EEM 2024
EUROPEAN ELASTIN MEETING
REIMS

28-31 October 2024
Reims, France

12th European Elastin Meeting

Book of abstracts

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Opening session

Paradigm or paradox: What (we think) we know about elastin.

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Our understanding of elastin has advanced beyond a "dense material in elastic tissues." Initially thought to be an inert structural protein, we now know elastin has many biological properties outside of its mechanical function. Perhaps more than most extracellular matrix proteins, model building has been essential in explaining elastin characteristics. These instructive models provide a theoretical foundation that guides research in our field. In the Structure of Scientific Revolution, Thomas Kuhn describes essential models like this as paradigms: "universally recognized scientific achievements that, for a time, provide model problems and solutions to the scientific community." It must be remembered that a paradigm need not, and in fact never does, explain all the facts with which it deals. Paradigms change as more knowledge contributes to a deeper understanding of the supporting model. This presentation addresses old questions with new data or altered perspectives. Many of the ideas that will be discussed are recognized as speculation, but it is hoped that they might supply a framework for further discussion and investigation.

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Youth session

Effects of solubilized elastin preparations on *in vitro* wound healing parameters

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Abstract

Biomaterials supplemented with solubilized elastin peptides may provide a platform for the development of biomimetic wound management therapies. In this study, solubilization of elastin fibres was performed by oxalic acid (OxA-ELN), potassium hydroxide (KOH-ELN) or digestion with elastase (Enz-ELN). Biological activity of obtained preparations was assessed using fibroblasts for extracellular matrix (ECM) remodelling (3 donors) and macrophages for immune response (4 donors).

Dermal fibroblasts showed that collagen deposition was not altered as assessed with Western blotting and Picrosirius Red staining. αSMA gene expression in fibroblasts was similarly low with RT-qPCR analysis for all preparations. αSMA protein expression was undetectable as measured by Western blotting and immunostaining. Flow cytometry showed that macrophages exposed to OxA-ELN and KOH-ELN preserved their initial M0-like phenotype, while Enz-ELN stimulated M1-like macrophage differentiation.

Further experiments will focus on assessment of elastogenesis and in vivo studies of the biomaterials in rats.

Acknowledgement: EU Horizon 2020, MSCA grant agreement No 955722.

Keywords: elastin peptides, wound healing, skin regeneration

* Speaker

Investigating the regulation of elastogenesis by studying gene expression and crosslinking in an *in vitro* model system

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Abstract

The precise regulation of gene expression and cross-linking is crucial for elastogenesis, the intricate process of elastin synthesis and assembly. This process involves transcription factors, signaling pathways, and microenvironmental cues that control the expression of elastogenesis-related genes. Elastin's unique cross-links are vital for vertebrate life, providing recoil to elastic fibers and enhancing structural integrity in dynamic tissues. Understanding the regulatory networks governing elastogenesis is key to tissue-specific elastin deposition.

This study utilized recombinant tropoelastin and lysyl oxidase variants to investigate elastogenesis. The results show that these proteins significantly enhance elastin production *in vitro* and stimulate the expression of related genes, facilitating the generation of extracellular matrices enriched with elastic fibers.

Additionally, the cross-linking capabilities of recombinant tropoelastin variants with pyrroloquinoline quinone were examined. Using surface plasmon resonance, the binding affinities of these variants with key elastogenesis proteins were elucidated, providing insights into the molecular interactions driving elastic fiber assembly.

This research improves knowledge of elastogenesis and helps create functional elastic materials for tissue engineering and regenerative medicine.

Keywords: Elastogenesis, cross, linking, tropoelastin

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Domain 36 of tropoelastin interacts with model membranes

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Abstract

Elastin is a major extracellular matrix protein. It can be found in the elastic fibers that endow tissues with elasticity and resilience (1). Domain 36 is highly conserved. It has a cluster of positively charged amino acids (-RKRK) at its end and a hydrophobic cluster at its beginning. This domain also contains the two cysteyl residues of tropoelastin. They are disulfide-bonded to form an intrachain loop structure (2).

As domain 36 is important to elastogenesis and because tropoelastin reside at the membrane periphery during this process, we hypothesized that domain 36 could interact with the membrane. To test this, molecular dynamics simulations of 1000 ns with 3 replicas were carried out and suggested that domain 36 could bind to a model membrane. This possibility was further confirmed by synchrotron radiation circular dichroism (SRCD) and orientated circular dichroism (OCD). Spectra demonstrated the interaction of domain 36 with a membrane.

(1) C.E.H. Schmelzer and L. Duca, Elastic fibers: formation, function, and fate during aging and disease. FEBS J. 289, 3704–3730, 2022. doi: 10.1111/febs.15899

(2) T.J. Broekelmann et al, Modification and functional inactivation of the tropoelastin carboxy-terminal domain in cross-linked elastin. Matrix Biol., 27, 631–639, 2008. doi: 10.1016/j.matbio.2008.06.001

Keywords: Elastin, lipids, molecular dynamics, circular dichroism, synchrotron radiation

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Understanding the vascular pathophysiology caused by the dominant C19F mutation in matrix Gla protein

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Abstract

In association with vascular calcification (VC), vascular smooth muscle cells (VSMCs) in arterial walls may transdifferentiate to chondrogenic cells. We previously reported that in matrix Gla protein (MGP)-deficient mice, VSMCs express chondrogenic markers only after the initiation of VC affecting the elastic laminae, suggesting VC might induce VSMC phenotypic switching. However, in a novel mouse model with the C19F dominant mutation in MGP causing skeletal dysplasia, we observed a proteoglycan-rich matrix in the arterial walls without VC. We hypothesize that MGP mutation may cause chondrogenic transdifferentiation(C/TD) of VSMCs independent of VC.

Aim

Investigate vascular pathologies in C19F MGP-expressing mice.

Methods

VSMC morphology and proteoglycan deposition in the arterial walls in control and mutant mice were examined by histology. Immunostaining was performed to detect ER stress and chondrogenic markers. Apoptosis was assessed by TUNEL assay.

Results/Conclusion

Mice expressing C19F-MGP exhibit no VC but show thickened arterial walls with a proteoglycan rich matrix. VSMCs are enlarged and undergo C/TD, confirmed by safranin O staining, and the upregulation of aggrecan and SOX9. Although ER stress is induced, increased apoptosis is not observed. Our findings suggest that MGP may regulate C/ TD of VSMCs and VC independent of each other.

Keywords: vascular smooth muscle cells, arterial wall, vascular calcification, matrix Gla protein, chondrogenic, trans differentiation, elastic laminae, phenotypic switching, ER stress, apoptosis

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Deciphering the dynamic behavior of elastin polypeptides through mesoscopic simulations

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Abstract

The extracellular matrix (ECM) plays an essential role in supporting tissues and organs. Technological limitations prevent its observation at the mesoscopic scale, therefore the precise organization of the ECM remains poorly known. Using physics engines such as Unity3D, and rigid body dynamics, the DURABIN demonstrator to simulate large biological macromolecules as dynamic chains of interacting rigid bodies has been developed. In this work, we used all-atom molecular dynamics simulations of different peptide fragments characteristic of tropoelastin. Starting from penta- and hexamer motifs, polymer-like motifs of these sequences used in biotechnological engineering were studied as well as three specific exons of tropoelastin. Analyses of simulations allowed to characterize their flexible and dynamic behaviors, their solvation and physicochemical properties of hydrophobicity and electrostatics. Finally, different clustering methods have been used to define the main conformations further used as primitives in the DURABIN mesoscope. The dynamics of rigid bodies could be applied for these characteristic patterns and the implementation of a library of fragments was proposed to allow the simulation of polymers under angle constraints, evaluating the possibilities of assembly or even behavior in crowded environments and thus making the virtual model of the ECM more realistic.

Keywords: Molecular dynamics, Mesoscopic simulations, Rigid bodies, Clustering, Elastin peptides, Elastomeric proteins, Elastin, ECM

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Pathogenic variation in fibrillin-2 confers susceptibility to spontaneous cerebrospinal fluid leaks through impairment of cellular adhesion

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Abstract

Spontaneous spinal cerebrospinal fluid leaks (ssCSFL) have a strong association with connective tissue diseases (CTDs) with altered elastic fiber formation/homeostasis including Marfan syndrome and Loeys-Dietz syndrome. Patients with ssCSFLs without a specific diagnosis often have nonspecific CTD manifestations, suggesting that mutations in extracellular matrix proteins underlie more common presentations of this condition. We report a case control study showing a significantly increased burden of rare variants in the gene encoding fibrillin-2, a basic structural element of elastic fibers, in patients with type 1b ssCSFLs. Two of these mutations reduce human dural fibroblast adhesion to fibrillin-2 fragments in vitro. One disrupts a known integrin binding motif in the TB4 domain; another affects a completely novel binding site in the TB7 domain. Mice harboring the fibrillin-2 TB4 variant (Fbn2 D1581V/+) and a mouse model of Marfan syndrome (Fbn1 C1041G/+) demonstrate a pronounced predisposition for dural rupture upon controlled leak induction. Single-nuclear RNA sequencing of Fbn2 D1581V/+ and Fbn1 C1041G/+ mouse dura demonstrates dysregulation of tropoelastin expression in dural fibroblasts. These data suggest that mutations in FBN2 that alter cellular adhesion and/or the synthetic repertoire of matrix elements can lead to increased susceptibility to ssCSFLs.

