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Aortic Smooth Muscle Cells from ApoE$^{(-/-)}$ Mice Secrete Biglycan with Hyperelongated Glycosaminoglycan Chains

Narin Osman[1,2], Robel Getachew[1] and Peter J Little*[1,2]

[1]*Discipline of Pharmacy, School of Medical Sciences and Diabetes Complications Group, Health Innovations Research Institute, RMIT University, Bundoora, VIC 3083, Australia*
[2]*Monash University, Departments of Medicine and Immunology, Central and Eastern Clinical School, Alfred Health, Melbourne, Victoria, 3181, Australia*

Abstract

Atherosclerosis is the major underlying process of cardiovascular disease. Atherosclerosis commences with an initial pre-inflammatory phase of the trapping and accumulation of lipids in the vessel wall and this is followed by an inflammatory response. The trapping of lipids occurs via binding to proteoglycans, specifically biglycan, with hyperelongated glycosaminoglycan (GAG) chains. Models have been developed to study the aetiology as well as the effectiveness of medical and experimental interventions in preventing atherosclerosis. ApoE is an apolipoprotein associated with removal of lipids from peripheral tissues. Disruption of the ApoE gene in C57BL/6 mice produces mice which are ApoE$^{-/-}$ (ApoE deficient) and show elevated plasma lipids and accelerated development of atherosclerosis which is exacerbated on a high fat diet. These mice are widely used in studies of atherosclerosis. We investigated the possibility that changes in the size of biglycan might participate in the development of atherosclerosis in ApoE$^{-/-}$ mice. We prepared Aortic Smooth Muscle Cell (ASMC) cultures by the digestion technique from ApoE$^{-/-}$ and ApoE$^{+/+}$ mice and studied the size of biglycan secreted by both cell types. The biglycan secreted by ASMCs for ApoE$^{-/-}$ mice was larger than that from ApoE$^{+/+}$ ASMCs. The difference was not eliminated by treatment of cells with a high concentration of Platelet Derived Growth Factor (PDGF) or by treatment with the PDGF antagonist, imatinib. The size difference was however observed in the small free GAG chains (xyloside GAGs) secreted in cells supplemented with exogenous xyloside as a cellular assay of GAG synthesizing capacity. This result suggests that there is a hyperactivity of the fundamental GAG synthesizing capacity in the ASMCs from ApoE$^{-/-}$ mice. These data suggest that hyperelongated biglycan might be contributing to lipid accumulation in ApoE$^{-/-}$ mice used in studies of atherosclerosis and that some of the medical interventions might have an action to reverse this hyperelongation response.

Keywords: Biglycan; Aortic smooth muscle cells; ApoE$^{-/-}$ mice; Glycosaminoglycans; Hyperelongation

Introduction

Atherosclerosis is the major pathology underlying cardiovascular disease which is the leading cause of premature mortality in developed countries [1,2]. Hence, atherosclerosis is a major area of research leading towards the identification of targets and potential therapeutic agents to prevent the onset and progression of the disease [3]. Animal models have been required to facilitate research around the aetiology and discovery of treatments for the prevention of atherosclerosis [4]. Animals and rodents in particular are resistant to the development of atherosclerosis even when fed pro-atherosclerotic modern Western diets. Accordingly, efforts have been directed at the development of modified rodent models which are susceptible to the development of atherosclerosis [4]. One of these approaches has been to exploit knowledge of lipid metabolism to genetically modify mice in a manner that leads to hypercholesterolaemia and exaggerated hypercholesterolaemia when animals are put on a high fat diet. In this context Apolipoprotein E (ApoE) is a 34 kDa glycoprotein and apolipoprotein which circulates in plasma in association with multiple lipoproteins and mediates removal of lipids from peripheral tissues. Interfering with the action of the ApoE leads to hyperlipidaemia and the accelerated development of atherosclerosis [5]. Genetic manipulation can disrupt the ApoE gene leading to the failure to produce and secrete ApoE. The ApoE$^{-/-}$ mouse (Apo E deficient or knockout mouse) is used as a model of hyperlipidaemia and atherosclerosis [6-9]. This work was undertaken using a standard ApoE$^{+/+}$ (C57BL/6) mouse strain which thus serves as the control for studies in ApoE$^{-/-}$ mice. When placed on a high fat Western diet ApoE$^{-/-}$ mice show extensive deposition of lipids in the vasculature and a strong inflammatory response and they thus serve as a model for drug development studies [6-9].

