Review

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Elastin peptides in aging and pathological conditions

Abstract: Elastin is the protein responsible for the resilience of vertebrate tissue. It is an extremely stable protein deposited during the early stages of life and experiencing almost no renewal. As a consequence, it can be considered that each individual has an elastin capital for life. Despite its extreme stability, elastin can be degraded by several enzymes termed elastases. Elastases are among the most aggressive proteases, and their presence is increased with age. As a consequence, elastin fragmentation resulting in the generation of elastin peptides is one of the hallmarks of aging. This review will examine their nature and further expose our current understanding of the role played by these peptides in aging and their contribution to tissue homeostasis and several pathologies.

Keywords: aging; amyloid; elastin; elastin peptides; receptors.

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Introduction

The resilience of vertebrate tissues is due to the presence of elastic fibers in their extracellular space (1). Elastic fibers are macromolecular assemblies whose main component is the elastin protein. The principal role of elastin is to provide resilience to tissues subject to repetitive distension and physical stress. As a consequence, elastic fibers, and thus elastin, are mostly found in tissues such as the arteries, lungs, ligaments, bladder, and skin (2). The general architecture of the elastic network depends on the specific tissue and the physical constraints it experiences. For instance, in the vessel wall, the elastin polymer forms concentric cylinders, called elastic lamellae, regularly arranged around the lumen of the vessel (3). The presence of these lamellae is essential for maintaining the strength, resilience, and structural integrity of the vascular wall. Indeed, *eln* knockout mice die rapidly after birth due to cardiovascular lesions (4).

Elastin synthesis (elastogenesis) is a highly regulated process beginning during gestation (5). In most tissues, elastin production begins around the time of mid-gestation and peaks near birth and during the early neonatal period. It then drops dramatically and is nearly totally repressed by maturity (2).

The first step of elastogenesis is the deposition of fibrillin-rich microfibrils in the pericellular space (5).

These structures serve as a molecular scaffold in which elastin will be deposited to form a mature elastic fiber (6). Besides the microfibrillar component (6, 7), the proper secretion and alignment of tropoelastin molecules and their correct assembly to form elastin necessitates the coordinated interplay of several key actors. In the reticulum and secretory pathways, newly produced tropoelastin molecules are associated with the elastin-binding protein (EBP) of the elastin receptor, allowing their delivery at the cell surface (8). There, following the desialylation of microfibrillar glycoconjugates, galactosyl moieties are exposed and interact with EBP (9), releasing tropoelastin at the cell surface where they interact with proteoglycans (10, 11) and form small aggregates associated with fibulin-4 and fibulin-5 (12, 13), which are thought to control tropoelastin aggregation. For instance, fibulin-5 can interact with both fibrillin and tropoelastin, and tethers the growing elastic fiber to the cell surface (14). When the aggregate reaches the appropriate size, it interacts with the microfibrillar component so that tropoelastin molecules can be aligned properly (15) to the nascent elastin fiber before the enzymatic activation of their lysyl residues by lysyl oxidase and lysyl oxidase-like proteins and the formation of elastin lysine-derived cross-links (16, 17). For a detailed review on elastogenesis, the reader is referred to refs. (5, 18).

Mature elastin is the major component of the elastic fiber. It is an insoluble, amorphous, hydrophobic, and extensively cross-linked polymer of tropoelastin molecules covalently bound to each other by bi- (lysinonorleucine), tri- (merodesmosine), and tetra-functional (desmosine and isodesmosine) cross-links (18). The nature of elastin chains and its high reticulation make elastin a remarkably stable molecule having a longevity comparable to the human lifespan (19).

Elastic fibers are thus long-lived structures. Their extreme longevity is explained both by the intrinsic resistance of elastin and by the presence of elastic fiber-associated proteins. For instance, fibrillins contribute to the mechanical behavior of elastic tissues by mediating elastic fiber tensile strength (20) and regulate tissue homeostasis by controlling transforming growth factor-β sequestration (21, 22).

