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Elastic Laminae in Vascular Development and Disease

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Abstract: The activities of vascular cells, including adhesion, proliferation, and migration, are mediated by extracellular matrix components, including collagen matrix and elastic fibers or laminae. Whereas the collagen matrix stimulates vascular cell adhesion, proliferation, and migration, the elastic laminae inhibit these activities. Coordinated regulation of cell activities by these matrix components is an essential process for controlling the development and remodeling of the vascular system. This article summarizes recent development on the role of arterial elastic laminae in regulating the development of smooth muscle-like cells from bone marrow-derived progenitor cells as well as in mediating cell adhesion, proliferation, and migration with a focus on the molecular mechanisms and physiological significance.

1 Introduction

The arterial wall is composed of extracellular matrix components, including collagen matrix and elastic laminae. These matrix components regulate vascular cell activities, including adhesion, proliferation, and migration, which are essential processes for vascular development and remodeling. While the collagen matrix stimulates cell adhesion, proliferation, and migration [1, 2], the elastic laminae negatively regulate these cell activities [3-5]. By exerting opposing effects, collagen matrix and elastic laminae coordinately control vascular morphogenesis during development and mediate pathological alterations in vascular disorders. The present article addresses the role of arterial elastic laminae in regulating the formation of smooth muscle-like cells from bone marrow-derived progenitor cells as well as vascular cell adhesion, proliferation, and migration with a focus on the associated molecular mechanisms.

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2 Elastic lamina-mediated formation of smooth muscle-like cells

2.1 Elastic laminae in development of vascular smooth muscle cells

Arterial smooth muscle cells (SMCs) are contractile cells that control the diameter of arteries and the rate of blood flow. Under physiological conditions, these cells reside primarily within the media, but not within the intima and adventitia of arteries, suggesting the presence of specific cues in the media for inducing the formation and maintaining the phenotype of SMCs. A unique feature of the medial structure is the presence of elastic laminae, an extracellular matrix constituent composed of primarily the protein elastin. Extracellular matrix plays an important role in regulating cell specification and differentiation during development [1, 2, 6]. Elastic laminae, the most abundant type of extracellular matrix in the arterial media, have been considered not only matrix components contributing to the mechanical strength and elasticity of the arterial wall [7-11], but also signaling elements regulating cell adhesion, proliferation, and migration [3, 4, 7, 9, 12-18]. Thus, elastic laminae possibly serve as a cue for regulating the formation and maintain the contractile phenotype of SMCs in the arterial media, contributing to vascular morphogenesis.

2.2 Elastic lamina-induced formation of SM α-actin filaments in CD34+ bone marrow cells

To test the role of elastic laminae in phenotype development of vascular SMCs, we have investigated whether arterial elastic laminae induce formation of SM-like cells from bone marrow-derived progenitor cells in the mouse. The bone marrow contains progenitor cells for vascular SMCs. These cells may be mobilized to the circulating blood, adhere to the wall of blood vessels, and transform to SM-like cells, contributing to SMC regeneration and intimal hyperplasia [19-24]. We have recently found that a large fraction of mouse bone marrow cells that express the transmembrane glycoprotein CD34, a hematopoietic stem and progenitor cell marker [25], co-express non-filamentous SM α actin [5]. The presence of SM α actin suggests that the CD34+ bone marrow cell population may contain SMC progenitor cells.

CD34+ bone marrow cells were isolated from the mouse femur and cultured on either elastic laminae from the media or collagen matrix from the adventitia of the mouse aorta. In the presence of elastic laminae, SM α actin filaments were found in 23 +/- 6%, 34 +/- 7%, and 44 +/- 8% of CD34+ bone marrow cells at day 5, 10, and 20, respectively (Figure 1). In contrast, a significantly lower fraction of CD34+ bone marrow cells expressed SM α actin filaments when cultured on collagen-dominant matrix specimens (5 +/- 3%, 8 +/- 3%, and 10 +/- 4% at day 5, 10, and 20, respectively). These observations suggest that the arterial elastic laminae stimulate the formation of SM α actin filaments in the CD34+ bone marrow cells.

To confirm the role of the arterial elastic laminae in regulating the formation of SM α actin filaments in CD34+ bone marrow cells, we transplanted GFP gene-transfected wild-type CD34+ bone marrow cells into the circulation of wild-type mice and measured the population size of SM α actin filament+ GFP cells within elastic lamina- and collagen-dominant matrix scaffolds implanted into the host aorta [5]. As shown in Fig. 2, transplanted GFP-CD34+ bone marrow cells migrated into the implanted matrix scaffolds. In the elastic lamina-dominant matrix scaffolds, 25 +/- 5 % and 37 +/- 9 % of GFP-CD34+ cells expressed SM α actin filaments at day 5 and 10, respectively, following matrix scaffold implantation and cell transplantation. In contrast, a significantly smaller population of GFP-CD34+ cells (7 +/- 3 % and 9 +/- 4 % at day 5 and 10, respectively) expressed SM α actin filaments in the collagen matrix-dominant scaffolds [5]. These observations confirmed the role of elastic laminae in regulating the formation of SM α actin filaments in CD34+ bone marrow cells.

2.3 Regulatory mechanisms for SM α-actin filament formation

The arterial elastic laminae can interact with circulating monocytes and activate a signaling mechanism involving a protein tyrosine phosphatase known as SH2 domain-containing protein tyrosine phosphatase (SHP)-1 [4]. The interaction of elastic laminae with monocytes induces activation of SHP-1 via the mediation of an inhibitory transmembrane receptor known as signal regulatory protein (SIRP) α [26-33]. Activated SHP-1 can dephosphorylate a number of mitogenic protein tyrosine kinases, including receptor tyrosine kinases [28, 34], Src [34], and JAKs [35, 36]. The consequence of SHP-1 activation is suppression of mitogenic activities, such as cell adhesion, proliferation, and migration [4, 37-45]. Since the suppression of mitogenic activities is associated with an increase in the density of contractile SMCs [46, 47], SHP-1 may enhance the formation of the SM α actin filaments in SM progenitor cells. Thus, the arterial elastic laminae may stimulate the formation of SM α actin filaments in CD34+ bone marrow cells via the mediation of SHP-1.