Atherosclerosis commences with the trapping of atherogenic cholesterol-rich lipoproteins in the vessel wall followed by a long slow unresolving inflammatory stage to form complex atherosclerotic plaques [3,10-12]; the clinical sequelae of a myocardial infarction or stroke occurs upon rupture of unstable plaques [13,14]. The initial phase of lipoprotein retention in the vessel wall occurs due to binding and retention of lipid by modified proteoglycans particularly, biglycan, with hyperelongated GAG chains [11,15-17]. This is the major mechanism underlying the early stages of human atherosclerosis [18]. Biglycan is a small leucine-rich Chondroitin Sulfate/Dermatan Sulfate (CS/DS) proteoglycan with a molecular weight of approximately 220 kDa [19]. Growth factor and hormone treatment of vascular smooth muscle cells leads to increased expression and secretion of biglycan core proteins and the biglycan molecules are larger because of activation of the synthetic processes in the Golgi apparatus and the resulting hyperelongation of the CS/DS GAG chains [20-25]. Proteoglycans such as biglycan with larger CS/DS GAG chains show enhanced binding to apolipoproteins in *in vitro* gel mobility shift assays [26]. Furthermore, isolated (chemically cleaved) GAG chains from biglycan from growth factor treated cells as well as the small free GAG chains that arise in cells supplemented with exogenous β-D-xyloside also show enhanced

***Corresponding author:** Peter J. Little, AM, Discipline of Pharmacy, School of Medical Sciences and Health Innovations Research Institute, RMIT University, Bundoora, Victoria 3083, Australia, Tel: +61 417 530 981; E-mail: peter.little@rmit.edu.au

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binding to LDL compared to the respective entities from untreated cells [26]. Treatment of cells with many mechanistic antagonists and cardiovascular drugs blocks this response [27-29] and furthermore, treatment of ApoE$^{-/-}$ mice with antagonists such as imatinib blocks PDGF-stimulated GAG hyperelongation *in vitro* and blocks lipid deposition in the vessel wall of mice *ex vivo* and *in vivo* [30,31]. Other drugs such as the angiotensin receptor blocker, telmisartan, reduce biglycan expression and lipid deposition in ApoE$^{-/-}$ mice [32]. Consistent with this work, the expression of the genes associated with GAG elongation and also biglycan core protein expression are increased in the related LDL Receptor deficient mouse model and the increase is associated with the onset of lipid deposition and elongated GAG chains on proteoglycans in their blood vessels [33].

Due to the convincing evidence for the role of hyperelongated biglycan in atherosclerosis, we considered if there might be any contribution of biglycan expression or hyperelongation to the aortic lipid deposition which is a feature of the ApoE$^{-/-}$ mouse fed a high fat diet. We addressed the question if changes in biglycan expression or structure might contribute to the enhanced lipid deposition observed in high fat fed ApoE$^{-/-}$ mice by determining if there is any difference in the biglycan secreted by Aortic Smooth Muscle Cells (ASMCs) from ApoE$^{-/-}$ mice *versus* their respective genetic controls ApoE$^{+/+}$ mice [6-9]. We prepared ASMCs from ApoE$^{-/-}$ and control ApoE$^{+/+}$ mice for the assessment of the size of biglycan secreted from these cells.

We report that the biglycan produced by ASMCs derived from ApoE$^{-/-}$ mouse aorta is larger than that from ApoE$^{+/+}$ mice and the difference persists when the cells are stimulated with PDGF or treated with the PDGF antagonist, imatinib. Furthermore, xyloside GAGs secreted by ASMCs derived from ApoE$^{-/-}$ compared to ApoE$^{+/+}$ mouse aorta are appreciably larger indicating that the biochemical mechanism(s) of GAG elongation in the Golgi is hyperactivated in the ASMCs derived from ApoE$^{-/-}$. These data indicate that hyperelongated biglycan may be making a previously unrecognised contribution to the development of atherosclerosis in ApoE$^{-/-}$mice.

