would be used in combination with a statin for a two pronged attack on the development of this life threatening disease.

### 5.3. Targeting the changes in PGs in the vessel wall

With the position of statins as the drug of first choice for the prevention of atherosclerosis, new therapeutic modalities must include the ability to lower cardiovascular risk on top of a statin such that dual therapy would be indicated. It is necessary to have a mechanism which is different from cholesterol lowering and most likely a vessel wall directed therapy addressing the disease process directly. The working hypothesis in the area of targeting galactosaminoglycan chain synthesis in that a statin would lower blood cholesterol and a “PG (galactosaminoglycan) inhibitor” would reduce the “stickiness” of the vessel wall. Proof of concept for this approach has been provided in two studies with imatinib (Ballinger et al., 2010; Getachew et al., 2010). Imatinib was developed as a tyrosine kinase Abl inhibitor for the treatment of Chronic Myeloid Leukaemia. Imatinib inhibits a very small number of tyrosine kinases including in the current context PDGF receptor tyrosine kinase (Getachew et al., 2010). Treatment with imatinib results in the inhibition of PDGF stimulated radiolabeled incorporation into PGs, elongation of galactosaminoglycan chain and reduced xyloside binding to LDL (Ballinger et al., 2010; Getachew et al., 2010). Furthermore, in an in vivo model of atherogenesis, high-fat fed apoE<sup>-/-</sup> mice, imatinib reduced lipid deposition in the vessel wall (Ballinger et al., 2010). The action of imatinib to reduce cholesterol deposition in the vessel wall, occurred without any effect on circulating lipid levels in the mice.

The optimum target in this area is the signalling pathways that controls the synthesis of galactosaminoglycan chains on biglycan (Ballinger et al., 2009; Burch et al., 2010; Burch et al., 2010; Burch et al., 2013; Little et al., 2013; Rostam et al., 2016; Yang et al., 2010). The actual enzymes which mediate galactosaminoglycan elongation (Izumikawa et al., 2011, 2012) are not targets themselves because those enzymes will be involved in multiple physiological processes and undoubtedly unwanted side effects would arise from targeting those enzymes. Furthermore, lipoprotein metabolism is very important in organs such as the liver and targeting those enzymes directly might perturb endogenous lipoprotein metabolism but targeting the signalling pathways is likely to be VSMC specific. In this review we have described in detail signalling pathways which mediate galactosaminoglycan hyperelongation in VSMCs and thus present as potential therapeutic targets (Burch et al., 2010; Burch et al., 2010; Burch et al., 2013; Kamato et al., 2016; Rostam et al., 2016).

## 6. Conclusions

Therapies for atherosclerosis have thus far targeted blood borne risk factors and blood pressure. Although this has produced some of the most efficacious cardiovascular therapies, specifically the cholesterol-lowering statins, there remains a large burden of potentially preventable atherosclerosis-related ischemic CVDs. An additional target is the disease process in the vessel wall, an area which had been extensively researched over the past three decades. There are practical and regulatory limitations in clinical studies based on vascular wall directed therapies but it is possible that great advances in imaging technologies may open up this area in a way that allows for the development of new classes of agents. Of the major areas of vascular wall directed therapies, this review proposes that a prime target is the potentially vascular-specific signalling pathways of vasoactive agonists that lead to an increase in the “stickiness” of vascular wall PGs for apolipoproteins on atherogenic LDL. The two critical processes of lipid retention and inflammation (represented by macrophage infiltration and foam cell formation) may pathologically be in series or parallel. Thus, if the processes are in series then slowing or preventing lipid retention will disrupt the atherosclerotic cascade. Hence, the combination of a statin to reduce blood cholesterol and an inhibitor of galactosaminoglycan hyperelongation aimed at stopping LDL accumulation in the vessel wall represents a potential strategy for attaining the requisite quantum

increase in efficacy required to prevent atherosclerosis and thus the burden of CVDs.