The CD34+ bone marrow cells as well as the CD34+ bone marrow cell-derived SM α actin filament+ cells expressed the protein tyrosine phosphatase SHP-1 (Fig. 3A, B). To demonstrate whether SHP-1 mediates the effect of the aortic elastic lamina-dominant matrix on the formation of SM α actin filaments in CD34+ bone marrow cells, we assessed the influence of elastic lamina-dominant matrix on the expression and phospho-

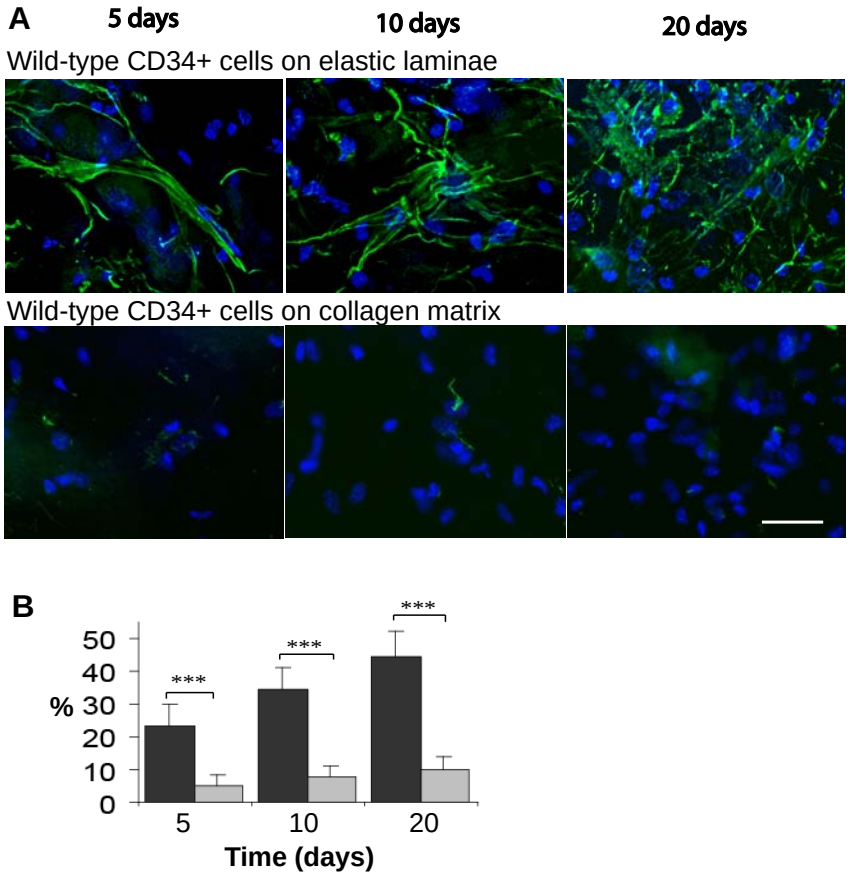

Figure 1: Influence of aortic elastic lamina- and collagen-dominant matrix on the formation of SM α actin filaments in CD34+ bone marrow cells in vitro. (**A**) Fluorescent micrographs showing that elastic lamina-dominant matrix stimulates the formation of SM α actin filaments in CD34+ bone marrow cells (row 1) compared to collagen-dominant matrix (row 2). Note that few cells expressed CD34 after day 5 of culture. Green: SM α actin. Blue: cell nuclei. Scale: 10 μm. (**B**) Percentage of SM α actin filament+ cells derived from wild-type CD34+ bone marrow cells cultured on aortic elastic lamina-dominant matrix specimens (black bars) and collagen-dominant matrix specimens (gray bars) in vitro. *** $p < 0.001$. ANOVA tests showed that changes in the percentage of SM α actin filament+ cells with time (5 to 20 days) were significant on both elastic lamina- and collagen-dominant matrix ($p < 0.001$). Note that when cultured on collagen-dominant matrix specimens, a small fraction of CD34+ cells exhibited actin filaments. However, these actin filaments were not as developed as those found in CD34+ cells cultured on elastic lamina-dominant matrix specimens. From ref. 5 with permission.

rylation of SHP-1 when collagen-dominant matrix was used as a control [5]. As shown in Fig. 3C, while the expression level of SHP-1 appeared similar between cells cultured on elastic lamina- and collagen-dominant matrix specimens, the elastic lamina-dominant matrix induced a noticeable increase in the relative phosphorylation of SHP-1 compared to the collagen matrix. These observations suggest that the aortic elastic lamina-dominant matrix stimulates the activation of SHP-1.

Since SHP-1 suppresses the activity of selected mitogenic protein tyrosine kinases, which are implicated in the negative regulation of SM α actin filament formation, we assessed the role of SHP-1 in mediating the formation of SM α actin filaments in CD34+ bone marrow cells by using a siRNA mediation approach [5]. The transfection

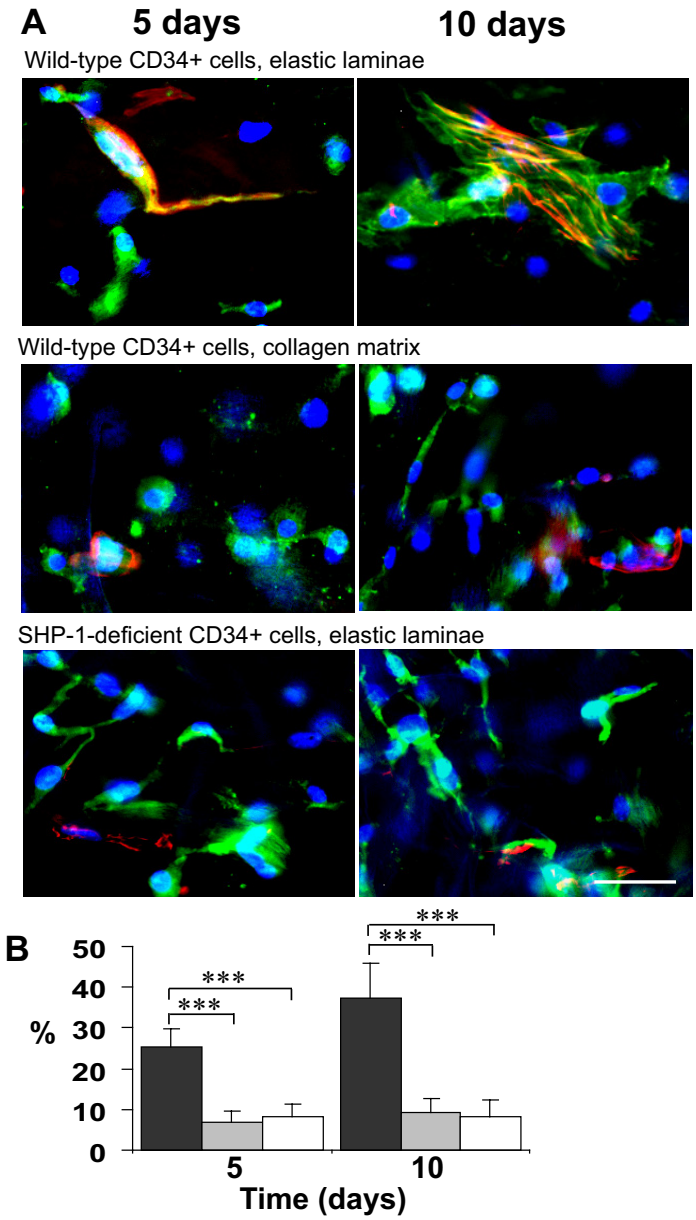

Figure 2: Influence of aortic elastic laminae and collagen matrix on the formation of SM α actin filaments in CD34+ bone marrow cells and role of SHP-1 in regulating the formation of SM α actin filaments *in vivo*. (**A**) Fluorescent micrographs demonstrating that elastic lamina-dominant matrix (row 1) stimulates the formation of SM α actin filaments (red) in transplanted GFP-CD34+ bone marrow cells (green) compared to collagen-dominant matrix (row 2) in matrix scaffolds implanted into the mouse aorta. Row 3 shows the influence of SHP-1 deficiency on the formation of SM α actin filaments (red) in transplanted GFP-CD34+ bone marrow cells (green) in elastic lamina-dominant matrix scaffolds. Blue: cell nuclei for all panels. Scale: 10 μm. (**B**) Percentage of SM α actin filament+ cells derived from transplanted wild-type GFP-CD34+ bone marrow cells in elastic lamina-dominant (black bars) and collagen matrix-dominant (gray bars) matrix scaffolds as well as from SHP-1-deficient GFP-CD34+ bone marrow cells in elastic lamina-dominant matrix scaffolds (white bars) *in vivo*. *** $p < 0.001$. From ref. 5 with permission.