Materials and Methods

Materials

Benzamidine hydrochloride, DEAE-Sephacel, proteinase K, chondroitin sulfate, PDGF-BB, β-D-xyloside, foetal bovine serum (FBS) and penicillin-streptomycin were from Sigma Aldrich, (St Louis, MO, USA). Imatinib mesylate (Novartis), Dulbecco's Modified Eagle Medium (DMEM) was from Invitrogen Corporation, USA. Sulfur-35 Na_2SO_4 ([^{35}S]-sulfate) (1494 Ci/mmol) was from PerkinElmer (Boston, MA, USA). Rainbow [^{14}C] methylated protein molecular weight markers were from Amersham Pharmacia Biotech, USA. Cetyl pyridinium chloride (CPC) was from Unilab Chemicals and Pharmaceuticals, Mumbai, India and other standard reagents were purchased from Sigma-Aldrich, Australia.

Generation of murine ASMC cultures by the enzymatic digestion technique

Male C57BL/6 (ApoE$^{+/+}$) and ApoE$^{-/-}$ mice were obtained from the Baker IDI Precinct Animal Facility, AMREP, Melbourne, Australia. Aortae from the cardiac root to the abdominal bifurcation were removed from the animals in a clean environment. The acquisition was approved by the AMREP Animal Ethics Committee. Under a stereo dissecting microscope, aortae were cleaned of all external fat. The cleaned aortae were transferred to a 35 mm diameter plastic dish and 2 ml collagenase (3 mg/mL) was added and incubated for 10 mins at 37°C in a 5% CO_2 water-jacketed incubator. Aortae were transferred to a clean sterile dish with media. The adventitia was removed and the remaining vessel transferred to a 35 mm dish with 1 ml each of collagenase and elastase (0.5 mg/mL) added. The vessel was cut into small pieces, incubated at 37°C then transferred to a tube and centrifuged for 5 mins at 1000 rpm. Supernatant was aspirated and cells were rinsed with 6 ml media into a 60 mm tissue culture dish. ASMCs were incubated and after two days media was changed then changed every 3-4 days. After several weeks sufficient cells were present to trypsinize and passage in the usual manner. Cultures were subsequently split (1:3) every second week and cells provided for experiments. The cells showed typical vascular smooth muscle cell morphology although somewhat more heterogeneous appearance compared to cells derived from rat aorta or human vascular explants. Occasionally, very small patches of endothelial cells (cobblestone morphology) were observed but these were rapidly overgrown by the vascular smooth muscle cells.

Quantitation of radiolabel incorporation into proteoglycans

Quiescent ASMCs in 24 well plates were treated in 5 mM glucose DMEM (0.5 ml), 0.1% FBS, 0.1% DMSO and imatinib as detailed in Results section and then exposed to [^{35}S]-sulfate (50 μCi/ml) with or without the presence of PDGF (50 ng/ml) for 24 hr. Secreted proteoglycans were harvested and [^{35}S]-sulfate incorporation into proteoglycans was quantitated using the CPC precipitation assay as described previously [26,28,34].

Biosynthesis of xyloside initiated GAG chains

Quiescent ASMCs from ApoE$^{+/+}$ and ApoE$^{-/-}$ mice were treated in DMEM (0.5 ml) (0.1% FBS) supplemented with xyloside (0.5 mM) under basal conditions for 4 hours prior to the addition of [^{35}S]-sulfate (50 μCi/ml) for a further 24 hours. Xyloside initiated GAG chains were harvested, isolated and quantified using SDS-PAGE.

SDS-PAGE determination of proteoglycan size

Proteoglycans labelled with [^{35}S]-sulfate were prepared for SDS-PAGE by isolation through DEAE sephacel anionic exchange mini columns. Samples were added to pre-equilibrated columns, then washed extensively with low salt buffer (8 M Urea, 0.25 M NaCl, 2 mM disodium EDTA, 0.5% Triton X-100). Proteoglycans were eluted with high salt buffer (8 M Urea, 3 M NaCl, 2 mM disodium EDTA, 0.5% Triton X-100) Aliquots (25,000 cpm) were precipitated (1.3% potassium acetate, 95% ethanol) and chondroitin sulfate was added as a cold carrier. Samples were resuspended in buffer (8M Urea, 2mM disodium EDTA, pH 7.5), to which an equal volume of sample buffer was added. Radiolabelled proteoglycans were separated on 4-13% acrylamide gels with a 3% stacking gel at 50V overnight. [^{14}C]-protein molecular weight markers were run simultaneously. Processed and dried gels were exposed to a phosphorimaging screen (Fuji Photo Film Co, Japan) for approximately 3 days, then scanned on a Bio-imaging analyser BAS-1000 MacBas (Fuji Photo Film Co, Japan).