## Acknowledgments

Funding supporting the studies in our laboratory and referenced in this review has been received from the National Health and Medical Research Council (#1022800) of Australia, National Heart Foundation of Australia (G09M4385), Diabetes Australia Research Trust (PJL and NO), academic support packages from RMIT University (PJL) and the University of Queensland (PJL) and Australian Government scholarships to post-graduate students, Malaysian Government Scholarship (MAR). We thank Professor Thomas N. Wight and his laboratory colleagues for introducing us to this area.

## Conflict of interests

Nothing to declare.

## References

- Aghamohammadzadeh, R., Ormandy, D., & Heagerty, A. M. (2015). Definition and epidemiology of arterial disease. In A. Berbari, & G. Mancina (Eds.), *Arterial disorders* (pp. 3–12). Springer.
- Anggraeni, V. Y., Emoto, N., Yagi, K., Mayasari, D. S., Nakayama, K., Izumikawa, T., ... Hirata, K. (2011). Correlation of C4ST-1 and ChGn-2 expression with chondroitin sulfate chain elongation in atherosclerosis. *Biochemical and Biophysical Research Communications* 406, 36–41.
- Ballinger, M. L., Ivey, M. E., Osman, N., Thomas, W. G., & Little, P. J. (2009). Endothelin-1 activates ETA receptors on human vascular smooth muscle cells to yield proteoglycans with increased binding to LDL. *Atherosclerosis* 205, 451–457.
- Ballinger, M. L., Nigro, J., Frontanilla, K. V., Dart, A. M., & Little, P. J. (2004). Regulation of glycosaminoglycan structure and atherogenesis. *Cellular and Molecular Life Sciences* 61, 1296–1306.
- Ballinger, M. L., Osman, N., Hashimura, K., de Hann, J., Jandeleit-Dahm, K., Allen, T. J., ... Little, P. J. (2010). Imatinib inhibits vascular smooth muscle proteoglycan synthesis and reduces LDL binding in vitro and aortic lipid deposition in vivo. *Journal of Cellular and Molecular Medicine* 14, 1408–1418.
- Barré, L., Venkatesan, N., Magdalou, J., Netter, P., Fournel-Gigleux, S., & Ouzzine, M. (2006). Evidence of calcium-dependent pathway in the regulation of human  $\beta$ 1, 3-glucuronosyltransferase-1 (GlcAT-I) gene expression: a key enzyme in proteoglycan synthesis. *The FASEB Journal* 20, 1692–1694.
- Boren, J., Olin, K., Lee, I., Chait, A., Wight, T. N., & Innerarity, T. L. (1998). Identification of the principal proteoglycan-binding site in LDL. A single-point mutation in apo-B100 severely affects proteoglycan interaction without affecting LDL receptor binding. *The Journal of Clinical Investigation* 101, 2658–2664.
- Borén, J., & Williams, K. J. (2016). The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Current Opinion in Lipidology* 27, 473–483.
- Brito, V., Mellal, K., Portelance, S. G., Pérez, A., Soto, Y., Ong, H., ... Vázquez, A. M. (2012). Induction of anti-anti-idiotypic antibodies against sulfated glycosaminoglycans reduces atherosclerosis in apolipoprotein E-deficient mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* 32, 2847–2854.
- Brito, V., Mellal, K., Zoccal, K. F., Soto, Y., Menard, L., Sarduy, R., ... Marleau, S. (2017). Atheroregressive potential of the treatment with a chimeric monoclonal antibody against sulfated glycosaminoglycans on pre-existing lesions in apolipoprotein E-deficient mice. *Frontiers in Pharmacology* 8, 782.
- Burch, M. L., Ballinger, M. L., Yang, S. N., Getachew, R., Itman, C., Loveland, K., ... Little, P. J. (2010a). Thrombin stimulation of proteoglycan synthesis in vascular smooth muscle is mediated by protease-activated receptor-1 transactivation of the transforming growth factor beta type I receptor. *The Journal of Biological Chemistry* 285, 26798–26805.