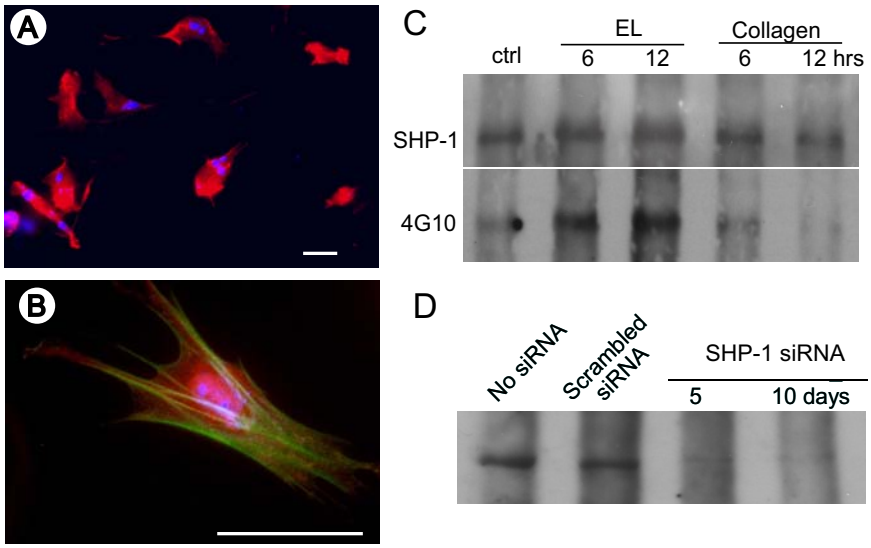

Figure 3: Expression of SHP-1 in CD34+ bone marrow cells and suppression of SHP-1 expression by siRNA transfection. (**A**) Fluorescent micrograph showing the expression of SHP-1 (red) in cultured CD34+ bone marrow cells at day 10. Scale: 10 μm. (**B**) Fluorescent micrograph showing the expression of SHP-1 (red) in a cultured CD34+ bone marrow cell expressing SM α actin filaments (green) at day 10. Blue: cell nuclei for both panel A and B. Scale: 10 μm. (**C**) Influence of aortic elastic lamina- and collagen-dominant matrix on the expression (upper) and phosphorylation (lower) of SHP-1 in CD34**+** bone marrow cells. 4G10: anti-phosphotyrosine antibody. ctrl: control cells cultured on plastic plates. EL: elastic lamina. (**D**) Suppression of SHP-1 expression with SHP-1-specific siRNA. From ref. 5 with permission.

of CD34+ bone marrow cells with SHP-1-specific siRNA induced a noticeable reduction in SHP-1 expression, while the transfection of scrambled siRNA did not influence the expression of SHP-1 (Fig. 3D). The suppression of SHP-1 expression by siRNA transfection resulted in a significant decrease in the formation of SM α actin filaments in the CD34+ bone marrow cells cultured on aortic elastic lamina-dominant matrix specimens compared to control cells transfected with scrambled siRNA (Fig. 4). These observations support the role of SHP-1 in mediating the effect of arterial elastic laminae on the formation of SM α actin filaments in CD34+ bone marrow cells.

We further tested the role of SHP-1 in regulating the formation of SM α actin filaments by using SHP-1-deficient and wild-type CD34+ bone marrow cells. As shown in Fig. 4, the population size of SM α actin filament+ cells in the SHP-1-deficient CD34+ bone marrow cells was significantly smaller than that in the wild-type CD34+ bone marrow cells cultured on aortic elastic lamina-dominant matrix specimens at day 5, 10, and 20. These observations confirmed the role of SHP-1 in mediating the effect of arterial elastic laminae on the formation of SM α actin filaments in CD34+ bone marrow cells.

To confirm the role of SHP-1 in mediating the formation of SM α actin filaments in CD34+ bone marrow cells residing in the arterial elastic lamina-dominant matrix scaffolds, we transplanted GFP gene-transfected SHP-1-deficient or wild-type CD34+ bone marrow cells into the circulation of wild-type mice and measured the population size of SM α actin filament+ GFP cells within the elastic lamina-dominant matrix scaffolds implanted into the host aorta [5]. As shown in Fig. 2, the population size of SM αgctin filament+ cells in SHP-1-deficient CD34+ bone marrow cells was significantly smaller than that in wild-type CD34+ bone marrow cells within the elastic lamina-dominant matrix scaffolds at day 5 and 10 after matrix scaffold implantation and cell transplantation. These observations confirmed the