The inevitable fragmentation of elastic fibers

Alteration of elastic fiber biology is one of the hallmarks of aging (23). Aged individuals experience slow and progressive alterations of the elastic functions of their organs, notably in the cardiovascular and respiratory systems. On the ground of these observations, Robert and colleagues (23) suggested that life expectancy could somehow be limited by elastin aging.

Age-related elastic fiber alterations are explained by the fixation of lipids, calcium, and further proteolytic degradation of elastic fibers. In response to these alterations, the elastic tissue adapts mechanically and biologically, resulting in important physiological changes. For instance, in the cardiovascular system, the vascular wall stiffens and blood pressure increases, resulting in higher heart fatigue (24). Thus, reduced tissue elasticity due to a compromised elastic fiber function becomes increasingly prevalent with age and contributes significantly to the burden of human morbidity and mortality (25).

Therefore, although considerably resistant, elastin degrades with age and the elastin stock of the individual progressively decreases. Elastic fiber degradation is due to the increased presence of elastase activities with age (26). For instance, matrix metalloproteinase-2 (MMP-2) activity is increasingly present in aged aortas (27).

Elastases are a class of enzymes known for their aggressiveness and their peculiar ability to degrade elastin, one of the most long-lived molecules of the body (19). They can be serine proteinases, cysteine proteinases, or MMPs. They are involved in a wide range of physiopathological processes involving matrix remodeling. Current data suggest that their activity is progressively augmented with age, notably in arterial walls (26).

The loss of elastin in these tissues is accompanied by both an important modification of their mechanical properties as well as the local release of elastin degradation products, termed elastin peptides (EPs). Usually, the organism compensates the loss of elastin by the synthesis of collagen, which further induces several complications. This point is well documented and the reader is referred to the work of Laurent and co-workers (28) for a comprehensive review on this topic. In this review, we will focus on the second, and often overlooked, consequence of elastin loss – the generation of EPs and the impact they have on tissue homeostasis and function.

EPs are generated following the action of elastases. Their concentration in the blood flow is usually low but can reach high values in aged subjects, notably in those developing pathologies where elastin is massively degraded (29). Unlike insoluble fibrous elastin, EPs can modulate the cellular physiology of numerous cells such as fibroblasts, smooth muscle cells, endothelial cells, monocytes/macrophages, and lymphocytes (30). They are sometimes termed elastokines, i.e., elastin-derived matrikines.

In this review, we will show that EPs define two distinct classes, bioactive peptides (elastokines) and amyloidogenic peptides. The nature, function, and physiopathological significance of these peptides will be discussed.

Structure of EPs

Elastokines

The observation that a mixture of elastin fragments, obtained by hydrolysis of human aorta and lung elastin with chemicals or by digestion with leukocyte elastase, showed biological activities fomented the research of potential active elastin fragments. Several EPs showing biological activity such as chemotaxis (31), vasorelaxation (32, 33), and platelet antiaggregation were identified. On this basis, several studies aimed at elucidating the structure-function relations of the active EPs were performed. Table 1 provides a detailed list of the most prominent elastokines together with their known biological activity.

Among the most studied peptides, particular attention was devoted to the hexapeptide VGVAPG. VGVAPG is located in the central region of the human tropoelastin polypeptide sequence, where it is repeated six times in the domain encoded by exon 24. This peptide was the first elastokine identified (31).

The conformational studies performed by circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies on VGVAPG in water have shown the presence of unordered and extended conformations in aqueous solutions. As a matter of fact, VGVAPG can adopt the extended left-handed helix poly-l-proline II (PPII) conformation while also being able to populate folded structures (34).

To better analyze the nature of VGVAPG folded conformations, the polypeptides were also studied in 2,2,2-trifluoroethanol (TFE). Although the use of TFE is not relevant from a biological point of view, its use is justified because this solvent could better mimic the potential receptor-binding site (35) and therefore stabilizes transient conformations such as the β-turn. These studies showed indeed that VGVAPG could adopt such conformations (36). Further, molecular dynamic simulations performed in explicit water (37) identified different clusters of structures distinguished in two main families: the more extended structures, corresponding to rather elongated conformations (Figure 1; conformers 8, 12, 14, and 16), and the more compact ones (Figure 1; conformers 2, 5, 7, 9, and 13). In particular, in the latter case, turn analysis showed the presence of an ideal type VIII β-turn, located along the GVAP residues, occurring at regular intervals of time, starting from 750 ps to the end of the simulation. Furthermore, a type IV β-turn occurred as a transient structure on VGVA, just preceding the formation of the turn along GVAP. This observation is consistent with the 'sliding β-turn' model proposed by Tamburro and coworkers (38) for elastin-derived polypeptide sequences, stating that the turns observed in elastin are rather labile and therefore can interconvert each other, giving rise to dynamic β-turns sliding along the chain that are responsible for the elasticity of the protein.