Results

To provide cells for the assessment of the relative size of the secreted proteoglycan, biglycan, we prepared cultures of ASMCs from ApoE$^{(-/-)}$ and ApoE$^{+/+}$ mice by standard techniques. Cells were passaged and extended to provide sufficient cells for experimentation as described in Methods. ASMCs were passaged and grown to confluency then serum deprived for 24 h in DMEM with 0.1 per cent serum. Cells were then treated with PDGF (50 ng/ml) in the presence of [^{35}S]-sulfate for 24 hr. The media

were harvested and secreted proteoglycans quantitated by the CPC precipitation method and isolated using DEAE ion exchange chromatography to produce fractions for size analysis by SDS-PAGE. The extent of [^{35}S]-sulfate incorporation was greater in ASMCs from ApoE$^{-/-}$ compared to ApoE$^{+/+}$ (Figure 1A). PDGF (50 ng/mL) stimulated an increase in [^{35}S]-sulfate incorporation in both cell types and the difference in the extent of incorporation was maintained (Figure 1A). To explore the mechanism of the greater incorporation in the ApoE$^{-/-}$ cells, we subjected the isolated proteoglycan fractions to SDS-PAGE to determine the average apparent size of the biglycan produced by each cell type. We specifically examined biglycan being quantitatively the most abundant proteoglycan secreted by passaged vascular smooth muscle cells and also the major lipid binding proteoglycan of most relevance to atherosclerosis [18,19]. Biglycan secreted by ASMCs from ApoE$^{-/-}$ was larger than biglycan from ApoE$^{+/+}$ ASMCs (approximately 220 versus 240 kDa) (Figure 1B).

To explore if the difference arose from a difference in the extent and autocrine action of secreted PDGF we used two techniques: we added saturating amounts of exogenous PDGF and also utilised the PDGF antagonist, imatinib [30,31,35]. Both cell types responded to stimulation with PDGF but the size difference was maintained in the biglycan harvested from both types of cells treated with PDGF (Figure 1B). We then explored the mechanism underlying the size difference examining the effect of the PDGF inhibitor imatinib on the size of the biglycan synthesized and secreted by ASMCs from ApoE$^{-/-}$ compared to ApoE$^{+/+}$ cells. Cells were grown to confluency, serum deprived for 24 h then labelled with [^{35}S]-sulfate in the presence and absence of imatinib (10 μM). The media was collected and proteoglycans isolated by DEAE ion exchange chromatography and sized by SDS-PAGE. The biglycan secreted by cells in the absence of imatinib was larger from ASMCs from ApoE$^{-/-}$ compared to ApoE$^{+/+}$ confirming the results shown in Fig 1B (Figure 2 lanes 1 and 2). The biglycan isolated from both cells types treated with imatinib was not affected by the imatinib treatment and the difference in the size of the biglycan was preserved (Figure 2 lanes 3 and 4). These data indicate that the mechanism determining the size difference does not involve an imatinib sensitive process, such as the increased secretion and autocrine action of PDGF, but is likely a more fundamental property of the GAG synthesizing capacity of the cells from the two mouse strains.

