— **Review** —

Role of Elastic Fibers on Cardiovascular Disease

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An important factor in the transition from an open to a closed circulatory system was a change in vessel wall that are dynamic structure composed of cells and extracellular matrix. The component of arterial wall in vertebrates that accounts for these properties is the elastic fiber network organized by medial smooth muscle cells. Elastin and elastin associated protein are synthesized and secreted by vascular smooth muscle cells and are the major extracellular matrix component deposited in the vascular wall. Pathological states related to hypertension or atherosclerosis is associated with vascular wall remodeling, which is deleterious for cardiovascular function. Elastic fiber may be key factors in the pathophysiology of hypertensive or atherosclerotic vascular remodeling. The well-known effects of cardiovascular disease on the deterioration and the promoted degradation of elastic fiber result to loss of arterial wall resilience. Recently, several studies have highlighted new roles for individual components of elastic fiber and their degraded products. This review describes current knowledge regarding components of elastic fibers and discusses relationship between their structural abnormalities and cardiovascular diseases.

Key words —— elastin, microfibrils, supravalvular aortic stenosis, elastic fiber assembly

INTRODUCTION

The extracellular matrix (ECM) plays a critical role in the development, growth and biomechanical properties of virtually every organ system. Elastin is the dominant extracellular matrix protein deposited in the arterial wall and can contribute up to 50% of its dry weight.[1)] The protein product of the elastin gene is synthesized by vascular smooth muscle cells and secreted as a tropoelastin monomer, that is soluble, non-glycosylated and highly hydrophobic. After posttranslational modifications, tropoelastin is crosslinked and organized into elastin polymers that form concentric rings of elastic lamellae around the medial layer of arteries. In arteries, elastin dictates tissue mechanics at low strains before stiffer collagen fibers are engaged.[2)] Elastin also confers elasticity, preventing dynamic tissue creep by stretching under load and recoiling to their original configurations after the load is released. In addition to the mechanical responsiveness, elastin is a potent autocrine regulator of vascular smooth muscle cells activity and this regulation is important for preventing fibrocellular pathology. The present review focuses on role of elastic fibers that affect the vascular functions.

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BIOLOGY OF ELASTIN

The heart and arteries are always active mechanically and seem to wear out more than any other system or organs. The potential energy accumulated in the stretching of the vessel wall during contraction of the heart (systole) is dissipated in elastic recoil of the wall during the period when the heart is inactive (diastole). This release of tension in the wall serves as an auxiliary pump, forcing the blood forward during diastole. Thus, near the heart the flow of blood is intermittent and it is elasticity of the wall. The wall of the large conducting vessels makes it possible to have a continuous flow with an intermittent pump. The normal human artery wall comprises an endothelial cell with a few underling smooth muscle cells (SMCs) which together make up tunica intima. The underlying tunica media contains SMCs and their associated matrix only and is sep-

arated from the intima by the internal elastic lamina and from the surrounding adventitia which contains fibroblasts, small feeding blood vessels, and adventitial nerves, by the external elastic lamina. In all blood vessels the components of connective tissue possess important structure and biomechanical functions. The lamellae of elastin are important in providing the elastomeric properties of the larger arteries and in the human aortic elastin represent 20–50% of the total dry weight.