Keywords: elastin, connective tissue disease, CSF leak, spinal CSF leak, human genetics, fibrillin-2, integrin, dura mater, mouse model

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Fundamental research session

Structure and interactions of the cross-linking enzyme lysyl oxidase and its propeptide

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Abstract

Lysyl oxidase (LOX) catalyzes the first step of collagen and elastin cross-linking, which contributes to the extracellular matrix (ECM) stiffness. LOX has also been detected in the nucleus of several cell types, acts on non-ECM substrates, and interacts with intracellular, membrane and ECM proteins. We have shown that LOX propeptide is intrinsically disordered, and expressed three recombinant forms of its catalytic domain in E. coli. They mostly contain b-strands and random coils and form oligomers of heterogeneous size, which we have characterized by dynamic light scattering, mass photometry, and negative staining electron microscopy (collaboration C. Baldock, Manchester, UK). Experiments with thioflavin T suggest that LOX could form amyloid-like fibrils. We have generated a 3D model of LOX catalytic domain (collaboration M. Dauchez, Reims, France). The catalytic site is located in a groove surrounded by two loops, which forms a hinge with a variable opening to accommodate the various sizes of LOX substrates. The 3D model exhibits hydrophobic protrusions, which could mediate the association of LOX with the cell membrane, close to collagen fibrillogenesis and elastogenesis sites.

Keywords: Lysyl oxidase, crosslinking, 3D structure, interaction network, extracellular matrix, elastin, collagens

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Uncovering the structural and physical basis for the mechanical properties of elastin

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Abstract

The biologically important mechanical properties of elastin include elastic recoil (the capacity of an elastic material to revert to its original size and shape once deformed), resilience (energy lost in extension-recoil cycles) and stress relaxation (work needed to keep an elastic material extended). However, how the sequence and structure of elastin and elastin-like peptides (ELPs) govern these properties is currently unknown. Recent studies from molecular dynamics (MD) simulations and NMR spectroscopy show that the hydrophobic effect and a high degree of conformational disorder maintain the equilibrium structural ensemble of ELPs, suggesting that these properties also govern protein elasticity. To test this hypothesis, we use massively-repeated, all-atom simulations and elastic network models of ELPs. Both equilibrium and non-equilibrium approaches are used to generate large conformational ensembles of these peptides under applied stress from which quantitative estimates of stiffness, resilience, and stress relaxation are computed. By providing direct quantitative relationships between stress, strain, and molecular structure, these studies afford insight into the structural and physicochemical basis for the viscoelastic properties of elastin and ELP-based materials.

Keywords: Elasticity, resilience, stress relaxation, molecular dynamics, conformational disorder, hydrophobic effect, elastin, like peptides

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Elastin recoil is driven by the hydrophobic effect

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Abstract

The basis for elastin's elasticity has been controversial for more than 50 years. Formed from a hydrophobic protein with an equivalent mass of water, the controversy is whether recoil is driven by entropy gain of the protein and/or the water. We have studied water ordering at the solvent:protein interface with 2H NMR and obtained a complete thermodynamic characterization by measuring elastin's length and volume as a function of force and temperature in water and with co-solvents (Jamhawi et. al., PNAS, 2024, 7982-7987).

When stretched, water is ordered proportional to the degree of stretching, the heat capacity increases, internal energy decreases and heat is released in excess of work showing that recoil is primarily driven by the hydrophobic effect rather than by configurational entropy as in rubber. Consistent with this conclusion are decreases in the thermodynamic signatures when co-solvents that alter the hydrophobic effect are introduced. We propose that recoil driven by the hydrophobic effect rather than by configurational entropy is responsible for elastin's great robustness and low elastic modulus. Unlike rubber, hardening from crystallization does not occur because water reorientation is facile and arteries readily expand when the heart contracts because elastin's entropic restoring force is opposed by a large heat loss.

Keywords: Recoil mechanism, NMR, thermodynamics

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Investigation of elastin structure and dynamics by solid state NMR in native hydrated extracellular matrix

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Abstract

Organization of elastin and its integration with collagen is crucial for the function of blood vessels constantly exposed to mechanical strain. Until now it was impossible to investigate molecular-level details of fully hydrated elastin enveloped by native extracellular matrix (ECM). We developed a method of production of cell-free elastin-enriched bVSMC ECM and combined it with isotope-labelled low temperature solid-state nuclear magnetic resonance spectroscopy (ssNMR). We used ssNMR methods that allow insight into elastin molecular dynamics and for the first time implemented the DUMBO INEPT method to a native hydrated protein. We used ^{13}C-proline labelling to study disordered regions in elastin in the same cell-free system and showed that Pro backbone carbon Cα correlated with type II β-turn, random coil and polyproline-II helix conformations that is consistent with the dynamic equilibrium between secondary structures in the sliding turn model of elasticity; we also observed Cβ and Cγ -exo and -endo conformations that suggested a twisted ring flip. ^{13}C-lysine labelling of elastin showed crosslinking signals corresponding to DES/IDES, MDES and potentially LNL within native elastin, demonstrating that ssNMR spectroscopy can be a highly useful probe of elastin molecular structure and dynamics, and, fundamentally, in intact ECM.

Keywords: elastin, native, hydrated, in vitro, solid state NMR, DUMBO, conformation, crosslink

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Identification of C5aR as a new interaction partner of membrane sialidase NEU1: potential implication of the elastin receptor complex in the regulation of the complement system

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Remodeling of elastic fibers leads to the production of elastin-derived peptides (EDP) that trigger biological effects through the elastin receptor complex (ERC). Data from the last decade have brought significant insights on the critical role played by its catalytic subunit, neuraminidase-1 (NEU1), in EDP-mediated biological effects in vascular diseases. We previously identified an action mechanism by which EDP binding to the ERC induces NEU1 catalytic activity and desialylation of key membrane glycoproteins, leading to modulation of major biological events and anticipating new biological functions for the ERC through NEU1. Here, we developed a Membrane Yeast Two Hybrid screening for the search of new interaction partners of membrane NEU1 from a human macrophage cDNA library. Several candidates were identified including the G protein-coupled receptor (GPCR) for C5a anaphylatoxin (C5aR). Interaction between NEU1 and C5aR was confirmed in HEK293 cells and results showed that NEU1 overexpression induces C5aR desialylation and increase in C5aR signaling following stimulation by its agonist. Importantly, cell preincubation with a bacterial neuraminidase recapitulated these effects. Together, these results demonstrate that NEU1 can physically interact with and desialylate GPCR, modulate GPCR signaling, and suggest that EDP may be potential modulators of the complement system.

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Characterization of new functional interactions of hemicentins within the elastin/fibrillin microfibril network

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Abstract

The elastic fiber/fibrillin microfibril network forms specialized cellular microenvironments endowing tissues with specific biomechanical properties. Hemicentins (HMCN1/HMCN2) are large 600 kDa proteins belonging to the fibulin family with largely unknown extracellular functions. Murine tissues were investigated by immunofluorescence (IF) and immunogold electron microscopy (EM) employing new specific antibodies raised against recombinant HMCN1 and HMCN2 protein fragments. Both HMCNs co-localize with FBN1 and FBN2 in different tissues. HMCN1 localizes to elastic fibers in the dermis while HMCN2 was also found to localize to the endomysium of skeletal muscle. Immunogold EM revealed that HMCN1 is targeted to fibrillin microfibrils and elastic fibers. Solid-phase interaction assays demonstrated that the fibulin-like module of HMCNs mediates the interaction with the N-terminal region of FBN1 and FBN2. Other elastic fiber network proteins such as LTBPs, FBLNs, LOXLs and tropoelastin also interact with the fibulin-module and co-localize with HMCNs. Transmission EM and echocardiography of adult Hmcn1-/- and Hmcn2-/- as well as Hmcn1-/-;Hmcn2-/- mice showed elastic fiber breaks and dilatations of the ascending aorta implicating that both HMCNs contribute to aortic wall stability. Our results give new insights into the mechanisms of elastogenesis and elastic fiber stability.

Keywords: Elastic fibers, Elastogenesis, Fibrillin, Fibulin, Hemicentin, Surface Plasmon Resonance, LTBP, Tropoelastin, LOXL

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New selective inhibition mechanism of cathepsin S elastolytic activity by exopolysaccharides from marine bacteria

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Abstract

During emphysema, large amounts of proteases are released by inflammatory cells leading to breakdown of elastin fibers, main components of lung tissue. Among these elastases, Cathepsin S (CatS) has been reported to play a key role in respiratory disorders. Thus, CatS is considered as a therapeutic target for the development of new inhibitors. Knowing that activity of CatS can be modulated by polysaccharides or glycosaminoglycans, we focused on a new source of exopolysaccharides (Infernan and Diabolican) produced by extremophilic marine bacteria (respectively *Alteromonas infernus* and *Vibrio diabolicus*). This study consists in evaluating the ability of low-molecular weight highly sulfated derivatives of Infernan and Diabolican to modulate the CatS elastolytic activity. We identified two potent and selective inhibitors of CatS. Interestingly, these two EPS only inhibit the CatS elastolytic activity without interfering with its other proteolytic activities. Molecular dynamics studies suggest that these two inhibitors are able to bind not only in the active site of CatS but also in a domain located outside the catalytic site (exosite), and that is crucial to the interaction between the enzyme and elastin. These results present a new mechanism of selective inhibition of the elastolytic activity of CatS and suggest that these two EPS would be promising candidates in CatS inhibition *in vivo*.

