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Glycosyltransferases, glycosylation and atherosclerosis

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Abstract Cardiovascular diseases arising from atherosclerosis are currently the leading cause of mortality worldwide. Leukocyte recruitment is a key step for the successful initiation of atherosclerosis and occurs predominantly in the inflamed endothelium. Leukocyte recruitment is mediated by a group of adhesive molecules and chemokine receptors, which are often glycosylated protein. Recent studies demonstrated that post-translational glycosylation by glycosyltransferases is necessary for adhesive molecules and chemokine receptors activities. Several glycosyltransferases, such as α2,3-sialyltransferases IV, α1,3-fucosyltransferases IV and VII, core 2 β1,6-*N*-acetylglucosaminyltransferase-I, are considered to participate in the synthesis of glycosylation for adhesive molecules and chemokine receptors, and the initiation of atherosclerotic lesions. In this review, we will discuss new data concerning the roles of different glycosyltransferases in atherogenesis. The knowledge of glycosyltransferases in atherogenesis offers the opportunity to develop novel therapeutic strategies.

Keywords Glycosyltransferases · Glycosylation · Atherosclerosis · Cell adhesion molecules · Chemokine receptors · Chemokines

Introduction

Cardiovascular diseases are currently the major cause of mortality worldwide, and atherosclerosis is the most dominant underlying pathology. Atherosclerosis is an inflammatory disease characteristic of the accumulation of leukocytes, smooth vascular cells and lipids in the arterial intima, leading to the formation of lesion that can contributes to arterial lumen narrowing [1–3]. Recruitment of leukocyte from blood stream into arterial intima is a crucial step for the accumulation of leukocytes in atherosclerotic lesion [4–6]. Leukocyte recruitment consists of a cascade of events: tethering, rolling, arrest (known as firm adhesion) and transmigration. In the beginning, free flowing leukocytes tether and roll along the endothelium. Tethering and rolling are mediated by a serial of selectins/ligands interaction. During rolling, Leukocytes are intimately engaged with endothelial cells, which gives endothelial cells bound chemokines the chance to bind to their specific chemokine receptors on leukocytes. This interaction triggers the activation of integrins, contributing to firm leukocyte adhesion to the endothelium and subsequent transmigration into subendothelial space. Therefore, adhesion molecules selectins/ligands and chemokine receptors/chemokines interactions regulate different steps in leukocyte recruitment: the former for tethering and rolling, the latter for firm adhesion. Interestingly, adhesion molecules and chemokine receptors are often glycoproteins. Notably, proper sugar chain structures are essential for the functions of adhesion molecules and chemokine receptors [7]. It is well-known that the formation of sugar chain structures is catalyzed by glycosyltransferases, so glycosyltransferases probably regulate the functions of adhesion molecules and chemokine receptors, and then affect different steps of leukocyte recruitment and the initiation of atherosclerosis. In this review, we hence will discuss new data concerning roles of different glycosyltransferases in atherogenesis.

Leukocyte recruitment

Leukocyte recruitment tends to occur at activated endothelium that corresponds to hemodynamic stress, such as inner

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curvatures and branch points of arteries [8]. This activation of endothelium contributes to increased permeability to lipoproteins and the accumulation of extracelluar matrix proteins that cause the trapping of atherogenic ApoB-containing lipoproteins within the arterial wall [9–12]. The retained lipoproteins become modified by local enzymes. Modified lipoproteins in turn amplify endothelial activation, and promote the recruitment of circulating leukocytes, because expression of adhesion molecules and chemokines are upregulated by modified lipoproteins on the surface of activated endothelial cells [13–17]. Different sets of adhesion molecules and chemokine receptors-chemokines axes are responsible for various steps of leukocyte recruitment, including six protein families: selectins, selectin ligands, integrins, immunoglobulins, chemokine receptors and chemokines (Table 1).