Elastin is the main component of elastic fiber, which provides resilience and elasticity to many tissues such as skin, lungs, ligaments, and arterial walls. Elastin fibers consist of two distinct components: elastin and microfibrils, whose components include fibrillin, microfibril-associated glycoprotein.[3–5] Elastin is secreted from cells as a soluble protein of approximately 70-kDa that undergoes a complex assembly process requiring the coordinate expression of microfibrillar proteins. Major types of domains are found in tropoelastin: hydrophobic domains rich in glycine (Gly), valine (Val), proline (Pro) and alanine (Ala); hydrophilic domains typical rich in lysine (Lys) and Ala involved in cross-linking.[6] The C-terminus of tropoelastin is highly basic amino acid residue (RKRK) and contains the only two cysteine (Cys) residues.[7, 8] The human single elastin gene has been localized to chromosome 7q11.1–21.1, spanning 45 kb.[9] The human gene has 34 exons with an intron: exon ratio of 20 : 1, indicating that relatively small exons are interspersed within large intron. Alu repeats are found in the human elastin gene at a frequency four times higher than elsewhere in the genome raising the possibility in this gene through recombination events in the general population[10, 11] and possibly contributing to diseases such as supravalvular aortic stenosis (SVAS). Some variation exists in the presence of exon homologues amongst species. For example, bovine exons 34 and 35 are absent from the human gene, while the human gene has the introduction of the unusual hydrophilic-encoding exon 26A, not described in any species.[10] Exon 36 is highly conserved amongst species and codes for the C-terminus in addition to a large 3-untranslated region suggesting the possibility of regulatory elements in this region. The decrease in elastin synthesis that occurs with age has been shown to result partly from the destabilization of mRNA.

The primary transcript of tropoelastin undergoes extensive alternative splicing resulting in the translation of multiple heterogeneous protein isoforms.[12] The alternative splicing of the elastin gene occurs in a cassette-like fashion in which an exon is either included or deleted in most cases. At least 11 human tropoelastin splice variants have been identified with six exons shown to be subject to alternative splicing; exons 22, 23, 24, 26A, and 32.[5, 10, 13] It has been reported that elastin gene exon 26A is always spliced out and that exon 32 is highly spliced out in human skin fibroblasts.[14, 15] Elastin gene exon 26A is a human-specific sequence and is rich in charge and polar amino acids. On the other hand, elastin gene exon 32 is a hydrophobic domain and is known to be a frameshift mutated exon in patients with cutis laxa (CL).[16] Although the heterogeneity of tropoelastin may play an important role in physiological functions, little is known about the elastic fiber formation of exon 26A or exon 32 missing tropoelastin.

ELASTIN SYNTHESIS[17]

Elastin expression in cultured SMC was enhanced by potent inhibitors of SMC proliferation including retinoic acid[18] and heparin.[19] While, elastin expression was reduced by potent stimulators of SMC proliferation like EGF,[20] angiotensin II[21] and high K concentration.[22] These results suggest that elastin expression and SMC proliferation are coupled tightly and inversely: potent stimulators of cell proliferation may potentially inhibit elastin expression and potent inhibitors for cell proliferation can stimulate elastin expression. Nitric oxide (NO), an endothelium-dependent relaxing factor, is continuously produced NO synthetase in endothelium. Endothelial dysfunction causes decreased NO release, resulting in a shared process involving the pathogenesis of atherosclerosis and neointima formation after angioplasty. We demonstrated that NO inhibited SMC proliferation, while NO enhanced expression of elastin.[23] On the other hand, endothelin-1, a potent vasoconstrictor, has been isolated from cultured porcine aortic endothelial cells. Endothelin-1 is known as a potent stimulator of cell proliferation and development of arterial diseases such as atherosclerosis. We demonstrated that endothelin-1 stimulated cell proliferation and endothelin-1 suppressed the elastin expression such as EGF, angiotensin II. The inhibition of elastin synthesis was completely prevented by an endothelin receptor antagonist. These results indicate that

endothelin-1 can modulate the elastin mRNA expression via an endothelin A receptor and that the regulator for elastin expression may play an important role in elastogenesis and SMC proliferation during the development of atherosclerosis.[24)]