Keywords: Lung, proteases, cathepsin S, elastin, exopolysaccharides, breakdown, elastases, glycosaminoglycans, inhibitors

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An experimentally determined conformational ensemble reveals atomic-level details of structure and disorder in tropoelastin

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Abstract

The underlying molecular mechanisms that drive elastin assembly and its function as an elastic polymer have been the subject of significant debate. This is largely due to an inability to obtain high resolution structural models of elastin and elastin's monomeric precursor tropoelastin. We have developed novel approaches to obtain near complete nuclear magnetic resonance (NMR) assignments of full-length human tropoelastin (hTE) and to model its dynamic structural ensemble with atomic-level precision. Experimentally determined hTE ensembles were generated using X-ray scattering data, site-specific secondary structure propensities from NMR chemical shifts and interatomic contact information from paramagnetic relaxation enhancement NMR. Our results provide strong evidence that hTE exists as a highly dynamic disordered ensemble that rapidly interconverts between a diverse set of 3D configurations. While our results suggest that hTE samples both highly expanded and collapsed conformations, the specific arrangements are far from random suggesting important roles for local conformational propensities in elastic fiber assembly and elastin function.

Keywords: elastin, tropoelastin, intrinsic disorder, NMR

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Elastogenesis and diseases session

Molecular mechanisms of accessory elastogenic proteins

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Abstract

Elastogenesis is a crucial process in the physiology of elastic tissues, including blood vessels, skin, and lungs. Elastic fiber abnormalities play a critical role in developing genetic and acquired diseases, leading, for example, to aneurysms in the aorta, laxity in the skin, or emphysema in the lungs. The formation of elastic fibers requires, besides the core elastin, several accessory fiber systems and proteins, including fibronectin, fibrillins, small fibulins, the latent TGF-β binding protein-4 (LTBP4), and microfibril-associated glycoprotein 4 (MFAP4). Each of these components has critical functions in elastogenesis, as is evident from the respective knockout mice. However, the detailed molecular roles and functional interactions are only beginning to emerge.

In this presentation, I will describe our new data on the interaction of fibulin-4 and LTBP-4 with cells, identify the cell receptors, and outline the functional consequence on elastogenesis. I will also present a high-resolution cryo-electron microscopic structure of MFAP4 and related functional analyses that shed light on the role of MFAP4 in elastogenesis. The data provide new information on elastic fiber formation and stability and its relevance to pathological aspects.

Keywords: Fibulin, 4, LTBP4, MFAP4, elastogenesis, cell receptors

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Pharmacotherapeutic strategies to prevent or cure elastic fiber damages and improve arterial elasticity

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Abstract

Elastic fibers provide the extensible tissues with elasticity which, in arteries, allows for appropriate hemodynamics and tissue perfusion. Unfortunately, aging or some pathologies feature different processes leading to elastic fiber damages, such as mechanical fatigue or proteases/anti-protease imbalance. Different strategies have been tested to limit these degradative processes. Pharmacotherapies, in vitro or in vivo in animals or humans, have revealed the relative efficiency of some drugs in enhancing elastic fiber neosynthesis, preventing/curing elastic fiber alteration, or restoring/improving arterial elasticity. This is of importance for limiting the impacts of arterial aging, genetic syndromes developmentally altering elastic fibers (Wiliams-Beuren, Marfan, ...), or conditions with later onset featuring arterial elastic fiber alterations (obstructive sleep apnea syndrome, ...). Some drugs targeted re-induction of elastin production (potassium channel openers), stimulation of elastin crosslinking by lysyl oxidases (dill extract), replacement of damaged elastic fibers or stimulation of elastin-binding cellular receptors (synthetic elastic protein), inhibition of TGF-signaling (losartan), ... Some representative results will be presented here, together with their limitations and potential for future treatments in the elderly or younger patients.

Keywords: Pharmacotherapies, arterial elastic fibers, elasticity, structure, function

* Speaker

Human C-peptide is a ligand of the elastin-receptor-complex

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Abstract

When insulin is excreted, it is always excreted together with C-peptide. I identified an elastin-receptor binding amino-acid motif xGxxPG in C-peptide. This receptor is involved in vascular remodelling, commonly activated by elastin-peptides with said motif. Systemic elastin-peptides may arise from smoking or from consuming elastin-rich processed red-meatproducts, therewith interfering vascular remodelling. Both excess elastin-peptides as well as excess C-peptide associate with core-manifestations of metabolic syndrome: insulin resistance, angiogenesis, and vascular remodelling. Excess elastin receptor-activity through excess activation (by systemic C-peptide, elastin-peptide, galectin-3, or others, identified herein as PREANS), is centrally involved in metabolic syndrome and T2D, inducing insulin resistance with excess and maladaptive macrovascular remodelling. Systemic C-peptide is increased in a "Western-diet" lifestyle, systemic elastin peptides are increased after smoking and possibly after elastin-consumption, and systemic galectin-3 increases in inflammation and obesity. As all these risk-factors associate with life-style mediated metabolic syndrome, I pose one up-stream cause: excess activation of ERC by PREANS stands central in causing metabolic syndrome.

Keywords: C peptide, elastin receptor, vascular diseases, PREANS

* Speaker

Loss of function variants of ADAMTSX involved in a new syndrome related to Marfan syndrome

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Abstract

We identified 3 heterozygous variants in the ADAMTSX* gene (* confidential name) including one de novo in pediatric cases with similar features. The 3 patients share craniofacial, skeletal, cardiovascular, and neurological features at the clinical level. This gene encodes ADAMTSX, an extracellular enzyme playing an important role in the extracellular matrix structure and homeostasis by interacting and cleaving ECM glycoproteins, such as fibrillin-1 and 2. Cellular and mouse models validate ADAMTSX as a candidate gene for this new syndrome. The patient's fibroblasts harboring a pathogenic variant in the ancillary domain of ADAMTSX result in a reduction of the cleavage of FBN1 and FBN2, being this phenotype rescued by conditioned media from control fibroblast. Another variant affecting ADAMTSX's prodomain results in the absence of the secreted protein, preventing its correct functionality. Altogether confirm these variants to be loss of function. Furthermore, the "ancillary domain" variant shows to be implied in the activation of the TGFβ signaling pathway by resulting in higher levels of SMAD2 phosphorylation. We demonstrated that AdamtsX-/- mice present ventricular septal defects remaining in the human phenotype. Altogether, with the validation of ADAMTSX, this study will give importance to the role of this metalloprotease in familial ascending aortic aneurysms.

Keywords: Aorta, ADAMTS, Marfan, patient cells, mouse model

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Cell-specific role of lysyl oxidase in the vasculature

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Abstract

Lysyl oxidase (LOX), the main enzyme responsible for crosslinking collagen and elastin, is indispensable as its deletion is incompatible with life. Furthermore, LOX mutations in humans cause familial thoracic aortic aneurysm and dissection. Using a novel conditional Lox mouse model, we sought to determine the cell-specific contribution of LOX to arterial integrity by deleting LOX specifically from endothelial cells (ECs) and smooth muscle cells (SMCs). While mice with EC-specific deletion of LOX were indistinguishable from wild-type (WT) littermates, mice lacking LOX in SMCs had 50% survival by 1 month of age, exhibited severe ascending aortic aneurysms extending through the aortic arch, and developed significant systolic hypertension with widened pulse pressure. Histologically, conduit arteries of SMC-Lox KO mice showed thickening of the media with increased number of SMC layers and fragmentation of elastic fibers while muscular arteries were intact. In conclusion, while EC-LOX may be dispensable, SMC-LOX is critical for arterial development and maintenance of conduit, but not resistance arteries possibly due to compensation by another family member or due to LOX secretion by a neighboring cell type. Future studies will focus on investigating the effect of LOX deletion on cell phenotype and on identifying alterations in signaling pathways that may be targeted therapeutically.

Keywords: Lysyl oxidase, aortic aneurysm, arterial development, vascular elastic fibers

* Speaker

Calcification of elastin: data from a cell-free model

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Abstract

It has been demonstrated that elastic fibres calcify in several pathologic conditions. To demonstrate that elastin degradation precedes and favours the mineralization process, we set up an in vitro cell-free model where calcification can be evaluated on coacervated elastin fibrils incubated in a phosphate fortified medium after organo-alkaline or bacterial enzymatic degradation (Int. J. Biol. Macromol. 2020;149: 693). Aim of the present study was to explore if: i) conditioned media of human dermal fibroblasts isolated from calcified and healthy tissues can degrade elastin fibrils favouring their calcification; ii) addition of heparan sulphate (HS) or of chondroitin sulphate (CS) during elastin coacervation can counteract the mineralization process. Results indicate that: i) conditioned media of pathologic fibroblasts have an increased proteolytic potential compared to control cells; ii) calcification is increased on fibrils degraded by pathologic conditioned media; iii) the presence of HS significantly reduces the calcification process in a dose-dependent manner, whereas CS does not exert any effect. Therefore, this in vitro cell-free model is suitable to demonstrate fibroblast proteolytic activities, and the extent of calcification that is related to the pathologic phenotype.