- Burch, M. L., Getachew, R., Osman, N., Febbraio, M. A., & Little, P. J. (2013). Thrombin-mediated proteoglycan synthesis utilizes both protein-tyrosine kinase and serine/threonine kinase receptor transactivation in vascular smooth muscle cells. *The Journal of Biological Chemistry* 288, 7410–7419.
- Burch, M. L., Yang, S. N., Ballinger, M. L., Getachew, R., Osman, N., & Little, P. J. (2010b). TGF-beta stimulates biglycan synthesis via p38 and ERK phosphorylation of the linker region of Smad2. *Cellular and Molecular Life Sciences* 67, 2077–2090.
- Burch, M. L., Zheng, W., & Little, P. J. (2011). Smad linker region phosphorylation in the regulation of extracellular matrix synthesis. *Cellular and Molecular Life Sciences* 68, 97–107.
- Cardoso, L. E., Little, P. J., Ballinger, M. L., Chan, C. K., Braun, K. R., Potter-Perigo, S., ... Wight, T. N. (2010). Platelet-derived growth factor differentially regulates the expression and post-translational modification of versican by arterial smooth muscle cells through distinct protein kinase C and extracellular signal-regulated kinase pathways. *The Journal of Biological Chemistry* 285, 6987–6995.
- Chang, M. Y., Potter-Perigo, S., Tsoi, C., Chait, A., & Wight, T. N. (2000). Oxidized low density lipoproteins regulate synthesis of monkey aortic smooth muscle cell

- proteoglycans that have enhanced native low density lipoprotein binding properties. *The Journal of Biological Chemistry* 275, 4766–4773.
- Chung, H., Ramachandran, R., Hollenberg, M. D., & Muruve, D. A. (2013). Proteinase-activated receptor-2 transactivation of epidermal growth factor receptor and transforming growth factor- $\beta$  receptor signaling pathways contributes to renal fibrosis. *The Journal of Biological Chemistry* 288, 37319–37331.
- Collins, R., Reith, C., Emberson, J., Armitage, J., Baigent, C., Blackwell, L., ... Peto, R. (2016). Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet* 388, 2532–2561.
- Daub, H., Weiss, F. U., Wallasch, C., & Ullrich, A. (1996). Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 379, 557–560.
- Davies, M. J. (1996). Stability and instability: Two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. *Circulation* 94, 2013–2020.
- Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. *Circulation* 109, III27–32.
- Delgado-Roche, L., Acosta, E., Soto, Y., Hernández-Matos, Y., Olivera, A., Fernández-Sánchez, E., & Vázquez, A. (2013). The treatment with an anti-glycosaminoglycan antibody reduces aortic oxidative stress in a rabbit model of atherosclerosis. *Free Radical Research* 47, 309–315.
- Delgado-Roche, L., Brito, V., Acosta, E., Pérez, A., Fernández, J. R., Hernández-Matos, Y., ... Marleau, S. (2015). Arresting progressive atherosclerosis by immunization with an anti-glycosaminoglycan monoclonal antibody in apolipoprotein E-deficient mice. *Free Radical Biology and Medicine* 89, 557–566.
- Derynck, R., Zhang, Y., & Feng, X. -H. (1998). Transcriptional activators of TGF- $\beta$  responses: Smads. *Cell* 95, 737–740.
- ten Dijke, P., Franzén, P., Yamashita, H., Ichijo, H., Heldin, C. -H., & Miyazono, K. (1994). Serine/threonine kinase receptors. *Progress in Growth Factor Research* 5, 55–72.
- Ehrenstein, M. R., Jury, E. C., & Mauri, C. (2005). Statins for atherosclerosis—as good as it gets? *The New England Journal of Medicine* 352, 73–75.
- Evers, M. R., Xia, G., Kang, H. -G., Schachner, M., & Baenziger, J. U. (2001). Molecular cloning and characterization of a dermatan-specific N-acetylgalactosamine 4-O-sulfotransferase. *The Journal of Biological Chemistry* 276, 36344–36353.