ELASTIC FIBER FORMATION

Elastin is secreted as a soluble protein of approximately 70 kDa referred to as tropoelastin, which has alternating hydrophobic and cross-linking domains. In the extracellular space, secreted tropoelastin molecules interact with 10–12 nm microfibrils, which are thought to facilitate cross-linking by aligning cross-linking domains between individual tropoelastin molecules. Previous studies have demonstrated a direct interaction between tropoelastin and microfibrils,[25–27)] and domain-mapping studies have localized the microfibril-binding domain to the carboxy-terminal region of tropoelastin.[28–30)] A member of the lysyl oxidase family of amine oxidases catalyzes the cross-linking reaction through oxidative deamination of the epsilon-amino group of specific Lys side chains with tropoelastin. There is substantial evidence that the hydrophobic domains are necessary for the self-aggregation of tropoelastin via coacervation, which is thought to concentrate and align tropoelastin molecules for cross-linking.[31)] Hydrophobic domains of tropoelastin are rich in amino acids such as Gly, Pro, Val, and leucine (Leu), which are present in a variety of tandem repeat sequences. The tandem repeats; *i.e.*, VGVAPG, GGLG(V/A) that resemble sequences found in other proteins to aggregate via β-sheet/β-turn structures,[32, 33)] contribute to the polymerization of elastin.[28, 34)] Recently, it has been reported that exon 16[35)] and 30,[28)] which encodes hydrophobic domain containing a tandem repeat sequence GGLG(V/A) is an important sequence for the assembly process. Our recently data showed that conditions favoring tropoelastin coacervation improved fibulin-5 binding.[36)] Fibrillin-1 has recently been reported to promote coacervation.[37)] Because fibulin-5 interacts with both fibrillin-1 and tropoelastins,[38, 39)] a fibulin-5–fibrillin-1 complex might cooperatively promote coacervation. Moreover, we reported that whole molecule of tropoelastin is necessary for the formation of cross-linking amino acids such as desmosine.[40)] Based on these observations, we hypothesize that conformational changes associated with coacervation of tropoelastin are necessary for fibulin-5 binding to tropoelastin. This hypothesis would predict that tropoelastin-fibulin-5 binding and tropoelastin coacervation are coupled molecular processes and that fibulin-5 binding facilitates the coacervation of tropoelastin and thereby enhances elastic fiber formation. This is exactly what our experiments demonstrated. Addition of fibulin-5 to tropoelastin indeed lowered its coacervation temperature consistent with enhanced coacervation of tropoelastin. It has been also reported that fibulin-5 interacts with lysyl oxidase like 1.[41)] Taken together, molecular interaction between tropoelastin and fibulin-5 may play a more positive role in the alignment and maturation of tropoelastin.

GENETIC DISORDER OF ELASTIC FIBERS AND VASCULAR DISEASE

Elastogenesis is a very complex process involving multiple hierarchical steps. Therefore, mutations in the genes controlling elastic fiber deposition might also participate in the development of cardiovascular diseases in general and hypertension in particular. The knowledge of the genes controlling elastogenesis has expanded in recent years, thanks to the studies of human genetic disorders of the elastic fiber and recent advances in targeted deletions of genes involved in elastogenesis in murine models. The fact that most of these mutations have been linked to abnormal vascular structure and, in some cases, with the development of hypertension, have raised the possibility to explore polymorphisms of these genes in relation to human essential hypertension.[42–44)] The elastin gene at least 3 clinical conditions have been linked to mutation or deletion of the elastin gene: autosomal dominant CL, SVAS, and Williams syndrome (WS). CL is primarily a cutaneous condition,[16, 45, 46)] while WS and SVAS are both disorders linked to alterations in the cardiovascular system.[46)] SVAS, an inherited obstructive arterial disease, may occur as an isolated condition[47)] or as part of the complex developmental syndrome, WS.[48)] Isolated SVAS is caused by a large spectrum of mutations including point mutations, translocations, and partial deletions within the elastin gene.[49–51)] Hypertension is present in a large proportion of SVAS and WS patients usually from early age.[52–54)] Development of elastin-null (ELN $-/-$)[55)] and hemizygous (ELN $+/-$) mice[44)] has reinforced the possible link between defective elastin

synthesis and hypertension. Elastin-null mice die of obstructive arterial disease due to SMC proliferation and reorganization in the absence of endothelial damage, thrombosis, or inflammation.[55] The arteries of ELN +/− mice exhibited several similarities with the human syndromes linked to elastin gene defects, such as thinner lamellae and increased number of lamellar units and are hypertensive from an early age.[44] When subjected to high pressures, large vessels from ELN +/− mice are stiffer than those from control animals, similar to the alterations observed in human essential hypertension.[56, 57] The vascular alterations in resistance arteries from ELN +/− mice have not been explored. The role of elastin in the maintenance of resistance artery structure and mechanics that we have recently reported[58] suggests the possibility that small artery narrowing might also contribute to the development of hypertension in these mice.