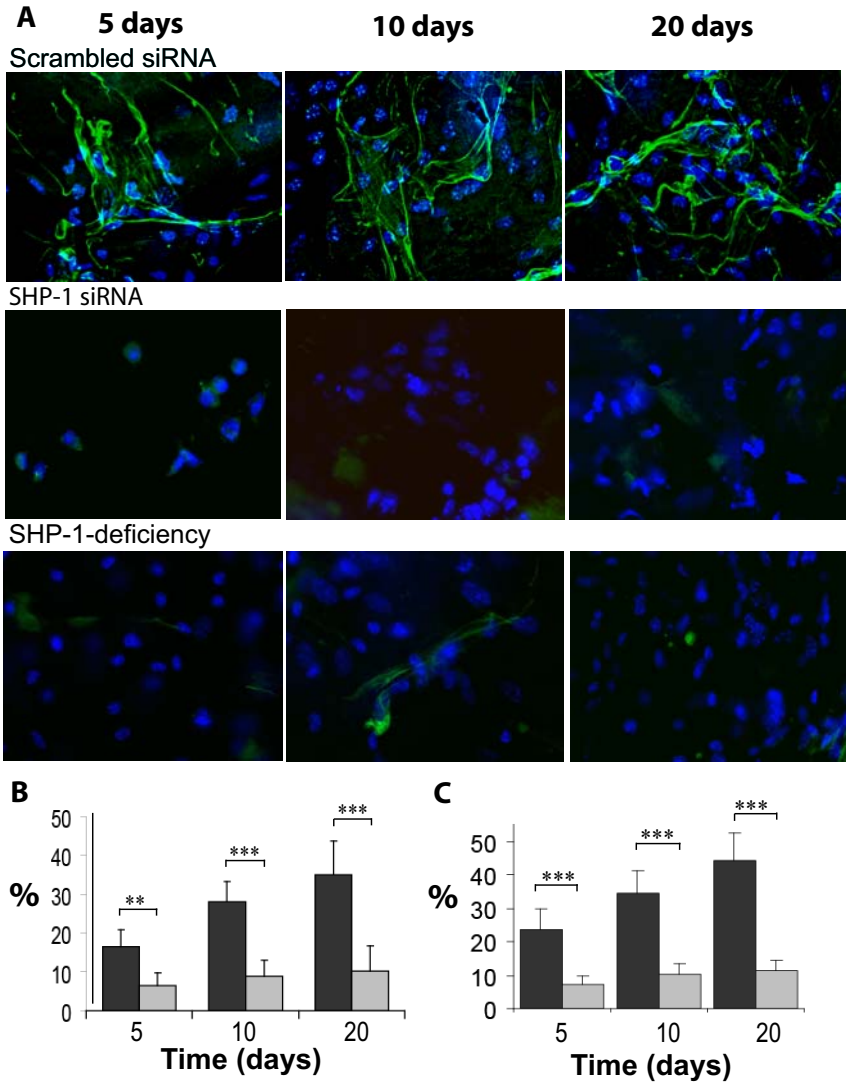

Figure 4: Influence of siRNA-mediated SHP-1 knockdown and transgenic SHP-1 deficiency on the formation of SM α actin filaments in CD34+ bone marrow cells *in vitro*. (**A**) Fluorescent micrographs demonstrating different levels of SM α actin filament formation in CD34+ bone marrow cells transfected with scrambled siRNA (row 1) and SHP-1-specific siRNA (row 2) as well as in SHP-1-deficient CD34+ cells (row 3). The CD34+ cells were cultured on aortic elastic lamina-dominant matrix specimens. Green: SM α actin. Blue: cell nuclei. Scale: 10 μm. (**B**) Percentage of SM α actin filament+ cells derived from CD34+ bone marrow cells transfected with scrambled siRNA (black bars) and SHP-1-specific siRNA (gray bars). ** $p < 0.01$, *** $p < 0.001$. ANOVA tests showed that changes in the percentage of SM α actin filament+ cells with time (5 to 20 days) were significant in CD34+ cells transfected with scrambled siRNA ($p < 0.01$), whereas changes in CD34+ cells transfected with SHP-1-specific siRNA were not significant ($p > 0.05$). (**C**) Percentage of SM α actin filament+ cells derived from wild-type CD34+ cells (black bars) and SHP-1-deficient CD34+ cells (gray bars). *** $p < 0.001$. ANOVA tests showed that changes in the percentage of SM α actin filament+ cells with time were significant in wild-type CD34+ cells ($p < 0.001$), whereas changes in SHP-1-deficient CD34+ cells were not significant ($p > 0.05$). Note that in the presence of SHP-1-specific siRNA or SHP-1 deficiency, a small fraction of CD34+ cells exhibited SM α actin filaments. However, these actin filaments were not as developed as those found in control CD34+ cells without SHP-1 siRNA or SHP-1 deficiency. From ref. 5 with permission.

role of SHP-1 in mediating the effect of arterial elastic laminae on the formation of SM α actin filaments in CD34+ bone marrow cells.

3 Elastic lamina-mediated suppression of leukocyte adhesion

3.1 Protective role of elastic laminae in inflammatory responses

Arterial elastic laminae have long been considered a structure that determines the strength and elasticity of blood vessels [8, 10-12, 48-50]. Recent studies, however, have demonstrated that arterial elastic laminae also participate in the regulation of arterial morphogenesis and pathogenesis [3-5, 14-16, 18, 51]. An important contribution of elastic laminae is to confine smooth muscle cells (SMCs) to the arterial media by inhibiting SMC proliferation [14, 15] and migration [3], thus preventing intimal hyperplasia under physiological conditions. Arterial elastic laminae also exhibit thrombosis-resistant properties [3, 4]. When implanted in an artery, elastic lamina scaffolds are associated with significantly lower leukocyte adhesion and thrombosis compared with collagen matrix scaffolds [3,4]. These observations suggest an inhibitory role for elastic laminae in regulating inflammatory responses relative to collagen matrix.

3.2 Inhibitory role of elastic laminae in regulating leukocyte adhesion

We used an in vivo matrix-based arterial reconstruction model to demonstrate the role of elastic laminae in preventing leukocyte transmigration through the arterial media [4]. As shown in Fig. 5, the majority of cells found in the matrix of aortic substitutes were CD11 b/c-positive leukocytes (predominantly monocytes/ macrophages and granulocytes), especially during the early period. While a large number of leukocytes migrated into the collagen-dominant adventitia, few leukocytes were found within the elastic lamina-dominant media of the matrix-based aortic substitutes. The density of leukocytes in the media was 58- to 70-fold lower than that in the adventitia from 1 to 30 days after surgery, while no significant difference was detected in the media between NaOH-treated (with an elastic lamina blood-contacting surface) and untreated matrix (with a basal lamina blood-contacting surface) substitutes. At the end of the elastic lamina-dominant media, leukocytes were not able to migrate into the gaps between the elastic laminae, even though the gaps were apparently larger than the diameter of leukocytes (Fig. 5A Day 10*). However, at locations with aneurysm-like changes (induced possibly by excessive mechanical stretch due to surgical damage to the adventitia), leukocytes migrated into the medial wall, where elastic laminae were largely destroyed (Fig. 5A Day 10**). These observations demonstrate that intact and NaOH-treated elastic laminae exert an inhibitory effect on leukocyte transmigration relative to the adventitial collagen matrix.

We carried out an in vitro monocyte adhesion assay to observe the inhibitory effect of elastic laminae on monocyte adhesion relative to that of the arterial basal lamina and adventitia. As shown in Fig. 6, the exposure of monocytes to NaOH-treated and untreated elastic laminae induced monocyte adhesion ranging from 9 to 20 cells/mm^2 and 13 to 16 cells/mm^2, respectively, from 3 to 24 hours. In contrast, exposure to the basal lamina and adventitia resulted in a more than 11- and 98-fold increase in monocyte adhesion, respectively, compared with elastic lamina. These observations verify the inhibitory role of elastic laminae on monocyte adhesion relative to collagen-containing matrix.

3.3 Regulatory mechanisms for the inhibitory role of elastic laminae

Leukocytes are known to express the inhibitory receptor SIRP α (also known as Src homology 2 domain-containing tyrosine phosphatase substrate-1), a transmembrane glycoprotein receptor that exerts an inhibitory effect on cell mitogenic [26, 27, 31, 38, 52, 53] and inflammatory [45, 54] activities. Upon ligand binding, SIRP α transmits inhibitory signals through tyrosine phosphorylation of its intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) [26,