Acknowledgements: Work supported by PXE Italia Odv

Keywords: Elastin, in vitro model, ectopic calcification, Heparan sulphate, Chondroitin sulphate

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Deletions upstream of Eln cause Elastin Insufficiency

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Abstract

Elastin haploinsufficiency is the predominant cause of non-syndromic congenital supravalvar aortic stenosis (SVAS). However, approximately 40-60% of patients with non-syndromic congenital SVAS have negative genetic testing. We previously identified a subset of patients with SVAS and deletions starting > 30 kb upstream of ELN. Fibroblasts from patients with upstream deletions have monoallelic expression of elastin suggesting these deletions cause elastin insufficiency and, consequently, SVAS. To investigate the causality, we generated a mouse model with the analogous Eln upstream deletion (UD) shared by all 4 patients (ElnUD/+ and ElnUD/UD). Echocardiogram of ElnUD/+ mice demonstrates an elongated ascending aorta with loss of the typical "candy cane" shape. ElnUD/+ and ElnUD/UD mice have more elastic lamellae and wall thickness in the ascending aorta as seen in Eln insufficiency. Microfil injections and microCT of the thoracic and cerebral vessels demonstrates globally elongated vessels and tortuosity in both ElnUD/+ and ElnUD/UD. These results suggest that deletion of a 180kb region 122.5 kb upstream of Eln in mice reduces, but does not completely abolish elastin expression.

Keywords: Supravalvar aortic stenosis, elastin Deficiency, transcription, diagnosis

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Lung Elastin Dysregulation Leads to Lung Emphysema via Induction of Cell Senescence

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Abstract

Loss of elastin and accumulation of senescent cells are major pathogenic processes in pulmonary emphysema (PE), but the link remains unknown. We examined whether reduced elastin levels lead to senescence of lung cells and whether this promotes lung changes. We combined studies of human-lung tissues and cells from patients with PE and studies of mice with elastase-induced PE or deletion of one elastin allele (ELN+/-). Treatment of healthy human cells with elastin siRNA induced senescence. This was attenuated when cells were seeded on an elastin-rich matrix before siRNA treatment. Cultured fibroblasts from Eln+/− mice exhibited a senescence phenotype which was also attenuated when cells were seeded on an elastin-rich matrix. Elastase-treated mice and Eln+/- mice showed increased markers of pulmonary senescence, accumulation of p16-expressing cells and development of PE. In p16-ATTAC mice activation of the ATTAC transgene prevented elastase-induced pulmonary functional and structural alterations. In Eln+/- mice, treatment with senolytic drugs decreased PE as assessed mechanically and histologically and increased lung elastin levels and pulmonary repair processes. Elastin loss or degradation is a inducer of lung cell senescence which underlies PE development. Targeting senescent cells is a new therapeutic strategy to induce pulmonary repair and stimulate elastogenesis in PE.

Keywords: Elastin, lung, senescence, pulmonary emphysema, senolytic drugs

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Biomaterials and repair

Engineering elastin: a biomimetic approach to materials design

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Abstract

Elastic fibers are key components of the extracellular matrix, providing elasticity and resilience to tissues like lungs, skin, and blood vessels. This study examines the complex process of elastogenesis, focusing on the assembly of elastic fibers and the cross-linking of tropoelastin onto fibrillin-rich scaffolds. Mature elastic fibers exhibit remarkable resistance to both intrinsic and extrinsic factors, maintaining their function throughout life. However, aging introduces changes such as enzymatic degradation, oxidative damage, glycation, calcification, and mechanical fatigue, impairing elastic fiber function and contributing to disease. This research explores the impact of these alterations, particularly in elastin-rich organs like skin, lungs, and the cardiovascular system. Building on new insights into elastogenesis and aging, we develop elastin-based biomaterials using advanced techniques such as electrospinning and bioprinting, aiming to create biomimetic materials with improved biocompatibility and mechanical properties for tissue repair.

Keywords: elastogenesis, hydroxylation, crosslinking, PTM, electrospinning, bioprinting

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Designing biomaterials that induce elastic fibre regeneration

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Elastic fibres play a critical role in tissue elasticity and resilience, but their regeneration following injury is limited, impairing tissue repair outcomes. Biomaterials containing elastin hold promise for addressing this issue by serving as elastic tissue replacements or enhancing elastin biosynthesis. Elastin degradation products, most likely through interaction with the elastin receptor complex, can stimulate elastin synthesis, cell proliferation, and chemotaxis. Elastin-based biomaterials can harness these biological effects when implanted. Two primary forms of tissue-derived elastin used in biomaterials are insoluble elastin, derived from tissues such as ligamentum nuchae, and solubilized elastin, obtained through hydrolysis of the insoluble fibres. Subcutaneous have demonstrated that biomaterials containing solubilized elastin support angiogenesis and elastic fibre neosynthesis.

A deeper understanding of elastin's synthesis at the cellular level is essential for developing biomaterials that not only trigger elastin production but also promote the formation of functional elastic fibre networks. Elastogenesis, the process of elastic fibre formation, can potentially be induced by elastin-related compounds and other extracellular matrix components. Biomaterials designed to stimulate this process must facilitate both the synthesis of elastin and the proper assembly of elastic fibres. Such advancements are crucial, especially for applications in dermal substitutes aimed at improving wound repair and tissue regeneration. Advancing the design of biomaterials that stimulate both elastin synthesis and elastic fibre formation holds great promise for enhancing tissue repair, particularly in applications like dermal substitutes for wound healing.

* Speaker

Multicomponent bioactive electrospun scaffold as versatile toolbox for biomedical applications

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Abstract

Multicomponent bioactive electrospun scaffolds represent a versatile and promising tool for biomedical applications, offering customizable design options, controlled release of bioactive molecules, enhanced biomimicry, and the potential for complex tissue engineering and regeneration. These scaffolds can mimic the ECM of native tissues more closely than single component scaffolds. By incorporating multiple bioactive components they can provide a microenvironment that better supports cell attachment, proliferation, and differentiation. In addition, by combining different polymers the mechanical properties can be tuned to those of specific tissues. The multifunctionality enhances their utility in applications ranging from wound healing and tissue engineering to drug delivery. Some examples of multicomponent electrospun scaffolds developed in our laboratory, consisting of synthetic polymers, biopolymers, peptides, inorganic components, natural products and drugs, will be presented. The promise and drawbacks that need to be overcome to unlock their full therapeutic potential in different biomedical applications will be highlighted.

Part of this study was carried out within the Agritech National Research Center, with funding from the European Union Next-Generation EU (PNRR) – M4C2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

Keywords: Electrospinning, elastin bioactive peptide, biomimetic scaffold

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Effect of elastin-derived peptides on tenocytes behaviour and collagen production

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Abstract

Elastin undergoes the elastin-derived peptides (EDPs) release with ageing. EDPs engage in biological activities. Elastin surrounds a subpopulation of tenocytes in tendons. This study aims to investigate the EDPs' effects on tenocytes and collagen production, providing insights into tendon repair.

Tenocytes were cultured in media containing VGVAPG (EDP) at gradient concentrations. Cell proliferation and migration were assessed by measuring Ki 67 protein and transwell assay. Collagen type I and III expression were evaluated at transcriptional and protein levels. Significant increases of Ki 67 protein and migration effects were observed, with optimal results at 100 μg/mL. EDPs also led to an increase in mRNA levels of collagen I and III. While the collagen type I protein was significantly increased, there was no significant change in type III.

The results show that EDPs could promote tendon repair by optimising cell behaviour and upregulating type I collagen production, thereby strengthening the matrix structure. Additionally, there was a positive correlation between EDP concentration and its biological effects. The differences in collagen types I and III protein levels may be due to different regulatory mechanisms requiring further research. Further understanding the interactions between EDPs and cell activity may provide a new target for alleviating tendon degeneration.

Keywords: Elastin derived peptides, tendon, matrix, collagen, tenocyte

* Speaker

Molecular interactions governing the assembly and phase behavior of elastin-like recombinamers

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Abstract

Biomolecular condensates are unique structures that form in living cells through a process known as liquid-liquid phase separation (LLPS), driven primarily by intrinsically disordered proteins (IDPs). Synthetic condensate engineering aims to develop novel bimolecular condensates by understanding the molecular relationships between amino acid sequences and the mechanical properties and assembly structures of the IDPs.

In this study, we focus on the formation of synthetic condensate using elastin-like recombinamers (ELRs) and examined the forces driving their coacervation, including simple coacervation (hydrophobic interactions) and complex coacervation (electrostatic interactions) at inter- and intramolecular levels.

An ELR library was created, featuring two highly charged monoblocks and a diblock. While the charged monoblocks could not coacervate alone, mixing them led to complex coacervation driven by hydrophobic and electrostatic interactions. Different physical techniques combined with confocal microscopy revealed the critical role of inter- and intramolecular interactions in coacervation.