An extensive structure-biological activity study was carried out by Brassart and co-workers (39) who analyzed by CD in water the structure of all the possible permutations of VGVAPG (VGVAPG, GVAPGV, VAPGVG,

Sequence	Biological effect	Cell type	References
VGVAPG	Chemotaxis	Monocyte, fibroblast, endothelial cell	(31, 39, 71)
$(VGVAPG)_3$	Proliferation	Smooth muscle cell	(67)
	Protease synthesis	Endothelial cell, fibroblast, HT1080	(69, 71)
	Angiogenesis	Endothelial cell	(71)
PGAIPG	Chemotaxis	Fibroblast, neutrophil	(120)
	Protease synthesis	Fibroblast	(39)
AGVPGLGVG	Chemotaxis	Macrophage	(62)
AGVPGFGVG	Chemotaxis	Macrophage, fibroblast	(40, 62)
Hydrolysates	Chemotaxis	Monocyte, macrophage	(64, 96)
	Proliferation	Smooth muscle cell, lymphocyte, endothelial cell	(66, 67, 88)
	Cell survival	Fibroblast	(75)
	Angiogenesis	Endothelial cell	(69, 71)
	Protease production	Monocyte, neutrophil, lymphocyte, fibroblast	(39, 76, 77, 87, 88)
	Reactive oxygen species production	Monocyte	(76, 77)

Table 1 The most prominent elastokines and their associated biological activities in normal cells.