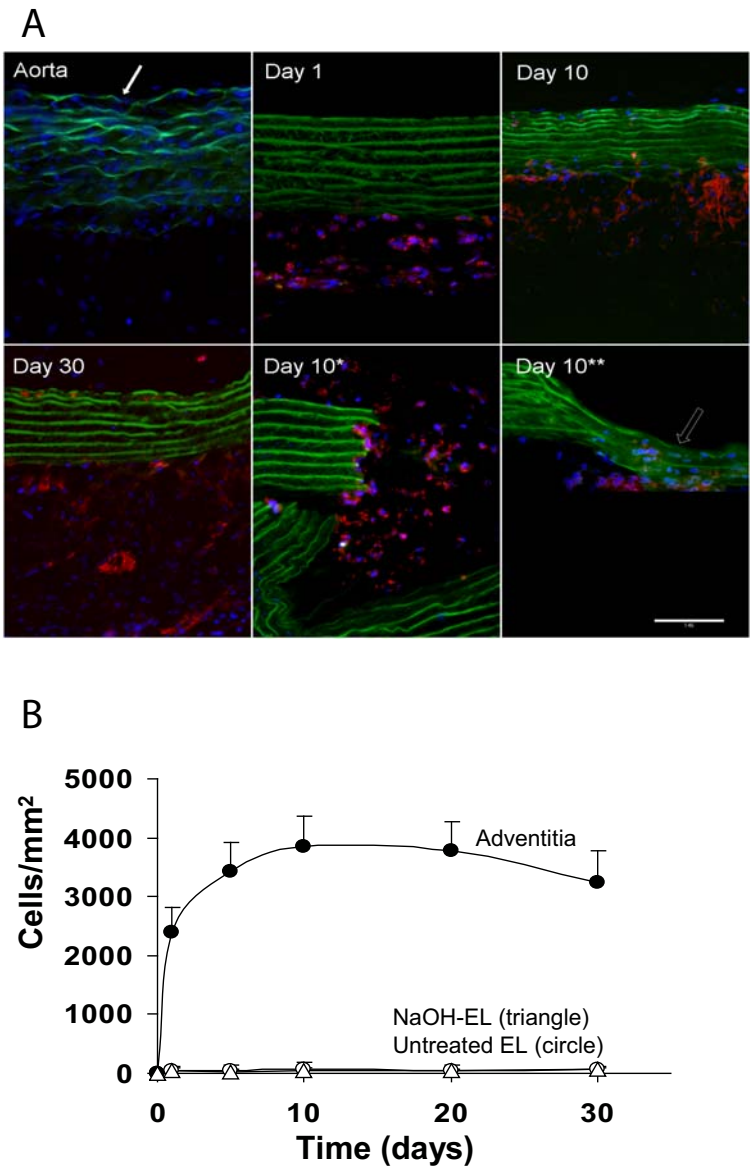

Figure 5: Leukocyte transmigration in the elastic lamina-dominant media and collagen-dominant adventitia of matrix-based aortic substitutes in vivo. (**A**) Transverse fluorescent micrographs showing the distribution of CD 11b/c-positive leukocytes in the media and adventitia of matrix-based aortic substitutes. Note that leukocytes did not migrate into the gaps between the elastic laminae at the end of the aortic matrix substitutes (Day 10*). However, leukocytes migrated into the media of aortic matrix substitutes when the elastic laminae were largely destroyed at locations with aneurysmatic changes (Day 10**). Red: antibody-labeled CD 11 b/c. Green: elastic laminae. Blue: Hoechst 33258-labeled cell nuclei. Solid arrow: blood-contacting surface of aortic matrix substitutes. Open arrow: aneurysmatic change. Scale: 100 μm. (**B**) Density of CD-11 b/c-positive cells within the elastic lamina-dominant media and collagen-dominant adventitia of NaOH-treated and untreated matrix scaffolds. Differences were significant ($p < 0.0001$) between elastic laminae and adventitia at all observation times except time 0, at which no CD 11 b/c-positive cells were found. No significant difference was detected between NaOH-treated and untreated elastic laminae at any observation time ($p > 0.05$). Means and standard deviations are presented ($n = 5$ for each group). From ref. 4 with permission.

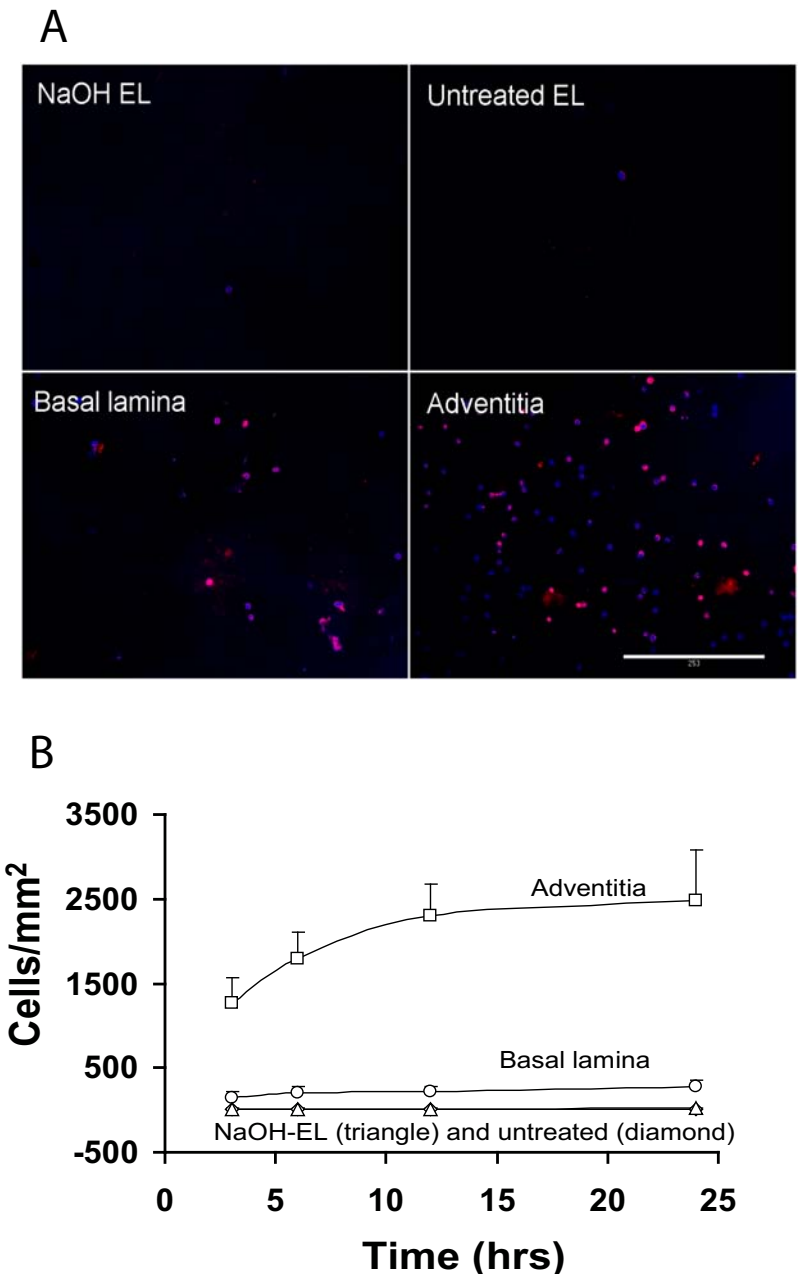

Figure 6: Monocyte adhesion to different matrix specimens in vitro. (**A**) En face fluorescent micrographs showing monocytes adhered to NaOH-treated and untreated elastic lamina, basal lamina, and adventitia. EL: elastic lamina. Scale: 100 μm. (**B**) Measurements of monocyte density on NaOH-treated and untreated elastic lamina, basal lamina, and adventitia. Differences were significant between elastic lamina and basal lamina as well as between elastic lamina and adventitia at all observations times ($p < 0.001$). Means and standard deviations are presented (n = 5 for each group). From ref. 4 with permission.