Our study highlights the interplay between self-organizing forces in forming complex hierarchical structures from IDPs. By fine-tuning inter- and intramolecular electrostatic interactions, we can control the formation and maturation of protein condensates.

Keywords: Biomolecular condensates, Liquid, Liquid phase separation (LLPS), Elastin-like recombinamers, Synthetic biology

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Tetra-peptide matrikines modulate epithelial cell migration and gene expression

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Abstract

We have previously shown that peptide matrikines GPKG (P1) and LSVD (P7), found in collagens and elastic fibre proteins respectively, can influence the transcription of genes involved in migration and circadian rhythms in human dermal fibroblasts. Here we test the hypothesis that these peptides can modulate behaviour and transcription in keratinocytes. Migration of an immortalised keratinocyte cell line (HaCaTs) in response to P1, P7 and P1+P7 was assessed by live cell imaging. P1, P7 and P1+P7 effects on the HaCat circadian clock were characterised by bioluminescence recording of cells containing a promoter-driven Bmal1-Luc. Finally, primary human keratinocytes (n=3, 36–61 yrs, female) were exposed to the peptide combination P1+P7 for 12 hours prior to RNASeq analysis. P7 (but not P1) significantly enhanced migration and promoter activity. P1+P7 enhanced migration only and, by RNASeq analysis, significantly upregulated expression of clock genes PER1, CRY1, and NR1D1, and enhanced pathways involved in proliferation, growth factor signalling and lipid metabolism. This study demonstrates that these matrikines can also affect the behaviour of both stromal and epithelial cells.

Keywords: Matrikines, peptides, skin, epithelial cells

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Post-modifications of recombinant elastin-like polypeptides towards bioactive (nano)materials and self-assemblies

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Abstract

Elastin-like polypeptides (ELPs) are thermo-responsive biopolymers whose primary sequence is derived from the natural extracellular matrix protein elastin. Genetically-engineered and produced recombinantly in heterologous hosts (typically Escherichia coli bacteria to ensure reasonable production yields), they are perfectly monodisperse macromolecules. Although powerful to yield ELPs with exact primary structures and lengths, protein engineering techniques present however some limitations, in particular lengthy bacterial cloning steps and limited chemical diversity due to few possible post-translational modifications in E. coli bacteria. My research activities are therefore dedicated to exploring a dual biotechnological and chemical approach, combining recombinant biosynthesis of ELPs with orthogonal chemical bioconjugation methods to enlarge the diversity of relevant ELP-based macromolecules and self-assemblies thereof for biomimetic, biological and/or biomedical applications.

Keywords: ELP bioconjugates, orthogonal post mofications, lipolypeptides, glycopolypeptides, self assembled particles

* Speaker

Advancing regenerative therapies: elastin-like recombinamer hydrogels for degenerative joint diseases

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Abstract

Degenerative joint disease, particularly in the knee, spans a range from focal cartilage defects to extensive diffuse cartilage lesions (DCL). These lesions, precursors to osteoarthritis (OA), pose significant treatment challenges due to the limited self-healing capacity of cartilage. Although current treatments with mesenchymal stem cells (MSC) show potential, they are limited by poor cell retention at the target site.

To address this, advanced cell microcarriers are being developed to enhance MSC spheroid localization and retention within cartilage lesions. Protein-engineered polymers create a spheroid nano-coating system incorporating cell-binding motifs (e.g., RGD), matrix metalloprotease (MMP)-sensitive sequences (e.g., GTAR), and specific binding sequences targeting hyaline cartilage components (e.g., collagen type II) into elastin-like recombinamers (ELRs). These ELRs are produced recombinantly and chemically modified with azide and cyclooctyne to enable a layer-by-layer spheroid nanocoating approach using click chemistry.

The ELR coatings demonstrated effective binding to articular cartilage, supporting cell viability, proliferation, and controlled release. This research represents a significant advancement in cell-based therapy for DCL, paving the way for a novel and more effective approach to cartilage regeneration.

Keywords: diffuse cartilage lesion, elastin like recombinamer, biomaterial

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Posters

P01. Reconsidering morphologic elastic lamellae damages and age-related aortic stiffness in humans

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Abstract

In aging human aorta elastic lamellae fragmentation is considered as morphological evidence of biomechanical weakening leading to mechanical load transfer to collagen and progressive increase of wall stiffness.These changes, considered post-translational modifications comprising enzymatic degradation (MMPs) follow increase in diameter, wall thickness and vessel length while associated with increase of collagen fibers and relative reduction of elastin the absolute amount of which remains unchanged like the number of elastic lamellae. The supposed weakening of elastin biomechanical properties is based on morphological evidences with the risk to confuse spatial distorsion with breacks/interrumptions and despite early and recent reports indicating that elastic fibers stiffen with age. Accurate multislice morphological procedures reveal progressive deposition of collagen fibers likely accounting for both anatomical changes and consequent distorsion/dispersion of the elastic fibers composing each single elastic lamella. All that shift attention from elastin degradation to remodelling of aortic wall by collagen. In fact end-group analysis of insoluble elastin network wall shows no evidence of relevant proteolytic attack.

Keywords: Human aorta aging, elastic lamellae fragmentation, age-related stiffness, elastic fiber remodeling

* Speaker

P02. Overexpression of the mutant allele in autosomal dominant cutis laxa is normalized by TGFβ1 treatment

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Abstract

Frameshift mutations in the last 4 exons of the elastin gene (ELN) cause autosomal dominant cutis laxa (ADCL). However, the precise molecular disease mechanisms of ADCL are not understood, and therefore no targeted treatment is available. We used dermal fibroblasts from four patients with ELN mutations in exon 34 or exon 30 and matching controls. Increased intracellular transforming growth factor-beta (TGFβ) signaling was found in patients with exon 30 mutations, despite unchanged extracellular TGFβ activity. TGFβ receptor 1 levels were increased at the protein and the RNA level. Patients with exon 34 mutations had normal TGFβ signaling. Thus, mutation-specific TGFβ signaling changes in ADCL patients may influence to disease severity. Elastin assays showed decreased elastin deposition in ADCL cells and long-term TGFβ treatment improved elastin deposition. Semi-quantitative RT-PCR experiments showed increased expression of the mutant compared to the wild-type allele in ADCL cells under baseline conditions. Long-term TGFβ1 treatment normalized this allelic imbalance in expression. Therefore, we conclude that increased TGFβ signaling is a protective mechanism in ADCL at the molecular level. Uncovering the nature of connections between allele-specific elastin expression and TGFβ may help developing targeted treatments for ADCL.

Keywords: elastin gene, mutation, autosomal dominant cutis laxa, transforming growth factor beta

* Speaker

P03. Deciphering the binding mechanisms of the elastin peptides to the human elastin binding protein

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Abstract

The extracellular matrix (ECM) plays an important role in maintaining tissue function, integrity and homeostasis, and provides architectural support for cells. Elastic fibres, composed of the protein elastin, are the main components of the ECM and are abundant in tissues subject to high mechanical stress, such as the skin, lungs and arteries. Elastin derived peptides (EDPs), formed as a result of elastin degradation, have been shown to play an important role in cellular physiology and the development of pulmonary, cardiovascular, neurodegenerative and metabolic diseases. The mechanisms by which EDPs bind to the elastin receptor complex (ERC) are thought to be at the root of these pathologies. ERC has been shown to play an active role in elastogenesis and to act as sensors of elastin degradation through its ability to bind EDPs. This heterotrimeric structure is composed of a peripheral subunit, elastin-binding protein (EBP), a protective protein/cathepsin A (PPCA) and, neuraminidase-1 (NEU-1) protein. While the sialidase activity of NEU-1 is essential for signal transduction and elastogenesis, the binding of EDPs to EBP is associated with pro-tumour effects. In order to better understand the role of EBP in the binding of elastin and its derivatives, the present study proposes to use a homology model of EBP, molecular docking experiments as well as extensive molecular dynamics simulations.

Keywords: elastin derived peptides, homology modelling, molecular docking, molecular dynamics

* Speaker

P04. Elastin-derived peptides drive macrophages into an alternative M2 polarization state in atherosclerosis

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Abstract

Macrophages play a key role in the development of atherosclerosis, one of the leading causes of myocardial infarction or stroke. These macrophages have the potential to respond to microenvironmental signals, notably by a polarization toward a pro-inflammatory M1 phenotype or an anti-inflammatory M2 phenotype. Elastin is one of the main components of the extracellular matrix, and this protein can be degraded in the atherosclerotic plaque by various proteases, including cathepsin S. In this study, we demonstrate that peptides derived from elastin degradation by cathepsin S are able to induce (i) a decrease in the production of pro-inflammatory cytokines, (ii) an increase in the production of anti-inflammatory cytokines and (iii) an expression of surface markers associated with a M2 phenotype. Furthermore, our data show that the endocytosis of oxidized LDL by macrophages is decreased after elastin derived peptide treatment, suggesting a polarization towards a M2 phenotype. In conclusion, our results indicate that peptides derived from elastin degradation by cathepsin S may induce macrophage polarization towards an anti-inflammatory M2 phenotype.