During inflammation, interaction of selectins and their ligands are responsible for the initial stage tethering and rolling of leukocyte recruitment [18, 19]. Selectins consist of three subfamily members: P-, E- and L-selectin. The featured structure of selectins includes N-terminal C-type lectins domain. Owing to C-type lectins, selectins can recognize and bind to specific carbohydrate motif on selectin ligands, such as P-selectin glycoprotein ligand-1 (PSGL-1), CD44, CD43 and others [20, 21]. P-selectin is considered as a predominant leukocyte rolling receptor on inflamed endothelium *in vivo*, since leukocyte rolling was almost completely absent from P-selectin deficient mice [22]. E-selectin is not expressed under normal situations, but induced under the inflammatory situations [23]. Animal studies showed that intervention of E-, P-selectins/ligands interaction dramatically suppresses the formation of atherosclerotic lesions in $ApoE^{-/-}$ or $LDLR^{-/-}$ mice, such as genetic deletion of PSGL-1 [24], P-selectin [25, 26], E- and P-selectin [27], or blocking of PSGL-1 or P-selectin with monoclonal antibody [28]. Conversely, L-selectin is constitutively expressed on leukocytes. L-selectin deficiency increases size, but does not change cellular composition in atherosclerotic lesions [29], suggesting that L-selectin protects from early atherosclerosis. Therefore, three selectins mediate leukocyte rolling on inflammatory sites, but not all selectins promote atherogenesis, indicating that there exist distinct effects to three selectins.

During rolling, endothelial cell surface bound chemokines recognize and bind to their respective receptors on leukocytes, leading to the activation of integrins on leukocytes (Table 1) [30, 31]. As a consequence, activated integrins interact with immunoglolubins, such as intercellular adhesion molecules-1

Table 1 Main adhesion molecule and chemokine receptor-chemokine pairs in leukocyte recruitment

Adhesion molecule / chemokine receptor	Other name	Ligands	Functions	Cell distribution	
				Endothelium	Leukocyte
Selectins					
E-selectin	CD62E	PSGL-1, CD15s (sLe^X), ESL-1	Tethering/rolling	+	
P-selectin	CD62P	PSGL-1, CD15s (sLe^X)	Tethering/rolling	+	
L-selectin	CD62L	PSGL-1, CD15s (sLe^X)	Tethering/rolling		+
Selectin ligands					
E-selectin ligand-1	ESL-1	E-selectin	Tethering/rolling		+
P-selectin glycoprotein ligand-1	PSGL-1, CD162	E-, P-, L-selectin	Tethering/rolling		+
Chemokine receptors					
CCR2	CD192, MCP-1 Receptor	CCL2	Firm adhesion		+
CCR5	CD195	CCL5	Firm adhesion		+
CXCR3	CD183	CXCL10	Firm adhesion		+
CX3CR1	Fractalkine receptor	CX3CL1	Firm adhesion		+
Intregrins					
Integrin α4/β1	CD49d/CD29, VLA4	VCAM-1	Firm adhesion	+	
Integrin αL/β2	CD11a/CD18, LFA1	ICAM-1, 2, 3	Firm adhesion		+
Integrin αM/β2	CD11b/CD18, Mac1	ICAM-1, 2, 3	Firm adhesion		+
Integrin αX/β2	CD11c/CD18	ICAM-1	Firm adhesion		+
Integrin αD/β2	CD11d/CD18	ICAM-3, VCAM-1	Firm adhesion		+
Immunoglolubins					
ICAM-1	CD54	LFA1, Mac1, αX/β2	Firm adhesion	+	+
VCAM-1	CD106	α4/β1, αD/β2	Firm adhesion	+	
PECAM-1	CD31	PECAM-1, αV/β3	Leukocyte transmigration	+	+

(ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1), which mediates firm leukocytes adhesion and subsequent migration into arterial intima. Studies have demonstrated that main chemokine receptor-chemokine axes participating in above processes are CCR2- CCL2, CCR5-CCL5, CXCR3–CXCL10, CX3CR1-CX3CL1 [32–35]. Deficiency of CCR2 reduced the development only at advanced stages of atherosclerotic plaque in ApoE$^{-/-}$ mice. In contrast, deficiency of CXCR3 reduced the development only at early stages of atherosclerotic plaque in ApoE$^{-/-}$ mice [31, 36]. Double knockout of CCR2 and CXCR3 displays reduced atherosclerotic lesion at both early and advanced stages, but without additive or synergetic effects [36]. Similarly, deletion of CCR5 [37, 38], CX3CR1 [39–41] or CX3CL1 [42] in ApoE$^{-/-}$ mice developed less atherosclerotic lesion compared with control ApoE$^{-/-}$ mice. In addition, disruption of integrins/immunoglolubins interaction protects against the development of atherosclerotic lesion, such as deletion of CD18 (β_2 subunit of integrin) [43] or ICAM-1 [25], VCAM-1 [8], blockade of β_1 subunit of integrin (VLA-4) [44].

Although previous studies provide apparent function redundancy in leukocyte recruitment and atherosclerotic development, cell adhesion molecules and chemokine receptors are also glycoproteins, which suggests that other regulatory mechanisms, such as post-translational glycosylations, maybe contribute to their activities.