- Falk, E. (1989). Morphologic features of unstable atherothrombotic plaques underlying acute coronary syndromes. *The American Journal of Cardiology* 63, 114E–120E.
- Fernández-Ruiz, I. (2016). Immune system and cardiovascular disease. *Nature research*.
- Figuerola, J. E., & Vijayagopal, P. (2002). Angiotensin II stimulates synthesis of vascular smooth muscle cell proteoglycans with enhanced low density lipoprotein binding properties. *Atherosclerosis* 162, 261–268.
- Flood, C., Gustafsson, M., Pitas, R. E., Arnaboldi, L., Walzem, R. L., & Boren, J. (2004). Molecular mechanism for changes in proteoglycan binding on compositional changes of the core and the surface of low-density lipoprotein-containing human apolipoprotein B100. *Arteriosclerosis, Thrombosis, and Vascular Biology* 24, 564–570.
- Ford, I., Murray, H., McCowan, C., & Packard, C. J. (2016). Long-term safety and efficacy of lowering low-density lipoprotein cholesterol with statin therapy: 20-year follow-up of West of Scotland Coronary Prevention Study. *Circulation* 133, 1073–1080.
- Getachew, R., Ballinger, M. L., Burch, M. L., Little, P. J., & Osman, N. (2010a). Characterisation of Ki11502 as a potent inhibitor of PDGF  $\beta$  receptor-mediated proteoglycan synthesis in vascular smooth muscle cells. *European Journal of Pharmacology* 626, 186–192.
- Getachew, R., Ballinger, M. L., Burch, M. L., Reid, J. J., Khachigian, L. M., Wight, T. N., ... Osman, N. (2010b). PDGF  $\beta$ -receptor kinase activity and ERK1/2 mediate glycosaminoglycan elongation on biglycan and increases binding to LDL. *Endocrinology* 151, 4356–4367.
- Gigli, M., Ghiselli, G., Torri, G., Naggi, A., & Rizzo, V. (1993). A comparative study of low-density lipoprotein interaction with glycosaminoglycans. *Biochimica et Biophysica Acta* 1167, 211–217.
- Gilman, A. G. (1987). G proteins: Transducers of receptor-generated signals. *Annual Review of Biochemistry* 56, 615–649.
- Gotoh, M., Sato, T., Akashima, T., Iwasaki, H., Kameyama, A., Mochizuki, H., ... Kikuchi, N. (2002). Enzymatic synthesis of chondroitin with a novel chondroitin sulfate N-acetylgalactosaminyltransferase that transfers N-acetylgalactosamine to glucuronic acid in initiation and elongation of chondroitin sulfate synthesis. *The Journal of Biological Chemistry* 277, 38189–38196.
- Götting, C., Kuhn, J., & Kleesiek, K. (2007). Human xylosyltransferases in health and disease. *Cellular and Molecular Life Sciences* 64, 1498–1517.
- Haberland, M. E., Olch, C. L., & Fogelman, A. M. (1984). Role of lysines in mediating interaction of modified low density lipoproteins with the scavenger receptor of human monocyte macrophages. *The Journal of Biological Chemistry* 259, 11305–11311.
- Heldin, C. H., & Westermark, B. (1999). Mechanism of action and in vivo role of platelet-derived growth factor. *Physiological Reviews* 79, 1283–1316.
- Hu, B., Shi, C., Tian, Y., Zhang, Y., Xu, C., Chen, H., ... Yuan, W. (2015). TGF- $\beta$  induces up-regulation of chondroitin sulfate synthase 1 (CHSY1) in nucleus pulposus cells through MAPK signaling. *Cellular Physiology and Biochemistry* 37, 793–804.
- Hubbard, S. R., & Till, J. H. (2000). Protein tyrosine kinase structure and function. *Annual Review of Biochemistry* 69, 373–398.
- Iverius, P. H. (1972). The interaction between human plasma lipoproteins and connective tissue glycosaminoglycans. *The Journal of Biological Chemistry* 247, 2607–2613.