27, 29, 31, 32, 38, 52, 53]. The phosphorylation of the ITIM initiates the recruitment of Src homology 2 domain-containing protein tyrosine phosphatase (SHP)-1 to SIRP α, which is known as a substrate of SHP-1 [29, 32]. The recruitment of SHP-1 also localizes and activates SHP-1 [28, 33, 55], which in turn dephosphorylates protein kinases, possibly including receptor tyrosine kinases [28, 33], the Src family protein tyrosine kinases [34], phosphatidylinositol 3-kinase [34], and the Janus family tyrosine kinases [35, 36]. These activities potentially suppress inflammatory and mitogenic responses [37, 39, 56, 57]. Since the inhibitory effect of elastic laminae coincides with the activity of the inhibitory receptor in leukocytes, it is conceivable that, upon contacting leukocytes, elastic laminae may interact with SIRP α and activate SHP-1, leading to the inhibition of leukocyte adhesion.

Tyrosine phosphorylation of SIRP α is required for its inhibitory effect and for the recruitment and activation of SHP-1. To test whether the interaction of monocytes with matrix components induces SIRP α phosphorylation and SHP-1 recruitment, we examined co-immunoprecipitation of SIRP α with SHP-1 and the relative phosphorylation of these molecules in monocytes reacted with NaOH-treated and untreated elastic laminae, basal lamina, and adventitia [4]. As shown in Fig. 7A, the exposure of monocytes to NaOH-treated and untreated elastic laminae induced an apparent increase in the relative phosphorylation of SIRP α. Phosphorylated SIRP α was co-immunoprecipitated with SHP-1, demonstrating recruitment of SHP-1 to SIRP α. Recruited SHP-1 was also phosphorylated. In contrast, the relative phosphorylation of SIRP α in monocytes reacted with the basal lamina and adventitia was not as apparent as that observed in cells reacted with elastic laminae (Fig. 7B). Little SHP-1 recruitment was found in monocytes cultured on the basal lamina and adventitia. These observations suggest that the exposure of monocytes to elastic laminae stimulates the activation of SIRP α, which induces the recruitment and phosphorylation of SHP-1.

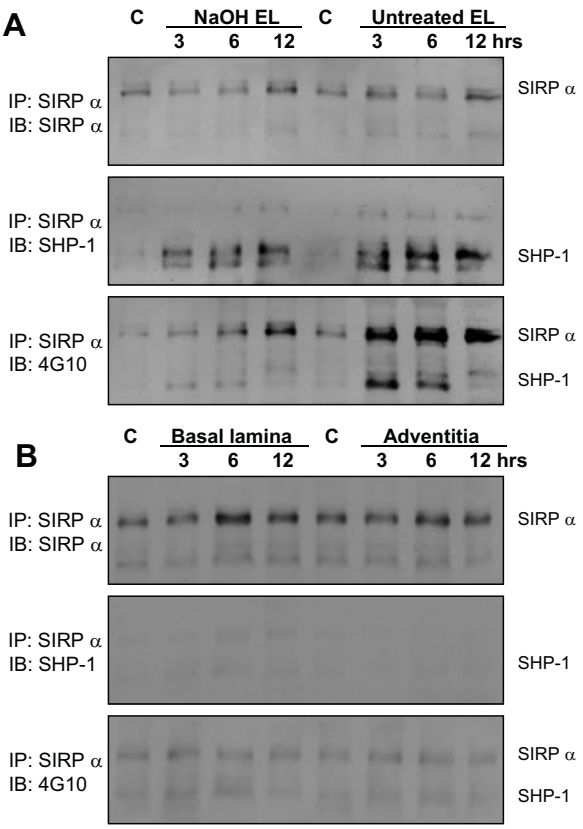

Figure 7: Co-immunoprecipitation and relative phosphorylation of SIRP α and SHP-1 in monocytes exposed to NaOH-treated and untreated elastic laminae (panel A) as well as to basal lamina and adventitia (panel B). Two IP/IB tests were conducted for each matrix specimen. C: control without exposure to a matrix specimen. EL: elastic lamina. IP: immunoprecipitation. IB: immunoblotting. 4G10: anti-phosphotyrosine antibody. From ref. 4 with permission.

We used a siRNA approach to knockdown the expression of SIRP α and SHP-1 and thus to demonstrate the role of these molecules in mediating the inhibitory effect of elastic laminae on monocyte adhesion [4]. As shown in Fig. 8A, transfection with SIRP α-specific siRNA apparently reduced the expression of SIRP α in monocytes. Such a treatment diminished the inhibitory effect of elastic laminae on monocyte adhesion, resulting in a significant increase in the density of monocytes on the surface of NaOH-treated and untreated elastic laminae (Fig. 8B). However, such a treatment did not significantly influence

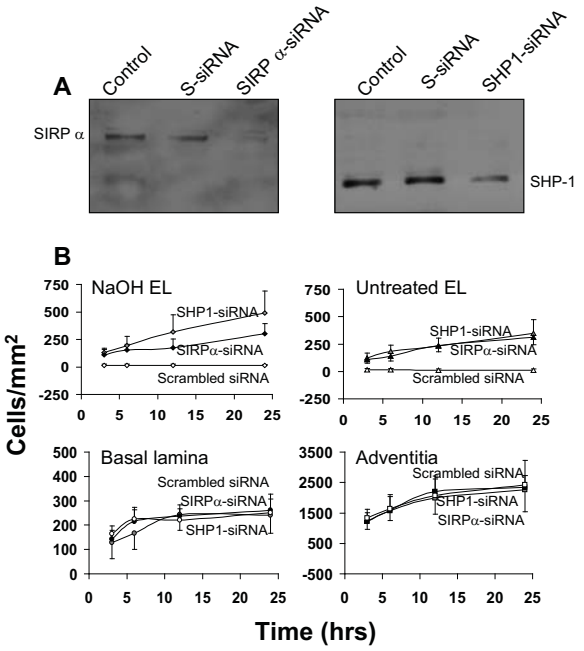

Figure 8: Influence of SIRP α- and SHP-1-specific siRNAs on monocyte adhesion to elastic laminae. (**A**) Knockdown of the relative expression of SIRP α and SHP-1 by transfection with SIRP α- and SHP-1-specific siRNA, respectively. Two tests were conducted for each siRNA treatment. Control: cells without siRNA. S-siRNA: scrambled siRNA. (**B**) Measurements of monocyte density on NaOH-treated and untreated elastic lamina, basal lamina, and adventitia in the presence of scrambled siRNA and SIRP α- and SHP-1-specific siRNA. The transfection with a SIRP α- or SHP-1-specific siRNA induced a significant increase in monocyte adhesion to NaOH-treated and untreated elastic lamina at all observations times ($p < 0.001$). In contrast, such a treatment did not induce significant changes in monocyte adhesion to basal lamina and adventitia ($p > 0.05$ for all observation times). Means and standard deviations are presented (n = 5 for each group). EL: elastic lamina. From ref. 4 with permission.

monocyte adhesion to the basal lamina and adventitia (Fig. 8B). Similar results were observed for the transfection with SHP-1-specific siRNA. These observations suggest that SIRP α and SHP-1 serve as potential mediators for the inhibitory effect of elastic laminae on monocyte adhesion.