Differences in biglycan size may arise from increased expression of the genes associated with GAG elongation in the Golgi apparatus [36,37]. GAG chains are mostly synthesized in the Golgi apparatus where GAG elongation and modifying enzymes are located [36,37]. The first step in the biochemical synthesis of a GAG chain is the addition of a xylose moiety to a serine reside in the core protein by the action of a xylosyl transferase in the endoplasmic reticulum [28,36-39]. The linker region and GAG chains are then extended from this initial xylose in the Golgi apparatus [28]. However, if cells are supplemented with exogenous xyloside then the xyloside acts as a "false acceptor" for subsequent residues and the synthesis of free GAG chains (xyloside GAGs) occurs, albeit generating short GAG chains relative to those synthesized on core proteins [28]. The synthesis of xyloside GAGs serves as a cellular assay for GAG synthesizing capacity and we have previously reported that this process responds to growth factors and inhibitors [28,40-42]. We grew ASMCs to confluency, serum-deprived the cells for 24 h then provided the cells with exogenous xyloside (0.5 mM) and [^{35}S]-sulfate for a further 24 h. We harvested the media, isolated the radiolabelled PGs and xyloside GAGs by CPC precipitation and DEAE- sephacel ion exchange chromatography then subjected the xyloside GAGs to size analysis using a 20% SDS-PAGE. The average size of the xyloside GAGs was increased from approximately 30 kDa in the product secreted from the ASMCs from the ApoE$^{+/+}$ strain to almost 40 kDa in the xyloside GAGs secreted from the ASMCs from the ApoE$^{-/-}$ mice (Figure 3). These data indicate that the GAG synthesizing capacity is higher in Golgi apparatus of the ASMCs derived from ApoE$^{(-/-)}$ compared to the ApoE$^{+/+}$ mouse strain.

Discussion

In this study we observed that the molecular size of the CS/DS lipid-binding proteoglycan, biglycan, synthesized and secreted by ASMCs, was larger in cells prepared from the aorta of ApoE$^{-/-}$ compared to those prepared from their control ApoE$^{+/+}$mouse strain. The difference was maintained when cells were stimulated with a high concentration of exogenous PDGF and was not abolished in the presence of the PDGF receptor antagonist imatinib. Furthermore,

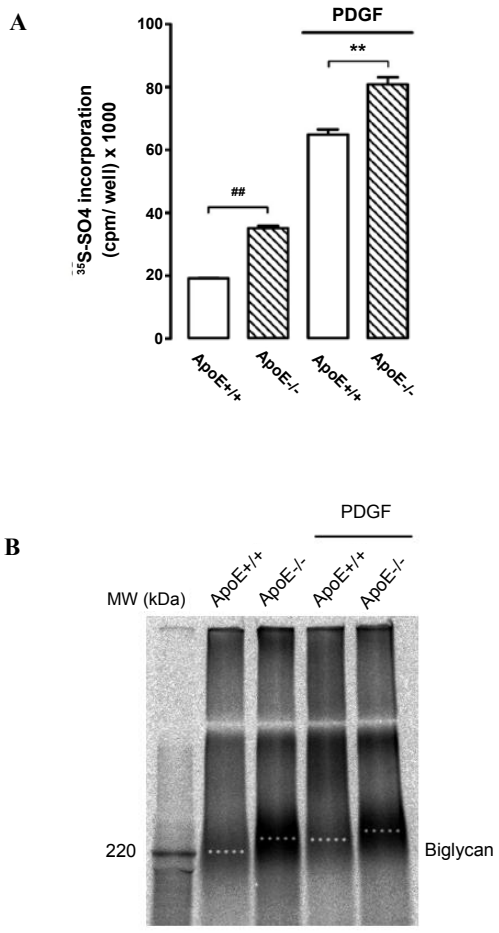

Figure 1: Biglycan synthesised byASMCs from ApoE$^{-/-}$ mice and ApoE$^{+/+}$ differs in size. (A) Measurement of radiolabel incorporation into proteoglycans. ASMCs isolated from from ApoE$^{+/+}$ and ApoE$^{-/-}$ mice mice were treated with and without PDGF (50 ng/ml) in the presence of [^{35}S]-SO_4 (50 μCi/ml) for 24 hr. Media containing secreted biglycan was blotted onto chromatography paper and precipitated via cetylpyridinium chloride. Results are expressed as the mean ± S.E.M of normalized data, ##P<0.01 untreated control samples, ** P < 0.01 PDGF treatments using a one-way ANOVA. (B) PDGF stimulates an increase in biglycan size. Secreted proteoglycans from ASMCs from ApoE$^{+/+}$ and ApoE$^{-/-}$ mice treated as detailed above were isolated using ion exchange chromatography (DEAE Sephacel) and concentrated by ethanol precipitation. Proteoglycans were separated on a 10% polyacrylamide gel. Gel was performed twice from two separate experiments, with a representative shown.