- Izumikawa, T., Koike, T., & Kitagawa, H. (2012). Chondroitin 4-O-sulfotransferase-2 regulates the number of chondroitin sulfate chains initiated by chondroitin N-acetylgalactosaminyltransferase-1. *Biochemical Journal* 441, 697–705.
- Izumikawa, T., Koike, T., Shiozawa, S., Sugahara, K., Tamura, J., & Kitagawa, H. (2008). Identification of chondroitin sulfate glucuronyltransferase as chondroitin synthase-3 involved in chondroitin polymerization: Chondroitin polymerization is achieved by multiple enzyme complexes consisting of chondroitin synthase family members. *The Journal of Biological Chemistry* 283, 11396–11406.
- Izumikawa, T., Okuura, Y., Koike, T., Sakoda, N., & Kitagawa, H. (2011). Chondroitin 4-O-sulfotransferase-1 regulates the chain length of chondroitin sulfate in co-operation with chondroitin N-acetylgalactosaminyltransferase-2. *Biochemical Journal* 434, 321–331.
- Izumikawa, T., Uyama, T., Okuura, Y., Sugahara, K., & Kitagawa, H. (2007). Involvement of chondroitin sulfate synthase-3 (chondroitin synthase-2) in chondroitin polymerization through its interaction with chondroitin synthase-1 or chondroitin-polymerizing factor. *The Biochemical Journal* 403, 545–552.
- Kamato, D., Bhaskarala, V. V., Mantri, N., Oh, T. G., Ling, D., Janke, R., ... Osman, N. (2017). RNA sequencing to determine the contribution of kinase receptor transactivation to G protein coupled receptor signalling in vascular smooth muscle cells. *PLoS One* 12, e0180842.
- Kamato, D., Burch, M. L., Osman, N., Zheng, W., & Little, P. J. (2013a). Therapeutic implications of endothelin and thrombin G-protein-coupled receptor transactivation of tyrosine and serine/threonine kinase cell surface receptors. *The Journal of Pharmacy and Pharmacology* 65, 465–473.
- Kamato, D., Burch, M. L., Piva, T. J., Rezaei, H. B., Rostam, M. A., Xu, S., ... Osman, N. (2013b). Transforming growth factor- $\beta$  signalling: Role and consequences of Smad linker region phosphorylation. *Cellular Signalling* 25, 2017–2024.
- Kamato, D., Rostam, M. A., Bernard, R., Piva, T. J., Mantri, N., Guidone, D., ... Little, P. J. (2015). The expansion of GPCR transactivation-dependent signalling to include serine/threonine kinase receptors represents a new cell signalling frontier. *Cellular and Molecular Life Sciences* 72, 799–808.
- Kamato, D., Rostam, M. A., Piva, T. J., Babaahmadi Rezaei, H., Getachew, R., Thach, L., ... Osman, N. (2014). Transforming growth factor  $\beta$ -mediated site-specific Smad linker region phosphorylation in vascular endothelial cells. *The Journal of Pharmacy and Pharmacology* 66, 1722–1733.
- Kamato, D., Thach, L., Getachew, R., Burch, M., Hollenberg, M. D., Zheng, W., ... Osman, N. (2016). Protease activated receptor-1 mediated dual kinase receptor transactivation stimulates the expression of glycosaminoglycan synthesizing genes. *Cellular Signalling* 28, 110–119.
- Karamanos, N. K., Syrokou, A., Vanky, P., Nurminen, M., & Hjerpe, A. (1994). Determination of 24 variously sulfated galactosaminoglycan- and hyaluronan-derived disaccharides by high-performance liquid chromatography. *Analytical Biochemistry* 221, 189–199.
- Kijani, S., Vázquez, A. M., Levin, M., Borén, J., & Fogelstrand, P. (2017). Intimal hyperplasia induced by vascular intervention causes lipoprotein retention and accelerated atherosclerosis. *Physiological Reports* 5, e13334.
- Kjellén, L., & Lindahl, U. (1991). Proteoglycans: Structures and interactions. *Annual Review of Biochemistry* 60, 443–475.