Mutations in the genes coding for the microfibrillar proteins, fibrillin-1 and fibrillin-2 have been linked to Marfan syndrome.[59−61] The major source of morbidity and mortality in this syndrome are the cardiovascular abnormalities involving aortic dilatation and dissection, which indicates the importance of fibrillins to vessel wall structure. There are some reports showing an association between aneurismal disease, systolic blood pressure, and polymorphic variation in the fibrillin-1 gene.[62, 63] A more recent report suggests an important role for fibrillin-1 genotype in cardiovascular risk associated with large-artery stiffening and pulse pressure elevation in individuals with coronary disease.[64] Therefore, the contribution of fibrillin-1 to the pathogenesis of vascular defects in human hypertension is a possibility that deserves further investigation. Fibrillin-deficient mouse models have also been developed. These studies have demonstrated that the absence of normal levels of fibrillin-1[65] and fibrillin-2[66] is incompatible with the proper function of mature elastic fibres. Fibrillin-1 deficient mice develop cardiovascular abnormalities similar to those found in the Marfan syndrome, such as aneurisms.[65] As in the elastin deficient murine model, SMC from fibrillin-1 deficient mice also show a change from quiescent contractile state to synthetic proliferative phenotype. This signaling pathway may be the basis of the fragility of the vascular wall that accompanies Marfan syndrome, as this phenotypic change is associated with a subsequent release of matrix metalloproteinases (MMPs) that degrade the vascular wall.[67]

The fibulins are a family of seven ECM proteins that contain EGF-like modules and a distinctive C-terminal domain.[68−70] Fibulin-5 [also known as developmental arteries and neural crest EGF-like (DANCE)] is an ECM protein expressed in large vessels during embryogenesis as well as in adult life. Inactivation of the fibulin-5 gene in mice results in a generalized elastinopathy affecting those organs with abundant elastic fibers, such as skin, lung and blood vessels. The aorta of fibulin-5 deficient mice presents abnormal morphology and altered elastic lamella.[38, 71] In addition, it has been reported that loss of compliance in fibulin-5 −/− aorta is similar to those observed in aged human arteries.[57, 72] However, the fact that abnormal elastin organization was found soon after birth, suggest an underlying developmental defect rather than an ageing process.[38] An interesting finding is that elastic fiber alterations were accompanied by a significant increase in systolic blood pressure and pulse pressure. These results indicate that the pulse dampening effect of the elastic arteries was disturbed owing to loss of elasticity in the aortic wall in mutant mice.[38] Fibulin-5 also seem to be an important inhibitor of SMC proliferation and migration, as demonstrated by the exaggerated remodeling of the carotid artery upon vessel ligation in fibulin-5 −/− mice compared to fibulin-5 +/+ controls.[73]

Decreased lysyl oxidase (LOX) levels have been detected in human hypertension.[74] Therefore, it could be argued that defective cross-linking of elastin due to alteration in LOX might cause a decline in the resilience of arterial wall and participate in the development of hypertension. However, all 5 LOX isoenzymes are likely to use both elastin and collagens as their substrates and a decrease in LOX would also reduce tensile strength of the arterial wall. In fact, mice with inactivation of the LOX gene (LOX −/−) develop aortic aneurisms and perinatal death.[75] The lethality associated with the lack of only 1 isoenzyme, was unexpected. It is possible that LOX deficiency cannot be compensated for by other isoenzymes in terms of its contribution to the development and maintenance of the structure and mechanical properties of the aortic wall. These findings suggest that alterations in LOX activity may also play a critical role in human cardiovascular diseases, but its implication in the development of vascular structural abnormalities in hypertension remains to be determined.