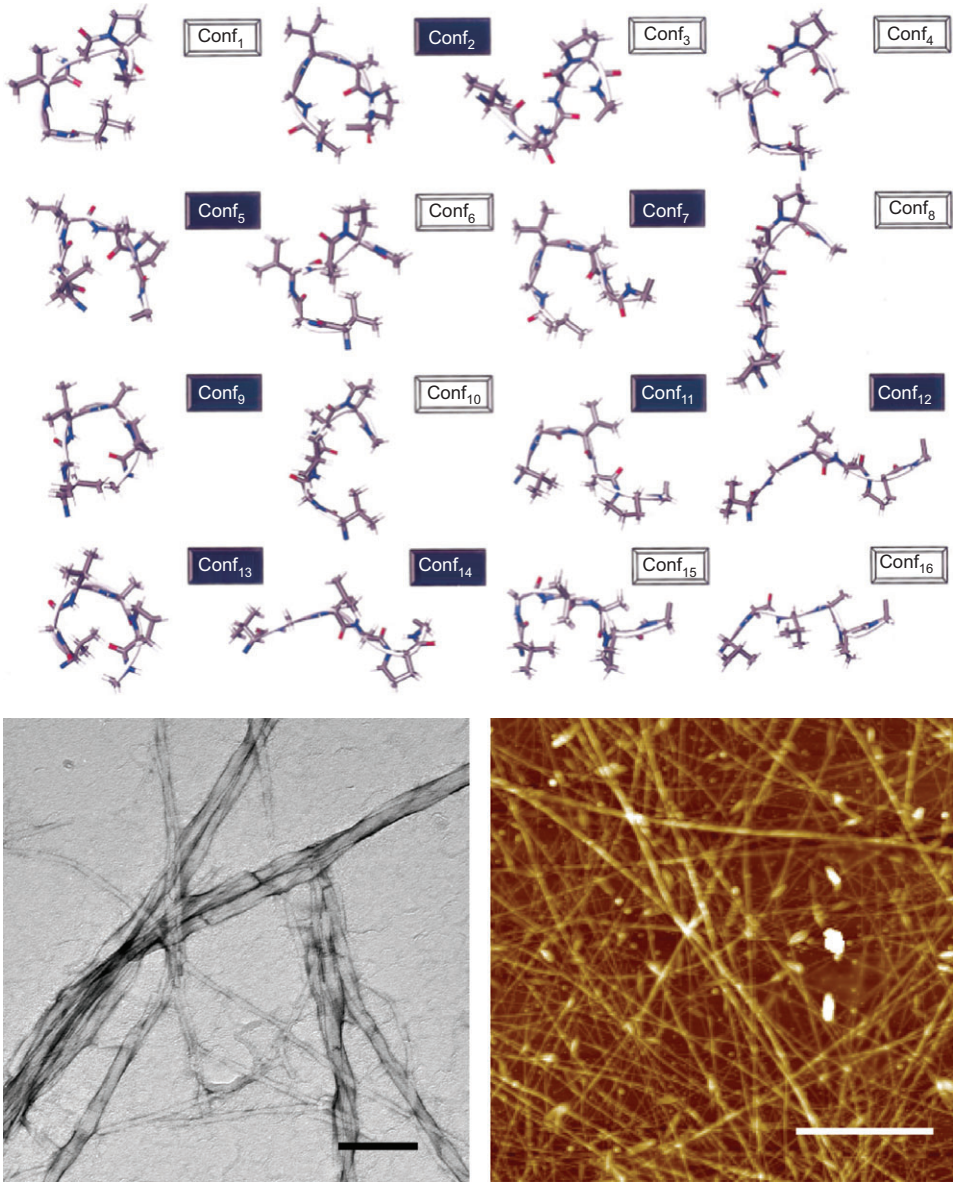

Figure 1 Structural diversity of elastokines and amyloidogenic elastin peptides.
Top image: representative structures of isolated VGVAPG obtained from molecular dynamic trajectories starting with fully extended conformations. Black labels stand for conformers presenting a prolyl residue in *trans* conformation, while the white ones have a *cis* conformation. [Reproduced from ref. (37) with permission]. Bottom images: supramolecular organization of amyloid-like fibrils of elastin-derived polypeptides. Left: TEM of EX30 fibrils (bar, 200 nm); right: AFM of EX32 fibrils. Bar, 2.5 μm. [Reproduced from ref. (119) with permission.]

APGVGV, PGVGVA, GVGVAP) as well as of two additional hexapeptides (LGTIPG, PGAIPG). Quite interestingly, only sequences harboring the GXXPG motif were able to upregulate pro-MMP-1 production in cultured human skin fibroblasts. The biological activity of these sequences was correlated to the possible presence of a type VIII β-turn at the GXXP sequence in all the active peptides (GVAPGV, VGVAPG, LGTIPG, PGAIPG). Because the GVGVAP sequence was inactive despite the presence of a GXXP motif, it was suggested that the presence of the glycine residue after the GXXP turn was a necessary condition for having a biological activity.

Besides the VGVAPG sequence, other chemotactic peptides were identified, such as GFGVG (40), PGAIPG (41), YGVG, and GLVPG (34). Furthermore, peptide sequences containing the XGXPG pattern (VGVPG, VGAPG) as well as GVAP, GGVPG were analyzed for potential chemotactic and chemokinetic activities (42). The conformational analysis of these peptides in aqueous solution revealed the absence of ordered conformations. However, in TFE,

a complex conformational picture comprising unordered together with folded structures was proposed. As a matter of fact, CD and NMR studies showed that GVAPG preferred unstructured conformations, while the other peptides may assume folded conformations in organic solvents. All these peptides but GGVPG showed chemotactic activity for monocytes. The chemotactic activity of VGVPG, VGAPG, and VGVAPG was inhibited by lactose, while the chemotaxis of peptide GVAPG was insensitive to lactose, suggesting the existence of different chemotactic receptors (36).