Glycosyltransferase-mediated glycosylation of adhesion molecules

Adhesion molecules and chemokine receptors belong to a group of glycoproteins where protein function depends on their proper post-translational glycosylation [45, 7, 46]. Glycosylation is performed by glycosyltransferases in rough endoplasmic reticulum and Golgi apparatus. Glycosyltransferases catalyze the transfer of specific glycosyl groups to acceptor substrates. Here we clarify biosynthetic pathway of glycosyltransferases-mediated glycosylation as an example of protein PSGL-1 [47, 45]. PSGL-1 is a protein of core 2 decorated O-glycan tipped with a tetrasaccharide sialyl Lewis X (sLe^X) moiety (Fig. 1). The first step of O-glycan biosynthesis is the addition of galactosamine (GalNAc) to serine or threonine residues at protein, which is catalysed by polypeptide GalNAc transferase (ppGalNAcT). The addition of galactose (Gal) to GalNAc in β1-3 linkage initiates the core 1 extension, which is catalysed by core 1 β1,3 galactosyltransferase (core 1 β1,3 GalT), or else the addition of acetylglucosamine (GlcNAc) to GalNAc in β1-6 linkage gives rise to the core 2 extension, which is catalysed by core 2 β1,6 galactosyltransferase I (core 2 β1,6 GlcNAcT-I). Extension of core 2 structure is followed by alternate addition of galactose and acetylglucosamine mediated by β1,4

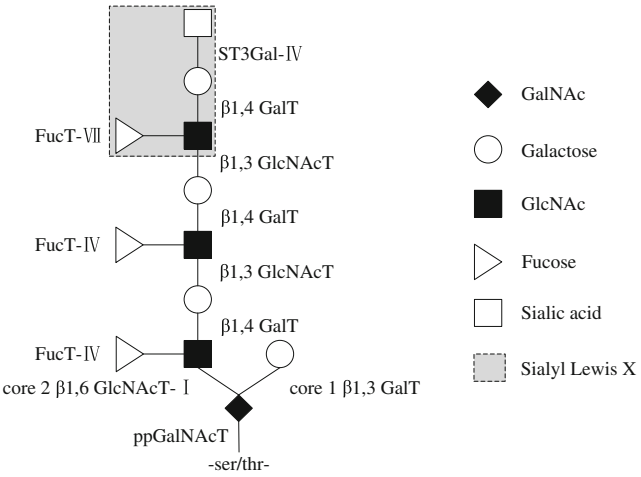

Fig. 1 Biosynthetic pathway of core 2 modified O-glycan terminated with the sialyl Lewis X (sLe^X)

galactosyltransferase (β1,4 GalT) and β1,3-*N*-acetylglucosaminyltransferase (β1,3 GlcNAcT), respectively. During extension, α1,3-fucosyltransferase IV (FucT-IV) catalyzes fucosylation of GlcNAc in α1-3 linkage. Elongation of core 2 structure is terminated by the transfer of sialic acid (known as *N*-acetylneuraminic acid) to galactose in α2-3 linkage, which is catalyzed by α2,3-sialyltransferase IV (ST3Gal-IV). At last, the transfer of fucose to the penultimate GlcNAc mediated by α1,3-fucosyltransferase VII (FucT-VII) leads to the formation sLe^X at the end of core 2 modified O-glycan. Similar glycosylated process probably exists in chemokine receptor, such as CCR5, because sialylated O-glycan regulated its activities [48]. In the following section, the role of glycosyltransferases in the development of atherosclerosis is discussed.

Inhibition of glycosyltransferases suppresses the development of atherosclerosis

α2,3-sialyltransferase IV (ST3Gal-IV)

α2,3-sialyltransferase IV (ST3Gal-IV) is a major enzyme for transferring sialic acid to terminal galactose residue to form tetrasaccharide sLeX at core 2 modified O-glycan [49]. ST3Gal-IV deficiency in ApoE-/- mice drastically reduced the size, stage and inflammatory cell content of atherosclerotic plaques compared to control ApoE-/- mice, suggesting that ST3Gal-IV plays an important role in the pathogenesis of atherosclerosis [50]. Analysis of atheroprotection of ST3Gal-IV deficiency reveals that ST3Gal-IVdeficiency hinders the formation of sugar-chain structure sLeX of CCR5, leading to the reduced binding of CCL5 to CCR5 in leukocytes, not CCL2 to CCR2, and the subsequently impaired CCL5-triggered integrin activation. As a consequence, CCL5-triggered leukocytes arrest (known as firm adhesion) and