Keywords: Elastin, cathepsin S, macrophages, atherosclerosis

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P05. Elastin-derived peptides favor type 2 innate lymphoid cells in COPD

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Abstract

Chronic obstructive pulmonary disease (COPD) is a condition characterized by chronic airway inflammation and obstruction. Although the involvement of immune cells in COPD pathogenesis is well established, the contribution of innate lymphoid cells (ILCs) remains poorly understood. ILCs are a type of innate immune cells that participate in tissue remodeling processes, but their specific role in COPD has not been fully elucidated. During COPD, the breakdown of pulmonary elastin generates elastin peptides that elicit biological activities on immune cells. This study aimed to investigate the presence of ILC in patients with COPD and examine the impact of elastin peptides on their functionality. Our findings revealed an elevated proportion of ILC2 in the peripheral blood of patients with COPD, and a general activation of ILC as indicated by an increase in their cytokine secretion capacity. Notably, our study demonstrated that serum from patients with COPD promotes ILC2 phenotype, likely due to the elevated concentration of IL-5, a cytokine known to favor ILC2 activation. Furthermore, we uncovered that this increase in IL-5 secretion is partially attributed macrophages upon stimulation by elastin peptides, suggesting an indirect role of elastin peptides on ILC in COPD. These findings provide insights into the interplay between elastin breakdown, immune cells, and disease progression.

Keywords: Elastin, derived peptides, innate immunity, COPD

* Speaker

P06. Evolutionary constraints on positional sequence, collective properties and sequence 'style' of tropoelastins dictated by fundamental requirements for formation and functionality of the extracellular elastin matrix

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Abstract

Previous studies of how the unusual properties of polymeric elastin arise from the sequence of tropoelastin have depended primarily on molecular biological and biophysical data. This study takes a unique alternative approach, using a database of Amniote tropoelastin sequences curated from > 80 representative species to identify characteristics that are conserved over > 300 myrs of evolution, presumably as a requirement for the fundamental properties of elastic matrices. Conserved characteristics include preservation not only of regions of linear or positional sequence, but also of collective or compositional characteristics derived from the sequence, but not strictly dependent on positional sequence. A plausible overall consensus sequence for Amniote tropoelastins allowed quantification of residue-by-residue, domain-by-domain and region-by-region levels of sequence conservation. Motif analysis indicated hPGhGG (with frequent mutations, insertions and deletions) as the underlying repeating unit of hydrophobic domains in all Amniote tropoelastins. The data identify significant evolutionary constraints dictated by fundamental requirements for formation and functionality of the extracellular elastin matrix. Mutations/polymorphisms in human tropoelastin affecting such well-conserved characteristics might be expected to have phenotypic consequences.

Keywords: tropoelastin, phylogeny, evolution, conservation, sequence, motifs

* Speaker

P07. Exploring the interactions of elastin peptides with model membranes by molecular dynamics simulations

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Abstract

Elastin is a major extracellular matrix protein. It can be found in the elastic fibers that endow tissues with elasticity and resilience (1). Elastin deteriorates with time, which can cause or contribute to pathological disorders and function loss (2). Among the hydrophobic peptides resulting from elastin cleavage, XGXXPG or XGXPGXGXG motif-bearing elastin derived peptides (EDPs) have biological activities due to their ability to bind to specific cell surface receptors and EDPs could interact directly with cell membranes (1). If so, this could affect their biological effect, or even have an impact on some pathologies.

To test this possibility, molecular dynamics simulations of 1000 ns with 3 replicas were carried out and suggested that some elastin peptides could interact with the membrane. Analyses were carried out on the peptide conformations adopted during the trajectories, as well as on the percentage of time spent at the membrane to determine if and how they would interact with lipids. Of the ten peptides tested, five appear to interact with the membrane.

(1) C.E.H. Schmelzer and L. Duca, "Elastic fibers: formation, function, and fate during aging and disease," FEBS J., 289, 3704–3730, 2022, doi: 10.1111/febs.15899.
(2) A. Heinz, "Elastic fibers during aging and disease," Ageing Res. Rev., 66, 101255, 2021, doi: 10.1016/j.arr.2021.101255.

Keywords: Elastin peptides, lipids, molecular dynamics

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P08. Identification of C5aR as a new interaction partner of membrane sialidase NEU1: potential implication of the elastin receptor complex in the regulation of the complement system

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Remodeling of elastic fibers leads to the production of elastin-derived peptides (EDP) that trigger biological effects through the elastin receptor complex (ERC). Data from the last decade have brought significant insights on the critical role played by its catalytic subunit, neuraminidase-1 (NEU1), in EDP-mediated biological effects in vascular diseases. We previously identified an action mechanism by which EDP binding to the ERC induces NEU1 catalytic activity and desialylation of key membrane glycoproteins, leading to modulation of major biological events and anticipating new biological functions for the ERC through NEU1. Here, we developed a Membrane Yeast Two Hybrid screening for the search of new interaction partners of membrane NEU1 from a human macrophage cDNA library. Several candidates were identified including the G protein-coupled receptor (GPCR) for C5a anaphylatoxin (C5aR). Interaction between NEU1 and C5aR was confirmed in HEK293 cells and results showed that NEU1 overexpression induces C5aR desialylation and increase in C5aR signaling following stimulation by its agonist. Importantly, cell preincubation with a bacterial neuraminidase recapitulated these effects. Together, these results demonstrate that NEU1 can physically interact with and desialylate GPCR, modulate GPCR signaling, and suggest that EDP may be potential modulators of the complement system.

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P09. Metabolic syndrome-associated murine aortic wall stiffening is associated with premature elastic fibers aging

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Abstract

Type 2 diabetes (T2D) is a major public health issue and remains one of the deadliest diseases worldwide. In 2022, 6.7 million T2D patients died prematurely from vascular complications. Diabetes increases the risk of myocardial infarction or stroke eightfold. Identifying the molecular factors involved in cardiovascular complications is crucial for prevention. Our hypothesis suggests that aging-related factors appear prematurely as diabetes progresses.

We studied the extracellular matrix (ECM), essential for vascular homeostasis, focusing on the collagen and elastic fibers in diabetic mice (6 months) compared to nondiabetic mice (6 and 20 months). The results revealed increased protease activity, leading to collagen accumulation and excessive elastic fiber degradation. This process generates elastin-derived peptides, a marker of premature ECM aging. These changes contribute to vascular rigidity, often linked to conditions like hypertension and atherosclerosis. In conclusion, diabetic mice at 6 months show ECM wear similar to 20-month-old mice, suggesting accelerated aortic remodeling, which may explain the early onset of cardiovascular diseases and premature death in T2D patients.

Keywords: Metabolic syndrome, aortic wall stiffening, premature elastic fibers aging

* Speaker

P10. Expanding the mutational and phenotypic spectrum in elastin-driven disease through a gene-first approach

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Abstract

Elastin is essential in the extracellular matrix and is known to be associated with vascular, skin, and pulmonary conditions. Despite sufficient evidence for clinically significant impact of elastin haploinsufficency, ELN does not demonstrate loss-of-function constraint, with prevalent missense variation across the molecule. Previously, our partners at Geisinger used MyCode data to conduct a PheWAS that revealed increased rates of large arterial dissection (corrected p< 1x10-9) in this population. However, many participants' records were absent any vascular imaging records, suggesting a need for prospective investigation.

Eighteen individuals with relevant variants of unknown significance were enrolled in a deep phenotyping study. To date, evaluated individuals have displayed near complete penetrance for vascular features, with much of their pathology not captured in the previous medical record. Relevant findings include defects of arterial size in (72%), dissections and aneurysm (22%), tortuosity (56%) and hypertension (67%). 100% of the individuals displayed at least one of these phenotypes. While ongoing, this study shows that ELN variation has a wider mutational spectrum than previously thought, with even missense variants conferring risk for vascular and other outcomes.

Keywords: genotype first, variants of unknown significance, aneurysm, stenosis

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P11. Sialic acids cleavage induced by elastin-derived peptides impairs the interaction between insulin and its receptor

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Abstract

The insulin receptor (IR) plays an important role in insulin signal transduction, the defect of which is believed to be the root cause of type 2 diabetes. In 3T3-L1 adipocytes as in other cell types, the mature IR is a heterotetrameric cell surface glycoprotein composed of two α subunits and two β subunits. Our objective is to understand how the desialylation of N-glycan chains plays a major role in the function of the IR. Using the 3T3-L1 adipocyte line, we show that removal of the sialic acid from N-glycan chains (N893 and N908), induced by the elastin receptor complex (ERC) and elastin derived-peptides (EDPs), leads to a decrease in the autophosphorylation activity of the insulin receptor. We demonstrate by molecular dynamics approaches that the absence of sialic acids on one of these two sites is sufficient to generate local and general modifications of the structure of the IR. Biochemical approaches highlight a decrease in the interaction between insulin and its receptor when ERC sialidase activity is induced by EDPs. Therefore, desialylation by EDPs is synonymous with a decrease of IR sensitivity in adipocytes and could thus be a potential source of insulin resistance associated with diabetic conditions.