- Libby, P. (2002). Inflammation in atherosclerosis. *Nature* 420, 868–874.
- Little, P. J., Ballinger, M. L., Burch, M. L., & Osman, N. (2008a). Biosynthesis of natural and hyperelongated chondroitin sulfate glycosaminoglycans: New insights into an elusive process. *Open Biochemistry Journal* 2, 135–142.
- Little, P. J., Burch, M. L., Getachew, R., Al-aryahi, S., & Osman, N. (2010). Endothelin-1 stimulation of proteoglycan synthesis in vascular smooth muscle is mediated by endothelin receptor transactivation of the transforming growth factor- $\beta$  type I receptor. *Journal of Cardiovascular Pharmacology* 56, 360–368.
- Little, P. J., Chait, A., & Bobik, A. (2011). Cellular and cytokine-based inflammatory processes as novel therapeutic targets for the prevention and treatment of atherosclerosis. *Pharmacology & Therapeutics* 131, 255–268.
- Little, P. J., Getachew, R., Rezaei, H. B., Sanchez-Guerrero, E., Khachigian, L. M., Wang, H., ... Osman, N. (2012). Genistein inhibits PDGF-stimulated proteoglycan synthesis in vascular smooth muscle without blocking PDGF $\beta$  receptor phosphorylation. *Archives of Biochemistry and Biophysics* 525, 25–31.
- Little, P. J., Osman, N., & O'Brien, K. D. (2008b). Hyperelongated biglycan: The surreptitious initiator of atherosclerosis. *Current Opinion in Lipidology* 19, 448–454.
- Little, P. J., Rostam, M. A., Piva, T. J., Getachew, R., Kamato, D., Guidone, D., ... Osman, N. (2013). Suramin inhibits PDGF-stimulated receptor phosphorylation, proteoglycan synthesis and glycosaminoglycan hyperelongation in human vascular smooth muscle cells. *The Journal of Pharmacy and Pharmacology* 65, 1055–1063.
- Lloyd-Jones, D. M., Goff, D., & Stone, N. J. (2014). Statins, risk assessment, and the new American prevention guidelines. *Lancet* 383, 600–602.
- Malmström, A., Bartolini, B., Thelin, M. A., Pacheco, B., & Maccarana, M. (2012). Iduronic acid in chondroitin/dermatan sulfate: Biosynthesis and biological function. *Journal of Histochemistry & Cytochemistry* 60, 916–925.
- Malmström, A., & Fransson, L. (1975). Biosynthesis of dermatan sulfate. I. Formation of L-iduronic acid residues. *The Journal of Biological Chemistry* 250, 3419–3425.
- Massague, J., Seoane, J., & Wotton, D. (2005). Smad transcription factors. *Genes & Development* 19, 2783–2810.
- Matsuzaki, K. (2013). Smad phospho-isoforms direct context-dependent TGF- $\beta$  signaling. *Cytokine & Growth Factor Reviews* 24, 385–399.
- Mendis, S., Puska, P., & Norrving, B. (2011). *Global atlas on cardiovascular disease prevention and control*. World Health Organization.
- Nakashima, Y., Fujii, H., Sumiyoshi, S., Wight, T. N., & Sueishi, K. (2007). Early human atherosclerosis: Accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. *Arteriosclerosis, Thrombosis, and Vascular Biology* 27, 1159–1165.
- Nigro, J., Osman, N., Dart, A. M., & Little, P. J. (2006). Insulin resistance and atherosclerosis. *Endocrine Reviews* 27, 242–259.
- Oberkersch, R., Maccari, F., Bravo, A. I., Volpi, N., Gazzaniga, S., & Calabrese, G. C. (2014). Atheroprotective remodelling of vascular dermatan sulphate proteoglycans in response to hypercholesterolaemia in a rat model. *International Journal of Experimental Pathology* 95, 181–190.
- Ohira, T., & Iso, H. (2013). Cardiovascular disease epidemiology in Asia. *Circulation Journal* 77, 1646–1652.