Table 2 Roles of glycosyltransferases deficiencies in atherogenesis

Glycosyltransferase	Targeting protein	Recruitment process	Effect	References
ST3Gal-IV	CCR5	Firm adhesion	Reduced size, stage and inflammatory cell content of atherosclerotic lesions in ST3Gal-IV$^{-/-}$ mice	[50]
FucT-IV	PSGL-1	Rolling	Slightly reduced size of atherosclerotic lesions in FucT-IV$^{-/-}$ mice	[52]
FucT-VII	PSGL-1	Rolling	Dramatically reduced size, macrophage content of atherosclerotic lesions in FucT-VII$^{-/-}$ mice	[52, 53]
core 2 β1,6 GlcNAcT-I	PSGL-1	Rolling	Reduced size, macrophage content and increased collagen of atherosclerotic lesions in core 2 β1,6 GlcNAcT-I$^{-/-}$ mice	[57]

recruitment under inflammatory conditions was almost completely abrogated in *in vitro* or *in vivo* investigations. Moreover, tetrasaccharide sLeX is essential for functional selectin ligands, due to its mediating the binding of selectin ligands to their receptors selectins. Atheroprotection of ST3Gal-IV deficiency was partially attributable to reduced leukocytes rolling, since ST3Gal-IV deficient mice indeed show an impaired PGSL-1 dependent leukocyte rolling [51].

α2,3-Fucosyltransferases FucT-IV and FucT-VII

FucT-IV and FucT-VII are two major enzymes for transferring fucose to GlcNAc residue at core 2 modified O-glycan [45]. However, FucT-VII deficiency yields substantially reduced lesion size, but FucT-IV deficiency does slightly, indicating there exists the collaborative contributions of FucT-VII and FucT-IV in atherogenesis, in which FucT-VII plays an important role, but FucT-IV does a subsidiary role [52]. Further research verifies that during atherogenesis FucT-VII on leukocytes plays a more important role than that in endothelial cells [53]. Atheroprotection of FucT-VII deficiency correlates mainly with profound reduction in selectin ligands activities in leukocytes. This reduction contributes to decreased leukocytes rolling and recruitment under inflammatory conditions, such as shear show [52, 54, 55]. Selectin ligands activities, such as PGSL-1, depend primarily on FucT-VII, secondarily on FucT-IV from gene deficient mice, suggesting that FucT-VII contributes to, but FucT-IV does not, the synthesis of sLe^X determinants which provide main contribution to selectin ligands activities [56]. However, expression of selectin ligands is not influenced by FucT-VII [56, 52, 54]. These studies indicated that FucT-VII do not take part in the