ABNORMAL ELASTIC FIBER AND VASCULAR DISEASE

Atherosclerosis is associated with local accumulation of lipid and calcium in the atherosclerotic plaque. It has suggested that one of the mechanism involved in the deposition of lipids in the elastin may be an interaction of the elastin protein with serum low density lipoprotein (LDL) resulting in a transfer of cholesterol ester to the elastin.[76] Moreover, reduction of LDL is able to result from an impairment in the binding site involved in the direct chemical binding of lipids to the elastin protein molecule.[77] Previous studies indicated that local lipid deposition in the atherosclerotic plaque is due to alteration of the elastin fraction, both quantitatively and qualitatively[78] and close association with elastin modulates the response of SMCs to hyperlipidemia and suggest a role for elastin in the formation of foam cells of smooth muscle origin from atherosclerosis.[79] These findings suggest that functional defense action of elastin against cholesterol synthesis and SMC migration was disturbed by accumulation of lipid on elastic fiber and the deposition on elastic fiber induced change of elasticity or physiological function in the arterial wall.

On the other hand, vascular calcification occurs with increasing age and in association with a diverse range of diseases, including atherosclerosis, diabetes, and uremia. Additionally, vascular calcification is now known to be present in 80% of significant lesions and in at least 90% of patients with coronary artery disease. The value of pulse wave velocity (PWV), indicate of vascular elasticity, is positively associated with ageing, calcification and degree of atherosclerosis. Therefore, PWV was used clinical diagnosis in Japan and these phenomena may be suggested that calcification or calcium deposition cause to inhibit elastin synthesis. Our previous data showed that the decreased contents of the cross-links (desmosine) in the experimental atherosclerosis relates to down-regulation of lysyl oxidase and elastin maturation during arteriosclerosis with calcification.[80]

In atherosclerosis, abnormal cholesterol and calcium deposition in aortic elastin fiber was associated with alterations in the amino acid composition of elastin and cross-links. Recent studies suggest that arterial and vascular calcification occurs through highly regulated molecular processes characterized by expression of osteogenic proteins and matrix-degrading proteinases. There are reports that proteolutic enzymes expressed by activated macrophages and myofibroblast-like cells in atherosclerotic plaque progression and aortic valve disease. During atherogenesis, macrophage derived proteinase such as elastolytic cathepsins, elastase, or matrix metalloproteinase (MMP-2, -9, and -12) promote the degradation of elastic fiber, which favors calcification through an increase of elastin polarity that in turn enhances elastin affinity for calcium. Cardiovascular calcification commonly causes devastating complications, including plaque rupture and aortic valve stenosis, which currently have no suitable therapeutic alternative beyond valve replacement.

FUTURE PERSPECTIVES

The present review has highlighted some common features between patients with cardiovascular diseases and abnormal elastic fibers. Both show similar alterations in vascular wall architecture and mechanical performance also seems to be a common characteristic. These findings, together with the fact that intact elastic fibers are essential for the normal functioning of vascular mechanics, suggest the possibility that elastic fiber abnormalities during the fetal and early postnatal life might play a pivotal role in the development of cardiovascular diseases. The most consistent and well-reported changes are luminal enlargement with arterial wall thickening (remodeling) and a reduction of elastic properties (stiffening) at the level of large elastic arteries. The understanding of elastogenesis and prevention of elastic fiber degradation would be very important from the therapeutic point of view for cardiovascular diseases. For example, the effect of current antihypertensive treatments on elastic fiber deposition and metabolism needs attention. Furthermore, knowledge gained on the association between elastic fibers and cardiovascular diseases would allow for the development of new therapeutic strategies. Finally, in the light of the influence that minerals, vitamins, and dietary supplements exert on elastogenesis, the role of diet during pregnancy and in early infancy on elastic fiber deposition and vascular remodeling deserves to examined, especially since it implies the possibility of prevention of cardiovascular diseases.

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