- Ohtake, S., Ito, Y., Fukuta, M., & Habuchi, O. (2001). Human N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase cDNA is related to human B cell recombination activating gene-associated gene. *The Journal of Biological Chemistry* 276, 43894–43900.
- Olsson, U., Camejo, G., Hurt-Camejo, E., Elfsber, K., Wiklund, O., & Bondjers, G. (1997). Possible functional interactions of apolipoprotein B-100 segments that associate with cell proteoglycans and the ApoB/E receptor. *Arteriosclerosis, Thrombosis, and Vascular Biology* 17, 149–155.
- Osman, N., Getachew, R., Thach, L., Wang, H., Su, X., Zheng, W., & Little, P. J. (2014). Platelet-derived growth factor-stimulated versican synthesis but not glycosaminoglycan elongation in vascular smooth muscle is mediated via Akt phosphorylation. *Cellular Signalling* 26, 912–916.
- Pierce, K. L., Premont, R. T., & Lefkowitz, R. J. (2002). Seven-transmembrane receptors. *Nature Reviews Molecular Cell Biology* 3, 639–650.
- Prante, C., Milting, H., Kassner, A., Farr, M., Ambrosius, M., Schon, S., ... Gotting, C. (2007). Transforming growth factor beta1-regulated xylosyltransferase I activity in human cardiac fibroblasts and its impact for myocardial remodeling. *The Journal of Biological Chemistry* 282, 26441–26449.
- Rezaei, H. B., Kamato, D., Ansari, G., Osman, N., & Little, P. J. (2012). Cell biology of Smad2/3 linker region phosphorylation in vascular smooth muscle. *Clinical and Experimental Pharmacology & Physiology* 39, 661–667.
- Ross, R. (1999). Atherosclerosis—an inflammatory disease. *The New England Journal of Medicine* 340, 115–126.
- Rostam, M. A., Kamato, D., Piva, T. J., Zheng, W., Little, P. J., & Osman, N. (2016). The role of specific Smad linker region phosphorylation in TGF- $\beta$  mediated expression of glycosaminoglycan synthesizing enzymes in vascular smooth muscle. *Cellular Signalling* 28, 956–966.
- Sambandam, T., Baker, J. R., Christner, J. E., & Ekborg, S. L. (1991). Specificity of the low density lipoprotein-glycosaminoglycan interaction. *Arteriosclerosis and Thrombosis* 11, 561–568.
- Sarduy, R., Brito, V., Castillo, A., Soto, Y., Griñán, T., Marleau, S., & Vázquez, A. M. (2017). Dose-dependent induction of an idiotypic cascade by anti-glycosaminoglycan Monoclonal antibody in apoe $^{-/-}$  Mice: Association with atheroprotection. *Frontiers in Immunology* 8.
- Sato, T., Gotoh, M., Kiyohara, K., Akashima, T., Iwasaki, H., Kameyama, A., ... Narimatsu, H. (2003). Differential roles of two N-acetylgalactosaminyltransferases, CSGalNAcT-1, and a novel enzyme, CSGalNAcT-2. Initiation and elongation in synthesis of chondroitin sulfate. *The Journal of Biological Chemistry* 278, 3063–3071.
- Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. *Cell* 103, 211–225.
- Schmidt, A. F., Swerdlow, D. I., Holmes, M. V., Patel, R. S., Fairhurst-Hunter, Z., Lyall, D. M., ... Power, C. (2017). PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. *The Lancet Diabetes & Endocrinology* 5, 97–105.
- Schonherr, E., Jarvelainen, H. T., Kinsella, M. G., Sandell, L. J., & Wight, T. N. (1993). Platelet-derived growth factor and transforming growth factor-beta 1 differentially affect the synthesis of biglycan and decorin by monkey arterial smooth muscle cells. *Arteriosclerosis and Thrombosis* 13, 1026–1036.
- Schwenke, D. C., & Carew, T. E. (1989). 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, Thrombosis, and Vascular Biology* 9, 908–918.