To test whether components from elastic laminae bind to SIRP α, we prepared elastic lamina degradation peptides and examined co-immunoprecipitation of elastic lamina degradation peptides with SIRP α [4]. As shown in Fig. 9A, in monocytes reacted with elastic lamina degradation peptides (10 μg/ml), SIRP α could be co-immunoprecipitated with elastic lamina degradation peptides by using an anti-elastin antibody, which was shown to react with elastin peptides in an immunoblotting analysis. In a flow cytometry test, a treatment with an anti-SIRP α antibody (5 and 10 μg/ml), developed with the extracellular domain of SIRP α as an antigen, competitively reduced the binding of fluorescein-conjugated elastic lamina degradation peptides (10 μg/ml) to monocytes (Fig. 9B). The relative fluorescent intensity at the peak distribution of the tested monocytes, a relative index for the level of ligand binding, was reduced by 50 +/- 15% and 63 +/- 12% in the presence of 5 and 10 μg/ml anti-SIRP αgntibody, respectively (n = 3). In contrast, an anti-CD11b antibody did not apparently influence the binding of the elastic lamina degradation peptides. These observations suggest that elastic lamina degradation peptides can bind to SIRP α in monocytes.

To test whether elastic lamina degradation peptides influence the activity of SIRP α and SHP-1, we examined the relative phosphorylation of SIRP α and SHP-1 in the presence of elastic lamina degradation peptides [4]. As shown in Fig. 10, a treatment with elastic lamina degradation peptides (10 μg/ml) induced an increase in the relative level of SIRP α phosphorylation, and heavily phosphorylated SIRP α was associated with increased co-immunoprecipitation with SHP-1 in monocytes, suggesting that SIRP α phosphorylation enhanced SHP-1 recruitment. The recruitment of SHP-1 was associated with an apparent increase in the relative level of SHP-1 phosphorylation in the presence of elastic lamina degradation peptides. These observations suggest that elastic lamina degradation peptides exert an activating effect on SIRP α and SHP-1, which is similar to that of elastic laminae.

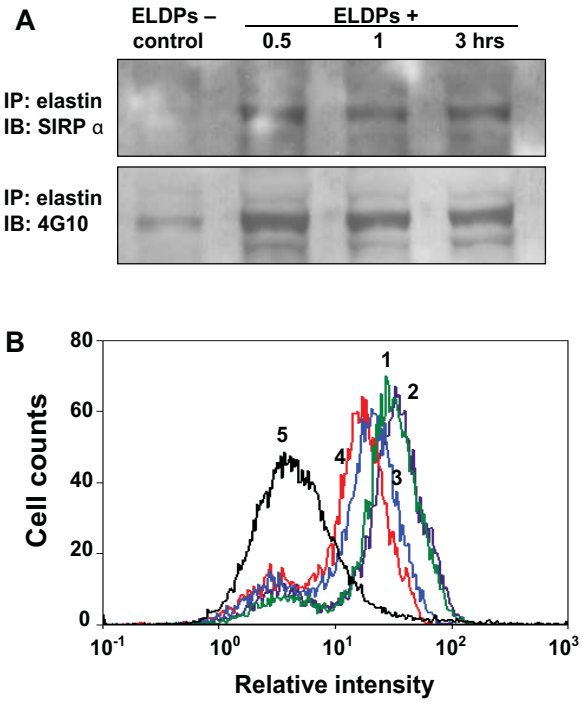

Figure 9: Binding of elastic lamina degradation peptides (ELDPs) to SIRP α. (**A**) Co-immunoprecipitation of ELDPs with SIRP α and phosphorylation of SIRP α. Two tests were conducted. IP: immunoprecipitation. IB: immunoblotting. (**B**) Influence of anti-SIRP α antibody on the binding of FITC-conjugated ELDPs to SIRP α. Curve 1: FITC-conjugated ELDPs 10 μg/ml. Curve 2: FITC-conjugated ELDPs 10 μg/ml with anti-CD11b antibody 10 μg/ml. Curve 3 and 4: FITC-conjugated ELDPs 10 μg/ml with anti-SIRP α antibody 5 and 10 μg/ml, respectively. Curve 5: control with an unrelated FITC-conjugated secondary antibody 10 μg/ml. Three cytometry tests were conducted. From ref. 4 with permission.

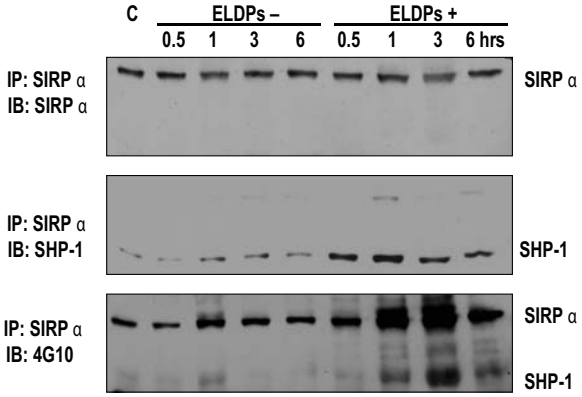

Figure 10: Co-immunoprecipitation and relative phosphorylation of SIRP α and SHP-1 in the presence of elastic lamina degradation peptides (ELDPs). Two tests were conducted. C: control monocytes without treatment with ELDPs. IB: immunoblotting. IP: immunoprecipitation. From ref. 4 with permission.

In summary, the present observations suggest that, compared with collagen-dominant matrix, arterial elastic laminae are resistant to monocyte adhesion. Such an effect is potentially mediated by the inhibitory receptor SIRP α and SHP-1. The interaction of elastic laminae with monocytes may activate SIRP α- and SHP-1-related signaling pathways that potentially suppress pro-adhesion mechanisms. The inhibitory effect of elastic laminae may potentially counterbalance the stimulatory effect of collagen matrix, contributing to coordinated regulation of inflammatory activities in the wall of arteries. The inhibitory feature renders elastic lamina a potential blood-contacting material for arterial reconstruction.

4 Elastic lamina-mediated inhibition of smooth muscle cell proliferation and migration

Smooth muscle cells reside within an elastic lamina-rich matrix in the arterial media. These cells are confined within the arterial media without a significant proliferative activity under physiological conditions. However, in the mouse model of elastin gene knockout, which is associated with impaired development of elastic fibers and laminae, SMCs exhibit a significantly increased proliferative activity in association with enhanced migration, resulting in profound intimal hyperplasia and arterial stenosis [15]. Similarly, incomplete development of arterial elastic laminae in human genetic disorders, such as Williams syndrome and supravalvular aortic stenosis, is associated with similar pathological changes in large arteries [18, 58, 59]. These observations

suggest a role for elastic laminae in inhibiting SMC proliferative activities.

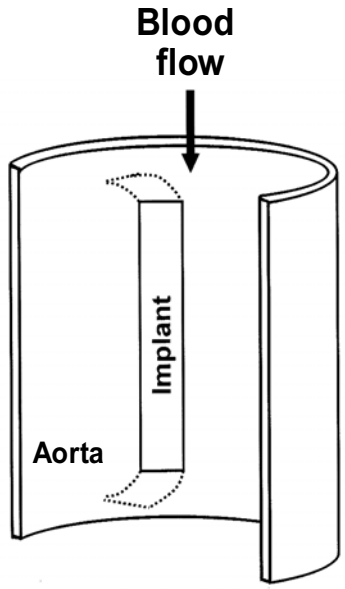

Figure 11: Schematic representation showing an aortic matrix strip implanted in the rat aorta. Three strips with distinct matrix surfaces, including the elastic lamina, basal lamina, and adventitia, were implanted in each host aorta. Arrow: blood flow direction. From ref. 3 with permission.