Another small but significant sequence among the elastin fragments is VVPQ, located in the domain encoded by exon 8 of human tropoelastin (43). This peptide, together with other peptides derived from thermolysin digestion of fibrous elastin, exhibits a mitogenic activity toward dermal fibroblasts *in vitro*. In water, its dominant conformation appears to be the unordered one. As expected, in TFE, spectroscopic data suggested the presence of more folded conformations. A model of VVPQ in TFE solution was obtained by simulated annealing structure calculations using NMR-derived constraints. The results indicated that an S-shaped folded conformation with an inverse γ-turn-like conformation in the V2-Q4 region was the most stable conformation for this peptide (43).

In summary, it should be emphasized that the conformational studies performed on EPs highlight, for the small peptides, a conformational picture very similar to those observed for longer ones containing them. As a consequence, the conformational space populated by elastokines in water is covering widely different structures, from the elongated structures (mainly PPII) to more folded conformations (β-turn) in rapid equilibrium between them. The main difference found is that the number of folded conformations (β- or γ-turns) that they could adopt is small, according to their reduced size, and this is probably the main reason for their specific activity.

Amyloidogenic EPs

The term amyloid originally referred to protein deposits accumulating into plaques *in vivo*. Only recently is it also used for intracellular aggregates and for fibrils deposited *in vitro*, even if the formation of amyloid-like fibrils is always associated to the onset of pathologies called amyloidosis affecting the central nervous system as well as peripheral tissues (44).

Amyloidoses are classified on the basis of the symptoms affecting organs, as primary or myeloma-related, acquired or secondary, or familial or senile. The altered function of the organ of interest is due to the precipitation of amyloid fibers in the extracellular matrix. The heart, kidney, and brain are among the most involved organs in amyloidogenesis (45). The brains of patients affected by Alzheimer's dementia are characterized by the presence of plaques that appear birefringent after staining with Congo red. The plaques are constituted by insoluble fibrils originated by the transformation of initially soluble protein or fragment of proteins to a totally insoluble matter that deposits *in situ* and alter tissue functions (46, 47). A common aspect to all amyloidogenic proteins is that these proteins, or fragments of them, become toxic for the organism only after they are released from the entire protein by means of enzymatic digestion.

An analogous mechanism was suggested by Tamburro and co-workers for elastin. As a matter of fact, they demonstrated that the polypeptide sequence encoded by exon 30 (EX30) of the human tropoelastin gene was able to give rise to amyloid-like fibers *in vitro* (48). Indeed, they suggested that the increased presence of elastase activities with age could accelerate the degradation of elastin by elastases, generating some elastin fragments with amyloidogenic properties (49). Interestingly, several reports have evidenced the presence of deposits containing elastin fragments, called elastotic material, in photodamaged skin, in atheromatous plaques, and in lung alveolae of patients with acute interstitial lung disease (49). Although the nature of these deposits has not yet been fully characterized, the elastotic material was shown to contain amyloid-like fibers.

Furthermore, it has been demonstrated that intramural amyloid deposits present in the arterial wall of elderly men do not react with any of the known or available antibodies to amyloid subtypes and that only a polyclonal antibody to human elastin marks this type of amyloid. On this basis, it was assumed that the precursor protein of this amyloid is derived from elastin (50). This finding could be considered the first *ex vivo* evidence for the possible involvement of elastin in amyloidogenesis.

However, Kozel et al. (51) suggested that EX30 plays a central role in determining the interaction of the C-terminal domain of tropoelastin with microfibrils, and that this interaction could be mediated by a mechanism of an amyloid-like self-aggregation route.

EX30 of human tropoelastin contains 13 glycyl residues and 12 hydrophobic residues and presents a motif of the general type, XGGZG (X, Z=V, L, A, I). The primary structure of EX30 is GLVGAAGLGGLGVGGLGVPGVGGLG. The motif is widely found in proteins such as collagens

IV and XVII, fibrillin 2 precursor, flagelliform silk protein, major ampullate gland dragline silk protein, major prion protein precursor, lamprin precursor, and amyloid A4 precursor protein binding. On this basis, it was hypothesized to be responsible for the amyloidogenic behavior of EX30. Consequently, an extensive screening of the polypeptide sequences encoded by the exons of human tropoelastin containing this motif was performed. Interestingly, this motif was found repeated along the primary structure of human tropoelastin in the following ways: XGGZG is tandemly repeated in EX30 (GLVGAAGLGGLGVGGLGVPGVGGLG) and EX32 (GAAGLGGVLGGAGQFPLG), and twice, even if interspersed with an alanine residue, in EX28 (GAAVPGVLGGLGALGGVGIPGGVV). It is present only once in EX16 and EX7.