Keywords: N-glycan, insulin receptor, elastin-derived peptides, insulin resistance, ligand receptor affinity

* Speaker

P12. Production of elastin-like polypeptides in fermenter for chemical post-modifications

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Abstract

Elastin-like polypeptides (ELPs) are protein-like polymers composed of (Val-Pro-Gly-Xaa-Gly) pentapeptide sequence repeats. They are widely explored for various biological and biomedical applications (e.g., tissue engineering or drug delivery) or for biotechnological applications (e.g., protein purification, hydrogels). Their design by genetic engineering and recombinant production allows an exquisite control over their macromolecular structure (sequence and length) at a level that could not be achieved using traditional polymerization methods. In addition, their lower critical solution temperature (LCST) phase behavior additionally allows their purification by a chromatography-free technique, and provides a means to control their self-assembly properties. Until now, LB medium was used, which was found to limit our production yields in fermenter. For this reason, the LB medium was replaced by ECPM1 medium, which is widely used to improve yields while preserving biochemical and physico-chemical properties of proteins. In this communication, we will report the optimization of the bioproduction of ELP(M1V3-n) with n=20,40,80 in fermenter using an ECPM1 medium and the process to purify the polypeptides.

Keywords: Elastin like polypeptides (ELPs), bioproduction in fermenter, ECPM1 medium, Met containing ELPs

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P13. Age- and sex-specific biomechanics and extracellular matrix remodeling of the ascending aorta in a mouse model of severe Marfan Syndrome

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Abstract

Thoracic aortic aneurysm (TAA) is associated with Marfan syndrome (MFS), a connective tissue disorder caused by mutations in fibrillin-1. Common findings in TAA include extracellular matrix (ECM) remodeling and altered mechanical properties. However, the time course of these changes over TAA progression and effects of sex have not been well documented. The aims of this study are to determine sex differences in the diameter dilatation, mechanical properties, and ECM remodeling over time in a severe mouse model (Fbn1ˆmgR/mgR) of MFS-associated TAA that has a shortened lifespan. Male and female Fbn1ˆmgR/mgR and wildtype (WT) mice were used at 1 – 4 months of age. In vivo diameters, ex vivo mechanical properties, and ECM organization and content of the ascending thoracic aorta were measured. We show that mechanical properties, such as structural and material stiffness, and ECM remodeling, such as elastin and collagen content, correlate with diameter dilatation during TAA progression. Male Fbn1ˆmgR/mgR mice have accelerated rates of diameter dilatation, stiffening, and ECM remodeling compared to female Fbn1ˆmgR/mgR mice that may contribute to their decreased lifespan. The correlation of mechanical properties and ECM remodeling with diameter dilatation suggest that they may be useful biomarkers for TAA progression.

Keywords: Marfan syndrome, aortic aneurysm, biomechanics, extracellular matrix

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P14. Carbamylation of elastic fibers is a molecular substratum of aortic stiffness

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Abstract

During its long lifespan, elastin is subjected to nonenzymatic post-translational modifications which alter its functional and structural properties and result in elastin molecular aging participating in the development of cardiovascular diseases. Carbamylation reaction corresponds to the binding of cyanate derived from the dissociation of urea to protein amino groups. The aim of this study was to determine whether vascular elastic fibers could be carbamylated, and if so, what would be the impact on the mechanical properties of the vascular wall. Our experiments showed that vascular elastin was carbamylated in vivo. Fiber morphology was unchanged after in vitro carbamylation, as well as its sensitivity to elastase degradation. Feeding ApoE−/− mice with cyanate-supplemented water led to an increased carbamylation rate associated with an increased stiffness of elastic fibers assessed by atomic force microscopy, whereas no fragmentation of elastic fibers was observed. Besides, this stiffness was also associated with an increase in aortic pulse wave velocity. These results provide evidence for the carbamylation of elastic fibers which results in an increase in their stiffness at the molecular level. These alterations may have significant consequences on the mechanical properties of the vascular wall suggesting a new role for carbamylation in cardiovascular diseases.

Keywords: Carbamylation, elastic fibers, stiffness, vascular wall

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P15. Microfibril-associated glycoprotein 4 forms octamers that mediate interactions with elastogenic proteins and cells

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Abstract

Microfibril-associated glycoprotein 4 (MFAP4) is a 36-kDa extracellular matrix protein with critical roles in organ fibrosis, chronic obstructive pulmonary disease, and cardiovascular disorders, including aortic aneurysms. MFAP4 multimerises and interacts with elastogenic proteins, including fibrillin-1 and tropoelastin, and with cells via integrins. Here, we present a cryo-electron microscopy structure of human MFAP4. In the presence of calcium, MFAP4 assembles as an octamer, where two sets of homodimers constitute the top and bottom halves of each octamer. Each homodimer is linked together by an intermolecular disulphide bond. A C34S missense mutation prevents disulphide-bond formation between monomers but does not prevent octamer assembly. The atomic model, built into the 3.55 Å cryo-EM map, suggests that salt-bridge interactions mediate homodimer assembly, while non-polar residues form the interface between octamer halves. In the absence of calcium, an MFAP4 octamer dissociates into two tetramers. Binding studies with fibrillin-1, tropoelastin, LTBP4, and small fibulins show that MFAP4 has multiple surfaces for protein-protein interactions, most of which depend upon MFAP4 octamer assembly. The C34S mutation does not affect these protein interactions or cell interactions. MFAP4 assemblies with fibrillin-1 abrogate MFAP4 interactions with cells.

Keywords: MFAP4, cryo-electron microscopy, elastogenic protein interactions

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P16. First-episode of psychosis during pregnancy is linked to alterations in placental elastic fiber expression and dynamics

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Abstract

Psychosis is a multifaceted clinical condition that leads to a disconnection from reality, as well as changes in behavior, sensory perception, and motor functions. Preliminary evidence supports that this condition has a significant impact in the placental tissue of pregnant women who developed a first episode of psychosis during pregnancy (FEP-PW), with enhanced oxidative stress, changes in the oxytocin and vasopressin pathways and evidence of different types of cell death such as apoptosis and ferroptosis. However, the effect of this condition on the extracellular matrix (ECM) of these placentas has not been explored yet. In this sense, the aim of the present study is to examine the possible pathophysiological role of elastic fibers and their precursors in the placentas of FEP-PW and compare them with the placentas of healthy pregnant women (HC-PW). Having this goal, we conducted immunohistochemical analysis and RT-qPCR to evaluate the protein and gene expression of tropoelastin (TE), fibrillin-1 (FBN-1), fibulins 4 (FBLN-4) and FBLN-5, LOX, and LOXL1, respectively, along with the MMP2 and MMP-9. Simultaneously, we measured the elastin content in this group of patients using histological methods, specifically orcein staining. Our results show that the placentas of FEP-PW exhibit increased content of elastin and their precursors when compared to those obtained from HC-PW.

Keywords: First episode of psychosis in pregnancy, placenta, elastic fibers, elastin precursors, matrix metalloproteinases

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P17. Fibulin-4 and LTBP-4 regulate elastogenesis via syndecan interaction

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Abstract

Elastogenesis is orchestrated by interactions among various extracellular matrix (ECM) proteins. Among these, fibulin-4 (FBLN4) and latent TGF-β binding protein-4 (LTBP4) play pivotal roles. Despite their recognized importance, the precise cell receptors and molecular pathways governing their participation in elastogenesis remain less understood. This study aims to elucidate these mechanisms using elastogenic dermal skin fibroblasts (NSF) and vascular smooth muscle cells (SMC). NSF and SMC interact with FBLN4 and LTBP4, with FBLN4 notably engaging as multimers. Cell interaction epitopes reside on FBLN4 within cbEGF2-3 and the C-terminal domain, and on LTBP4 in the N-terminal region. Cell binding to FBLN4 and LTBP4 was disrupted by heparin and attenuated by heparan sulfate or heparinase treatment, implicating heparan sulfate proteoglycans as essential cell surface receptors. Knockdown experiments and direct binding assays delineated syndecan-2 and -3 in mediating FBLN4 and LTBP4 interactions. Functionally, the interaction of FBLN4 and LTBP4 with syndecans stimulated tropoelastin assembly and elastic fiber formation through activation of focal adhesion kinase (FAK), RhoA, and ERK signaling. Inhibition of FAK, ERK1/2, or RhoA markedly attenuated tropoelastin assembly and elastic fiber formation, underscoring the significance of these pathways in elastogenesis.

Keywords: Elastogenesis, fibulin-4, LTBP-4, syndecan

* Speaker

P18. Minoxidil treatment improves vascular stiffness and cerebral vasculature perfusion and in an aged model of elastin haploinsufficiency - at a cost

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Abstract

Elastin haploinsufficiency exhibits an array of well characterized vascular phenotypes including large artery narrowing and vascular stiffness. Pharmacologic effects of minoxidil include increased elastin deposition and attenuation of vascular stiffness. As previously reported in treated younger mice, we confirm that minoxidil decreases systemic pressure and increases aortic compliance in the aged cohort, but exacts a toll on the heart in the form of hypertrophy. Similar to the aortic arch and emanating great vessels, the $Eln^{+/-}$ cerebrovasculature exhibits striking vascular similarities within the larger vessels of the brain, such as acute angles at branch points, tortuosity, and a dysmorphic appearance of the Circle of Willis. While a genotype effect in cerebral vessel caliber was not present, there was a combined mutant and control treatment effect on that feature. Unlike previous work that showed a functionally significant increase in cortical perfusion post-treatment, the aged cohort displayed a bimodal distribution in which a portion of mice responded similarly to the younger cohort with increased perfusion and the remaining group exhibiting diminished perfusion, possibly due to cardiac dysfunction.