We have used a model of arterial matrix scaffold implantation to assess the role of elastic laminae in regulating SMC proliferative activities and intimal hyperplasia, a pathological process involving SMC migration into the arterial intima [3]. In this model, matrix scaffolds were constructed from rat aortic specimens with various surface matrix components, including elastic lamina, basal lamina, and adventitial collagen matrix. The matrix scaffolds were implanted into the aorta of the recipient rat as shown in Fig. 11 and collected from the rat for pathological tests at day 5, 10, and 20. As shown in Fig. 12, the neointimal layer on the elastic lamina surface was significantly thinner than that on the basal lamina and adventitia. To further test the difference between the elastic laminae and collagen matrix, we implanted a matrix scaffold with two different surfaces: one side with elastic lamina and the other side with adventitial collagen matrix. As shown

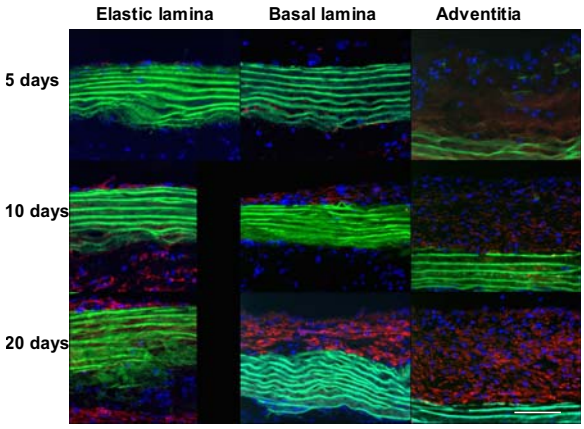

Figure 12: Fluorescent micrographs showing antibody-labeled SMC α actin (red in color) in implanted aortic matrix scaffolds with various surfaces, including the elastic lamina, basal lamina, and adventitia. Green: elastic laminae. Blue: cell nuclei. Arrow: Lumen surface. Scale: 100 μm. From ref. 3 with permission.

in Fig. 13A, the neointima on the elastic lamina surface was considerably thinner than that on the collagen matrix surface of the same matrix implant. When a matrix scaffold with an elastic lamina surface at both sides was implanted, the neointimal thickness was comparable at both sides (Fig. 13B). A BrdU assay demonstrated that the elastic lamina surface was associated with lower BrdU-incorporation than the basal lamina and adventitial surfaces at day 10, while that on the basal lamina was not significantly different from that on the adventitia (Fig. 14). Moreover, whereas a high density of SM α actin-positive cells appeared in the collagen-rich adventitia, few such cells were found in the elastic lamina-rich media (Fig. 12). These observations suggest that elastic laminae exert a negative effect on SMC proliferative activities and intimal hyperplasia compared to collagen-containing matrix. Such an inhibitory feature renders elastic lamina a potential material for constructing the blood-contacting surface of engineered blood vessels.

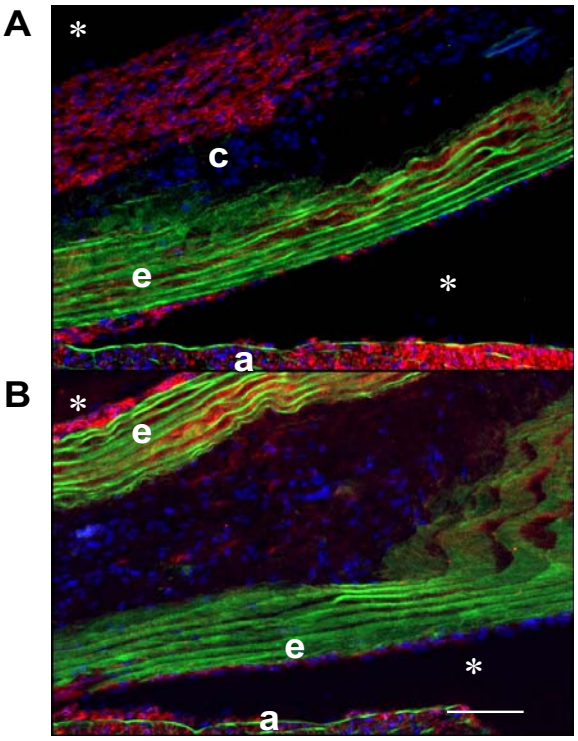

Figure 13: Fluorescent micrographs showing the influence of elastic laminae and adventitial collagen matrix on the formation of neointima. In panel A, a matrix scaffold was implanted into the rat aorta with an elastic lamina surface at one side and an adventitial collagen surface at the other. At day 20, the SMC-containing neointima (red in color) on the collagen surface (labeled c) was significantly thicker than that on the elastic lamina (labeled e) in the same implant. In panel B, a matrix scaffold was folded along the axial direction at the adventitial side, generating a scaffold with an elastic lamina surface on both sides. The thickness of neointima was comparable at both sides. The letter "a" represents the wall of the host aorta. Red: SMC αgctin. Green: elastic laminae. Blue: cell nuclei. *: lumen. Scale bar: 100 μm. From ref. 3 with permission.

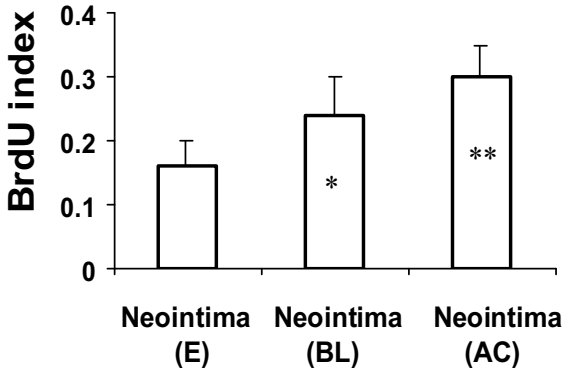

Figure 14: Cell proliferation, detected by BrdU incorporation, in the neointima of matrix implants with different surfaces, including elastic lamina (E), basal lamina (BL), and adventitial collagen matrix (AC) at day 10. BrdU index was defined as the ratio of BrdU-positive cells to the total cells. Means and standard deviation are presented. * and **: $p < 0.05$ and 0.01, respectively, for comparisons between the elastic lamina surface and basal lamina surface and between the elastic lamina surface and collagen surface (n = 5 each time). From ref. 3 with permission.

5 Concluding remarks

Arterial elastic laminae have long been considered an inert matrix structure that contributes to the stability, elasticity, and strength of the arterial wall. However, recent studies have demonstrated that elastic laminae can serve as a signaling element and participate in the regulation of vascular cell activities. In particular, elastic laminae regulate the formation of SM α-actin filaments in bone marrow-derived CD34+ cells. Such an observation provides insights into the mechanisms of vascular SMC development. Furthermore, elastin, the major component of elastic laminae, exerts an inhibitory effect on leukocyte adhesion, and SMC proliferation and migration. These observations suggest that elastic laminae serve as a matrix component that prevents arteries from inflammatory responses and confine SMCs within the arterial media under physiological conditions. Structural alterations and malfunction of elastic laminae may result in or enhance vascular disorders such as intimal hyperplasia and atherosclerosis.

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