Tamburro and co-workers have demonstrated that only the polypeptide sequences containing the XGGZG repeated motif, i.e., EX28, EX30, and EX32, were able to give rise to amyloid-like fibers *in vitro* (48, 52–54). The criteria adopted to assess the presence of amyloid fibrils were those commonly used in the literature (55). Structural studies on those amyloidogenic elastin polypeptides were performed in solution to resolve the molecular structure of the prefibrillar, soluble state, and in the solid state, to unravel the molecular structure of the fibrillar, insoluble state.

The experimental data obtained from CD, NMR, and FTIR spectroscopies evidenced that the solution state of those peptides was mostly populated by left-handed polyproline II helices with some unordered conformations, while in the fibrillar solid state cross-β structures were the dominant conformations for the amyloid-like fibrils (56, 57). The presence of transient and labile conformations, such as PPII and sliding β-sheet along the chain, quickly interconverting among themselves was also demonstrated by molecular dynamic simulations for elastin amyloidogenic peptides in the prefibrillar state.

Altogether, these data suggest that the EX30 peptide, when inserted into the protein, does not favor amyloid-like aggregation but rather participates in the formation of filaments and bundles of filaments typical of the elastic fiber. Consistently, Miao et al. (58) demonstrated that in a polypeptide sequence constituted by different tropoelastin domains, the substitution of the exon 24 domain with the exon 30 domain did not alter the coacervation properties. On the contrary, when the EX30 peptide is isolated from the protein, it leads to the amyloid-like fibril organization.

The supramolecular organization of elastin-derived amyloidogenic sequences is provided in Figure 1.

EPs as modulators of cell behavior

Biological effects of elastokines

EPs have been shown to regulate a plethora of biological activities such as cell chemotaxis (31, 34, 59–65), proliferation (66, 67), proteinase production (39, 68–72), tumor invasion (70, 73, 74), angiogenesis (69, 71), cell survival (75), reactive oxygen species production (76, 77), ion flux (78–81), and vasomotricity (33, 82, 83). Strikingly, some of their biological activities are extremely beneficial, such as protection of the heart against ischemia/reperfusion injury (71, 84) or tissue repair (85), while others are deleterious, such as their contributions to aortic abdominal aneurysms (60), calcification of vessel walls (86), hyperplastic neointimal formation (67), and more recently the progression of melanoma growth and invasion *in vivo* (74).

The most striking effect of EPs is the high chemotactic power they exert toward most cell types, notably inflammatory ones (31, 34, 77, 87, 88). EPs are therefore strongly associated with severe inflammatory disorders of elastic tissues, such as emphysema (61), atherosclerosis (89, 90), and aneurysms (60).

Receptors

These effects are mediated through the interaction of elastokines with elastin receptors such as the elastin complex receptor that includes a 67-kDa EBP, identified as an enzymatically inactive spliced variant of human β-galactosidase designated as spliced galactosidase (S-Gal), a neuraminidase (Neu-1), and a cathepsin A protective protein (91–93). Besides its elastin binding site, S-Gal also contains a β-galactosugar binding site whose occupancy causes its shedding from the cell surface (94), and therefore results in the inactivation of the elastin receptor complex (ERC).

The second elastin receptor identified is the $\alpha_v\beta_3$ integrin (95). This integrin binds many ligands, including those from the extracellular matrix (fibrinogen, tumstatin, MMP-2, u-PA, etc.). This recognition does not always require the presence of the classic integrin RGD motif that is absent in tropoelastin. Galectin-3, a multifunctional protein involved in cellular interactions, cell cycle, apoptosis, and intracellular trafficking, has also been suggested as an elastin receptor; however, the evidence remains mostly circumstantial (72). Finally, a lactose-insensitive elastin receptor has also been suggested (62). The nature of this receptor is still under investigation.