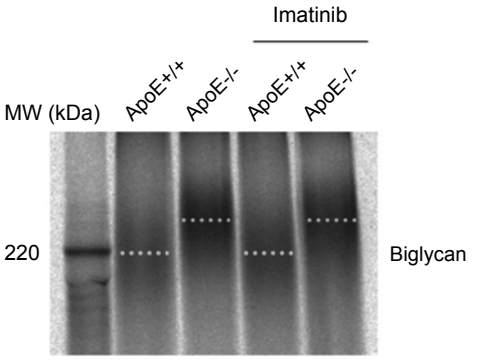

Figure 2: Biglycan synthesis in ASMCs from ApoE$^{+/+}$ and ApoE$^{-/-}$ mice is not sensitive to imatinib. ASMCs were treated with and imatinib (10 μM) in the presence of [^{35}S]-SO_4 (50 μCi/ml) for 24 hr. Isolated proteoglycans were separated by SDS-PAGE.

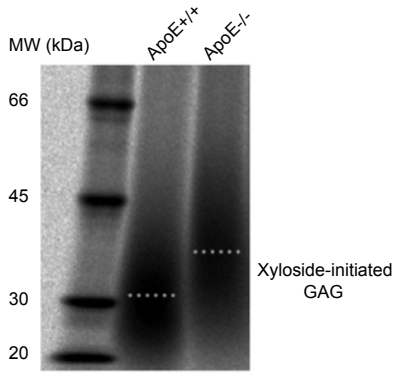

Figure 3: Xyloside initiated GAG chains. ASMCs from ApoE$^{+/+}$ and ApoE$^{(-/-)}$ mice were treated with xyloside (0.5 mmol/l) under basal conditions in the presence of [^{35}S]-SO_4 (50 μCi/ml) for 24 hr. Secreted proteoglycans were isolated and concentrated. GAG chains were cleaved from proteoglycan core proteins via a β-elimination reaction and were separated by SDS-PAGE on 4–13% acrylamide gradient gels. Dotted lines indicate middle of band.

using xyloside supplementation and the synthesis of xyloside GAGs as a measure of cellular GAG synthesizing capacity [28,36-39], we made the complementary observation that the xyloside GAGs were larger in ASMCs prepared from the aorta of ApoE$^{-/-}$ compared to their control ApoE$^{+/+}$ mouse strain. Biglycan is a major lipid binding proteoglycan and its form with hyperelongated GAG chains has been strongly implicated with the initiation of atherosclerosis both in animal models and human coronary artery disease [11,18,33]. Based on the substantial evidence that hyperelongated GAG chains demonstrate enhanced binding to LDL, the current data suggest that the relatively hyperelongated biglycan present in the aorta of ApoE$^{-/-}$ compared to their control ApoE$^{+/+}$ counterparts, may be making a previously unrecognised and unexpected contribution to the deposition of lipid in this very widely used model of atherosclerosis [43-48]. A further implication is that the mechanism underlying many of the experimental interventions showing reduced lipid deposition and accumulation in the aorta of high fat fed ApoE$^{-/-}$ mice may be due to a contributing inhibition of GAG synthesizing capacity and GAG hyperelongation in ApoE$^{-/-}$ mice [11,18,33].

The question arises as to what might be the cell biology underlying this observation. The most apparent possible mechanism involves the primary target of the genetic manipulation in the mice being the absence of ApoE in the ApoE$^{-/-}$ mice. This protein is secreted by vascular smooth muscle cells [49-51] so it is most likely that it is secreted by the ApoE$^{+/+}$ derived strain of ASMCs but will be absent in cultures of ApoE$^{-/-}$ ASMCs [51].

Surprisingly, ApoE has direct effects on the properties and behaviour of vascular smooth muscle cells [52,53]. For example, ApoE inhibits the actions of PDGF to stimulate proliferation and migration of vascular smooth muscle cells [52]. ApoE inhibits growth factor signalling [53]. ApoE competes with growth factors for binding to heparan sulfate proteoglycans (HSPGs) where the binding of growth factors to HSPGs and concomitantly to the growth factor receptor is a major pathway for growth factor signalling. If ApoE prevents growth factors binding to the HSPGs acting as co-receptors then this will inhibit signalling [54]. We have shown that multiple growth factors and hormones including PDGF, TGF-β, endothelin and thrombin stimulate the secretion of biglycan with longer GAG chains from human vascular smooth muscle cells [22,25,26,40]. So it is possible that in ASMCs from ApoE$^{-/-}$ mice, the secretion acts as an inhibitory pathway which is then absent in the ASMCs from ApoE$^{+/+}$ derived cells. To indirectly investigate this possibility we explored the effect of adding a growth factor and its inhibitor on the difference between the sizes of the biglycan between the two cell types.