- Shah, R. V., & Goldfine, A. B. (2012). Statins and risk of new-onset diabetes mellitus. *Circulation* 126, e282–e284.
- Skalen, K., Gustafsson, M., Rydberg, E. K., Hulten, L. M., Wiklund, O., Innerarity, T. L., & Boren, J. (2002). Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature* 417, 750–754.
- Soto, Y., Acosta, E., Delgado, L., Perez, A., Falcon, V., Becquer, M. A., ... Vazquez, A. M. (2012). Antiatherosclerotic effect of an antibody that binds to extracellular matrix glycosaminoglycans. *Arteriosclerosis, Thrombosis, and Vascular Biology* 32, 595–604.
- Survase, S., Ivey, M. E., Nigro, J., Osman, N., & Little, P. J. (2005). Actions of calcium channel blockers on vascular proteoglycan synthesis: Relationship to atherosclerosis. *Vascular Health and Risk Management* 1, 199–208.
- Tabas, I. (1999). Nonoxidative modifications of lipoproteins in atherogenesis. *Annual Review of Nutrition* 19, 123–139.
- Tan, N. Y., & Khachigian, L. M. (2009). Sp1 phosphorylation and its regulation of gene transcription. *Molecular and Cellular Biology* 29, 2483–2488.
- Taylor, F., Huffman, M. D., Macedo, A. F., Moore, T. H., Burke, M., Davey Smith, G., ... Ebrahim, S. (2013). Statins for the primary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews* 1, CD004816.
- Theocharis, A. D., Theocharis, D. A., De Luca, G., Hjerpe, A., & Karamanos, N. K. (2002). Compositional and structural alterations of chondroitin and dermatan sulfates during the progression of atherosclerosis and aneurysmal dilatation of the human abdominal aorta. *Biochimie* 84, 667–674.
- Tran-Lundmark, K., Tran, P.-K., Paulsson-Berne, G., Fridén, V., Soininen, R., Tryggvason, K., ... Hedin, U. (2008). Heparan sulfate in perlecan promotes mouse atherosclerosis. *Circulation Research* 103, 43–52.
- Uyama, T., Kitagawa, H., & Sugahara, K. (2007). Biosynthesis of glycosaminoglycans and proteoglycans. In J. P. Kamerling, G. J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, & A. G. J. Voragen (Eds.), *Comprehensive glycoscience*. Vol. 3. (pp. 79–104). Elsevier Science.
- Virmani, R., Kolodgie, F. D., Burke, A. P., Finn, A. V., Gold, H. K., Tulenko, T. N., ... Narula, J. (2005). Atherosclerotic plaque progression and vulnerability to rupture. *Arteriosclerosis, Thrombosis, and Vascular Biology* 25, 2054–2061.
- WHO (2017). In W. H. O. (WHO) (Ed.), *Cardiovascular diseases (CVDs) fact sheet*. Vol. 2017. World Health Organization (WHO).
- Witztum, J. L. (1994). The oxidation hypothesis of atherosclerosis [see comments]. *Lancet* 344, 793–795.
- Yadav, K., Sharma, M., & Ferdinand, K. C. (2016). Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors: Present perspectives and future horizons. *Nutrition, Metabolism, and Cardiovascular Diseases* 26, 853–862.
- Yamada, S., Sugahara, K., & Özbek, S. (2011). Evolution of glycosaminoglycans: Comparative biochemical study. *Communicative & Integrative Biology* 4, 150–158.
- Yang, S. N., Burch, M. L., Getachew, R., Ballinger, M. L., Osman, N., & Little, P. J. (2009). Growth factor-mediated hyper-elongation of glycosaminoglycan chains on biglycan requires transcription and translation. *Archives of Physiology and Biochemistry* 115, 147–154.
- Yang, S. N., Burch, M. L., Tannock, L. R., Evanko, S., Osman, N., & Little, P. J. (2010). Transforming growth factor-beta regulation of proteoglycan synthesis in vascular smooth muscle: contribution to lipid binding and accelerated atherosclerosis in diabetes. *Journal of Diabetes* 2, 233–242.