Signaling

These three elastin receptors are all expressed at the surface of various cell types, and the observed effects following elastokine/receptor interaction are highly dependent on cell type and therefore on the triggering of specific signaling cascade(s). For instance, activation of protein kinase G, cAMP, or cGMP production by EPs was reported to induce chemotaxis of monocytes (64, 76, 96). Moreover, activation of pertussis toxin-sensitive G proteins and the MEK/ERK signaling cascade were involved in EP-mediated effects on cell proliferation as observed in arterial smooth muscle cells (67). In turn, S-Gal occupancy by elastokines led to the activation of both NF-κB and p38 in melanoma cells (97).

Signaling following elastokine treatment is mostly described in human dermal fibroblasts. In this biological system, the observed effects have been linked to the ERC. Recent results have shown that the catalytic activity of Neu-1 is essential for signal transduction by the ERC (98). This is explained by the local conversion of GM_3 ganglioside into lactosylceramide, which is now regarded as the second messenger of the complex (99).

EPs as modulators of tissue homeostasis during aging

EPs can play various roles during aging. We will focus here on their contribution to tissue homeostasis in several pathologies linked to aging, ranging from lung and cardiovascular diseases to cancer progression.

Emphysema and chronic obstructive pulmonary disease

Emphysema and chronic obstructive pulmonary disease are inflammatory pathologies associated with massive degradation of lung elastin (100). *In vivo* experiments on a mouse model have established that EPs are the major actors driving the progression of the disease by the sustained recruitment of inflammatory cells (61).

In these pathologies, EPs seem to be involved at two critical levels. First, their generation following the action of locally released elastases contributes to the recruitment of macrophages that contribute to the progression of the disease by the action of their metalloelastase (61). Additionally, Lee and co-workers (101) have suggested that the continuous presence of EPs could also contribute to the generation of antielastin antibodies and an increased T-helper type 1 responses.

These findings suggest that when a critical level of EPs is reached, a vicious circle is set up. Indeed, EPs recruit inflammatory cells that release their elastases and thereby contribute to the generation of new EPs.

Abdominal aortic aneurysms

One of the most important pathologies affected by EPs is abdominal aortic aneurysm (AAA). It was shown that fragmentation of human aortic elastic fibers with age was correlated with an increase of elastase activity in the media (102). The generated EPs are responsible for a drastic phenotypic change of cells involved in the genesis of the pathology. In this pathology, the MMP-2 and elastase upregulation induced by mechanical stress leads to an increase in elastin degradation (103, 104) and consequently to EP production. Being chemotactic for most inflammatory cells, EPs attract these cells to their production site. It is important to note that elastolysis is mainly triggered by elastases from inflammatory cells that secrete neutrophil elastase, MMP, or cathepsins, which act individually or in concert to degrade elastin (105). As a consequence, in this context, EPs sustain their own production. This point is supported by the fact that anti-VGVAPG antibodies decreased the infiltration of monocytes in human AAA extracts (60), demonstrating that, in such an inflammatory disease, EPs play a central role.

Atherosclerosis

Atherosclerosis is a multifaceted, progressive, inflammatory disease that affects mainly large and medium-sized arteries. It is characterized by the formation and buildup of atherosclerotic plaques that consist of extracellular matrix degradation and remodeling, well-defined structure of lipids, necrotic cores, calcified regions, inflamed smooth muscle cells, endothelial cells, immune cells, and foam cells (106). Many studies have documented augmented elastase, collagenase, and gelatinase activities within atherosclerotic lesions. Conversely, elastin degradation is involved in the progression of atherosclerosis. Indeed, elastin degradation and/or degradation products increase during the progression of atherosclerosis, according to the presence of various kinds of proteases, including MMP-9 (107). It has been notably shown that generated EPs (108) contribute to the activation of monocytes/macrophages through the induction of their chemotaxis to

the chronic site of inflammation (atheroma) as well as to the oxidation of low-density lipoproteins by the activation of free radical production (89). This forms a vicious circle that contributes to the progression and chronicity of the atherosclerotic process.