This size difference might result from the action of growth factors, perhaps the autocrine action of secreted growth factor being greater in the cells from ApoE$^{-/-}$ mice. To address this question we stimulated cells with a maximal dose of PDGF (Figure 1A and B). The larger size of the biglycan from ApoE$^{-/-}$ cells persisted in the presence of PDGF. We also investigated the impact of adding a PDGF inhibitor to test the hypothesis that the difference occurs due to an autocrine action of PDGF which would be abolished by the inhibitor and render the secretion of equal sized biglycan molecules. We used imatinib which we have previously demonstrated inhibits GAG elongation *in vitro* and reduces lipid deposition in ApoE$^{-/-}$ mice *in vivo* [30,31]. However, the difference in the size of secreted biglycan in the two cells type was maintained in cells treated with imatinib. These data suggest that there is some fundamental difference in the GAG synthesizing capacity of the cells from the ApoE$^{-/-}$ compared to those from ApoE$^{+/+}$ ASMCs.

It is possible that the difference arises for fundamental alterations in the GAG synthesizing capacity in the cells. Early work on the cloning and characterisation of CS/DS GAG synthesizing enzymes has been complemented by recent work showing an association between the expression of GAG enzymes and lipid accumulation [33,55]. A multitude of enzymes have been cloned and characterised but the actual mechanism of CS/DS chain elongation and termination remains unknown [17]. Some of these enzymes work in pairs to affect monosaccharide addition and GAG elongation or otherwise sulfation and some can contribute to GAG elongation in the absence of their transferase activities [17]; in practice these enzymes are only capable of synthesizing relatively short GAG chains *in vitro*. Thus, some of these enzymes, notably chondroitin 4-O-sulfotransferase-1 (C4ST-1) and chondroitin N-acetylgalactosaminyltransferase-2 (ChGn-2), are involved in GAG synthesis [33] and we speculate that the increased expression of one or more of these enzymes mediates the hyperelongation response to growth factors such as TGF-β, PDGF and GPCR agonists such as thrombin and endothelin [22,25,26,40]. Thus we can speculate that ASMCs from the aorta of ApoE$^{-/-}$ compared to those prepared from their control ApoE$^{+/+}$ mice strain have a higher level of expression of one or more GAG synthesizing enzymes such that the capacity to synthesize GAGs in the Golgi is enhanced and

biglycan with longer GAG chains is synthesized and secreted. How this difference may result from a modification of the expression of the ApoE gene in these mice is difficult to understand at this stage. We are currently establishing assays for the expression of multiple GAG synthesizing enzymes and it will be interesting to test this hypothesis by analysis of gene expression in ASMCs from the two mouse strains.

A final consideration might be the effects of lipid levels on GAG hyperelongation. ApoE has an effect on lipid levels in vivo however this is unlikely to be a significant factor in a cell culture environment, nevertheless, examining the effects of lipid on GAG synthesis would be an interesting line of investigation. Furthermore, in relation to the effect of lipid levels on GAG elongation, it might be interesting to explore the properties of biglycan secreted by aortic smooth muscle cells from LDLR-deficient mice and their controls, with this being the other most widely used model of hyperlipidemia and atherosclerosis [33].

In conclusion, we have made the surprising observation that the size of the lipid-binding proteoglycan, biglycan, synthesized and secreted by ASMCs from ApoE$^{-/-}$ mice is larger than that from the relevant control strain of mice. As larger, so-called hyperelongated GAG chains, are associated with enhanced binding of apolipoproteins *in vitro* and lipid accumulation *in vivo* this suggests that this property of biglycan is making a contribution to the atherosclerosis that occurs in this mouse model. Our data did not support an autocrine action of PDGF but pointed to a fundamental alteration in the GAG synthesizing capacity of the cells. These data suggest that differences in the properties of biglycan might be contributing to the atherosclerosis that occurs in ApoE$^{-/-}$ mice and that inhibition of this effect might be contributing to the many studies in which lipid deposition is reduced by treatment of ApoE$^{-/-}$ mice with experimental and complimentary medicines as well as many cardiovascular drugs.

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