Keywords: Elastin, cerebrovasculature, minoxidil, aged, perfusion

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P19. N-linked glycans in short fibulins and LTBP-4 mediate matrix assembly and function

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Abstract

Elastogenesis requires various extracellular proteins, including fibulin-4 and -5, and the latent TGFβ binding protein-4 (LTBP-4) long and short isoforms (LTBP-4L and LTBP-4S). These proteins are N-linked glycosylated, but the role of glycosylation during elastic fiber formation has not been determined. Fibulin-5, but not fibulin-4 induces an extension and functional change of LTBP-4S, resulting in an increase in LTBP-4 assembly and tropoelastin deposition. This data together with published data, suggest the existence of two separate developmental axes in elastogenesis (fibulin-4-LTBP-4L and fibulin-5-LTBP-4S). We found that complete deglycosylation influences LTBP4 assembly as well as tropoelastin deposition.

Loss of N-linked glycans from LTBP4L resulted in a complete abrogation of its binding to fibulin-4 and its extension, whereas removal of N-linked glycans from fibulin-4 did not affect its binding or its ability to extend LTBP-4L. Fibulin-5 N-linked glycans promoted LTBP4S molecular extension, binding, LTBP4 assembly, and tropoelastin deposition. Nglycosylation mutants of fibulin-4, when endogenously expressed, enhanced elastin assembly. The data provide new mechanisms in elastogenesis.

Keywords: Short fibulins, LTBP-4, N-linked glycosylation, elastogenesis

- Speaker

P20. Mouse arterial wall imaging and analysis from synchrotron X-ray microtomography

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Abstract

Age-related diseases can be precipitated by metabolic disorder such as diabetes. In particular, vascular aging is characterized by profound modifications of large elastic arteries and vascular diseases involve remodeling of elastic lamellae in vessel walls. Our study aims to investigate arterial alterations due to aging and age-related diseases. Some characteristics of these alterations may help find biomarkers to forecast vascular outcome. In this study, we use synchrotron X-ray microtomography to acquire arterial wall images of mice of different ages and different health status. We segment and classify various regions of interest to analyze their modifications. We apply a methodological pipeline to extract the tunica media where elastic lamellae lie. We preprocess images to reduce noise. We further segment the lumen, which has a relatively clear boundary with the inner contour of the tunica media, by a co-occurrence texture analysis. Then, we compute a normal field to define image patches and orient them homogeneously to build a dataset used to train a Siamese neural network in order to find the outer contour of the tunica media. In this analysis, patches are labeled according to their similarities based on their spatial relations. We also propose a measure to evaluate the tortuosity of skeletonized elastic lamellae fragments to quantitatively compare their geometries in different health situations. The proposed pipeline leads to a segmentation precision higher than 90% on 2D cross-sections and the proposed measure shows a significant difference between the geometries of elastic lamellae in healthy and diabetic mice.

Our current and future works are the further segmentation and classification of the 5 medial lamellae by preprocessing and analyzing stacks of 2D images, extending segmentation methods to 3D, and finally analyzing how these structures are modified during aging and pathological processes.

Keywords: aorta, aging, synchrotron imaging, image analysis, AI

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P21. Effects of solubilized elastin preparations on *in vitro* wound healing parameters

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Abstract

Biomaterials supplemented with solubilized elastin peptides may provide a platform for the development of biomimetic wound management therapies. In this study, solubilization of elastin fibres was performed by oxalic acid (OxA-ELN), potassium hydroxide (KOH-ELN) or digestion with elastase (Enz-ELN). Biological activity of obtained preparations was assessed using fibroblasts for extracellular matrix (ECM) remodelling (3 donors) and macrophages for immune response (4 donors).

Dermal fibroblasts showed that collagen deposition was not altered as assessed with Western blotting and Picrosirius Red staining. αSMA gene expression in fibroblasts was similarly low with RT-qPCR analysis for all preparations. *α*SMA protein expression was undetectable as measured by Western blotting and immunostaining. Flow cytometry showed that macrophages exposed to OxA-ELN and KOH-ELN preserved their initial M0-like phenotype, while Enz-ELN stimulated M1-like macrophage differentiation.

Further experiments will focus on assessment of elastogenesis and in vivo studies of the biomaterials in rats.

Acknowledgement: EU Horizon 2020, MSCA grant agreement No 955722.

Keywords: elastin peptides, wound healing, skin regeneration

* Speaker

P22. Investigating the regulation of elastogenesis by studying gene expression and crosslinking in an *in vitro* model system

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Abstract

The precise regulation of gene expression and cross-linking is crucial for elastogenesis, the intricate process of elastin synthesis and assembly. This process involves transcription factors, signaling pathways, and microenvironmental cues that control the expression of elastogenesis-related genes. Elastin's unique cross-links are vital for vertebrate life, providing recoil to elastic fibers and enhancing structural integrity in dynamic tissues. Understanding the regulatory networks governing elastogenesis is key to tissue-specific elastin deposition.

This study utilized recombinant tropoelastin and lysyl oxidase variants to investigate elastogenesis. The results show that these proteins significantly enhance elastin production *in vitro* and stimulate the expression of related genes, facilitating the generation of extracellular matrices enriched with elastic fibers.

Additionally, the cross-linking capabilities of recombinant tropoelastin variants with pyrroloquinoline quinone were examined. Using surface plasmon resonance, the binding affinities of these variants with key elastogenesis proteins were elucidated, providing insights into the molecular interactions driving elastic fiber assembly.

This research improves knowledge of elastogenesis and helps create functional elastic materials for tissue engineering and regenerative medicine.

Keywords: Elastogenesis, cross, linking, tropoelastin

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P23. Understanding the vascular pathophysiology caused by the dominant C19F mutation in matrix Gla protein

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Abstract

In association with vascular calcification (VC), vascular smooth muscle cells (VSMCs) in arterial walls may transdifferentiate to chondrogenic cells. We previously reported that in matrix Gla protein (MGP)-deficient mice, VSMCs express chondrogenic markers only after the initiation of VC affecting the elastic laminae, suggesting VC might induce VSMC phenotypic switching. However, in a novel mouse model with the C19F dominant mutation in MGP causing skeletal dysplasia, we observed a proteoglycan-rich matrix in the arterial walls without VC. We hypothesize that MGP mutation may cause chondrogenic transdifferentiation(C/TD) of VSMCs independent of VC.

Aim

Investigate vascular pathologies in C19F MGP-expressing mice.

Methods

VSMC morphology and proteoglycan deposition in the arterial walls in control and mutant mice were examined by histology. Immunostaining was performed to detect ER stress and chondrogenic markers. Apoptosis was assessed by TUNEL assay.

Results/Conclusion

Mice expressing C19F-MGP exhibit no VC but show thickened arterial walls with a proteoglycan rich matrix. VSMCs are enlarged and undergo C/TD, confirmed by safranin O staining, and the upregulation of aggrecan and SOX9. Although ER stress is induced, increased apoptosis is not observed. Our findings suggest that MGP may regulate C/ TD of VSMCs and VC independent of each other.

Keywords: vascular smooth muscle cells, arterial wall, vascular calcification, matrix Gla protein, chondrogenic, trans differentiation, elastic laminae, phenotypic switching, ER stress, apoptosis

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P24. Pathogenic variation in fibrillin-2 confers susceptibility to spontaneous cerebrospinal fluid leaks through impairment of cellular adhesion

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Abstract

Spontaneous spinal cerebrospinal fluid leaks (ssCSFL) have a strong association with connective tissue diseases (CTDs) with altered elastic fiber formation/homeostasis including Marfan syndrome and Loeys-Dietz syndrome. Patients with ssCSFLs without a specific diagnosis often have nonspecific CTD manifestations, suggesting that mutations in extracellular matrix proteins underlie more common presentations of this condition. We report a case control study showing a significantly increased burden of rare variants in the gene encoding fibrillin-2, a basic structural element of elastic fibers, in patients with type 1b ssCSFLs. Two of these mutations reduce human dural fibroblast adhesion to fibrillin-2 fragments in vitro. One disrupts a known integrin binding motif in the TB4 domain; another affects a completely novel binding site in the TB7 domain. Mice harboring the fibrillin-2 TB4 variant (Fbn2 D1581V/+) and a mouse model of Marfan syndrome (Fbn1 C1041G/+) demonstrate a pronounced predisposition for dural rupture upon controlled leak induction. Single-nuclear RNA sequencing of Fbn2 D1581V/+ and Fbn1 C1041G/+ mouse dura demonstrates dysregulation of tropoelastin expression in dural fibroblasts. These data suggest that mutations in FBN2 that alter cellular adhesion and/or the synthetic repertoire of matrix elements can lead to increased susceptibility to ssCSFLs.

Keywords: elastin, connective tissue disease, CSF leak, spinal CSF leak, human genetics, fibrillin-2, integrin, dura mater, mouse model

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