Stromal aging and cancer

One of the most important risk factors for the development of cancer is aging (109). It has been shown that melanoma incidence increases in individuals older than 65 years. Moreover, aged patients have a worse prognosis. The central role of the extracellular matrix (110) as well as the accumulation of senescent cells in tissues may promote cancer progression in aged individuals (111). The occurrence of senescence gives rise to an important phenotypic shift (112–114). Especially, the ability of matrix degradation is enhanced in senescent fibroblasts in which uPA, tPA, stromelysin(s) (MMP-3), and collagenase (MMP-1) are upregulated. Moreover, elastases such as cathepsin K, MMP-2, and a 94-kDa metalloendopeptidase are also upregulated in senescent fibroblasts (115, 116). Importantly, such proteolytic cascades are not balanced by a parallel overexpression of inhibitors such as tissue inhibitors of MMPs or plasminogen activator inhibitors (117).

The generated EPs could play a role in amplifying the process of senescence, thereby leading to a higher content in senescence cells in elastin-rich tissues. During aging, dermal fibroblasts accumulate ceramides, which could lead to the induction of cell apoptosis thereby eliminating aged cells. However, several environmental factors could change cell fate and allow cells to accumulate ceramides without dying, thereby facilitating the increase of senescent cells. In this context, it has been shown that the interaction of EPs with the ERC protects dermal fibroblasts from ceramide-induced apoptosis, allowing the accumulation of ceramide in living cells (75). The signaling pathways involve in the activation of the PI3K-p110γ/Akt axis, which inhibits caspase-9 activation, inhibits Bad, and triggers Foxo3a sequestration by 14-3-3ζ. These signaling events could contribute to maintaining damaged or aged cells in tissues, which could ultimately favor the occurrence of cellular senescence.

Expert opinion and outlook

Since the pioneering work of Senior in the early 1980s (118), the literature related to EPs and their biological consequences has grown considerably. Nevertheless, the role played by EPs in the organism still remains poorly understood. This mainly comes from the fact that EPs have been shown to have both detrimental and beneficial effects depending on the considered biological effect. Altogether, our present knowledge of EP biology allows us to foresee that EPs may be related to a healing process sustained by inflammation and intense extracellular matrix remodeling. This point should be evaluated in the coming years.

In parallel, as they could constitute promising targets in therapeutic strategies, the characterization of EP conformations and dynamics, either isolated or bound to their cognate receptor, deserves further attention. A direct consequence of this work will be the determination of the ERC assembly mode and its structure-function relation. The operational mode of the ERC is slowly being revealed (99). Undoubtedly, the availability of tridimensional data on the structure of this receptor will open new perspectives in the elastin field.

Besides the elastokine-related research, more data have to be gathered concerning elastin amyloid-like structures. The question of whether these peculiar EPs can exist together with elastokines in body fluids has to be posed and raises the possibility that elastin amyloid-like structures could be deposited in aging tissues. The reality of these possible depositions and their physiopathological conditions has to be considered. This is a really challenging task.

Highlights

- Elastin degradation following the action of elastases is a fateful process.
- Elastin fragmentation results in a mixture of elastin peptides. As a consequence, the nature of elastin peptides produced *in vivo* is hardly understood. Thus, data are raised either using synthetic peptides derived from the known sequence of the elastin gene or using hydrolyzed elastin also termed soluble elastin.
- Elastin degradation products can be classed into two groups: circulating peptides presenting a biological activity (elastokines) and peptides having a propensity to form amyloid-like structures.
- Elastokines have numerous biological activities on both normal and tumoral cells.
- The biological activities of elastokines are mostly transduced by a specific receptor derived from the

lysosomal complex of β-galactosidase. This receptor is termed the elastin receptor complex and seems to be ubiquitously expressed by cells.
- Elastokines are thought to constitute strong tissue repair signals.
- Elastokines are potent survival-promoting molecules. As such, they could modulate tissue homeostasis by preventing the disposal of aged cells by apoptosis, thereby promoting overall tissue senescence.
- The biological role of elastin amyloid-like peptides is unknown. Their effective deposition in aging tissues is not yet proven and must be explored.
- The structure of the elastin receptor complex will permit to fully understand its functional mode and will open new therapeutic applications.

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