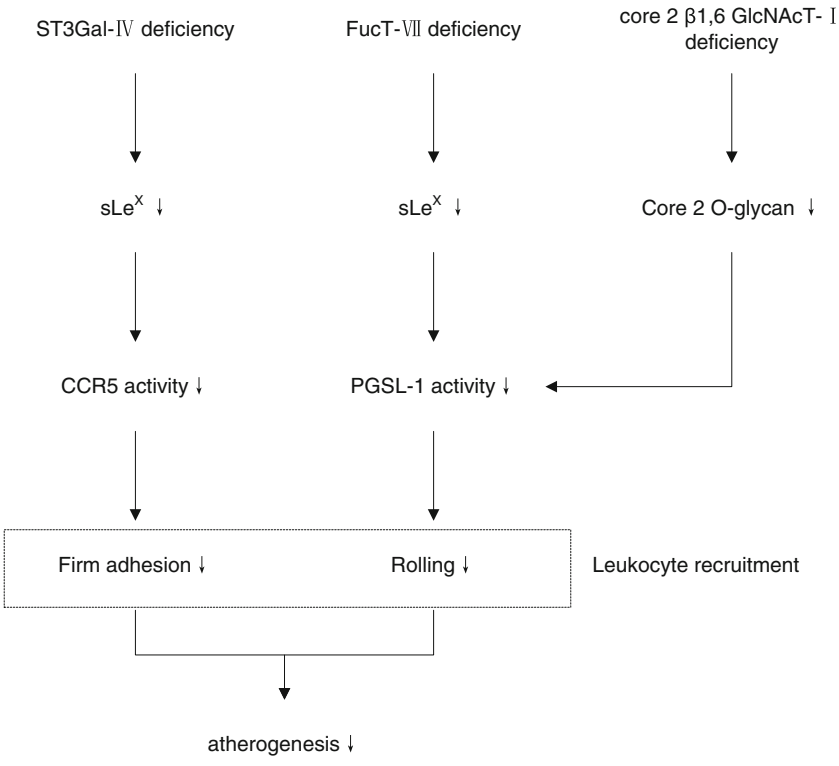

Fig. 2 Schematic representation of possible mechanisms of several glycosyltransferases in the inhibition of atherogenesis. ST3Gal-IV and FucT-VII prevent the formation of sugar-chain structures sialyl Lewis X (sLe^X), and core 2 β1,6 GlcNAcT-I stops the formation of sugar-chain structures core 2 O-glycan, all of which contribute to the reduced leukocyte recruitment such as rolling and firm adhesion and the sequent reduction of atherosclerotic lesions

biosynthesis of selectin ligands, but rather in activating them. Altogether, disruption of selectins/ligands axes through the genetic deletion of FucT-VII, necessary for posttranslational glycosylation of selectin ligands, leads to the reduction of atherosclerotic lesions.

Core 2 β1,6 galactosyltransferase I (core 2 β1,6 GlcNAcT-I)

Core 2 β1,6 GlcNAcT-I is the crucial branching enzyme in the biosynthesis of core 2 modified O-glycan and is critical for selectin ligands binding to selectins. Deficiency of systemic core 2 β1,6 GlcNAcT-I, or core 2 β1,6 GlcNAcT-I only in vascular endothelium, both reduced the size, macrophage and tissue factor content, necrotic area, but increased collagen content in atherosclerotic lesions, suggesting that Core 2 β1,6 GlcNAcT-I in both leukocytes and vascular endothelial cells contributes to the formation of atherosclerotic lesions [57]. Atheroprotection of Core 2 β1,6 GlcNAcT-I is mediated by reduced Ly-6C^{hi} monocytes rolling at inflammatory artery *via* disruption of selectin ligands PGSL-1 function. Supportive evidences for this hypothesis are provided by following observations: Selectin ligands, such as PGSL-1, CD43 and CD44, are glycoproteins modified by Core 2 β1,6 GlcNAcT-I [58]. However, CD43 deficiency displays no change in recruitment of leukocytes into inflammatory sites [59]. Another study confirmed that Core 2 β1,6 GlcNAcT-I deficiency did not affect CD44 activity [57]. In contrast, PGSL-1 deficiency inhibited recruitment of leukocytes into inflammatory sites [24], and core 2 O-glycan modification is critical for PGSL-1 binding to P-selectin [60, 56] (Table 2).

Future directions

As schematized in Fig. 2, several glycosyltransferases deficiency, such as ST3Gal-IV, FucT-VII, and core 2 β1,6 GlcNAcT-I, suppressed atherogenesis *via* the reduced leukocyte recruitment. Progresses have been made in the past about the roles of glycosyltransferases in atherogenesis, but more work is still needed. Firstly, it is still unclear whether an individual glycosyltransferase influences only single or multiple stages of leukocyte recruitment in atherosclerosis, since past studies were limited to investigate single stage of glycosyltransferases to leukocyte recruitment. Secondly, previous studies confirm that several glycosyltransferases, such as ST3Gal-IV, FucT-VII, and core 2 β1,6 GlcNAcT-I, play a key role in atherogenesis, but it is unknown whether other glycosyltransferases also take part in atherogenesis, because other glycosyltransferases expression in acute coronary syndrome patients are different from control patients, for example, ST6GalNacII and ST6GalNacIII are down-regulated, but ST6GalNAcI is up-regulated [61]. Thirdly, it is still unclear whether glycosyltransferases change any other leukocyte behaviors besides recruitment in atherogenesis, such as foam cell formation, macrophage transition between M1 (pro-inflammatory macrophage) and M2 (anti-inflammatory macrophage). For example, FucT-IV and FucT-VII expressions are reduced during macrophage-derived foam cell formation [62]. At last, previous researches focus on the role of glycosyltransferases in the initiation of atherosclerotic lesions. In the future, more studies are needed to determine whether inhibition of glycosyltransferases activities can improve the pre-existing lesions, since this is the stage at which clinically most patients are diagnosed with atherosclerosis. Anyway, previous studies suggest that at least ST3Gal-IV, FucT-VII, and core 2 β1,6 GlcNAcT-I may be hopeful therapeutic candidates for limiting the pathogenesis of atherosclerosis.

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Conflict of interest The authors have declared no conflict of interest.

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