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Vitamin K2-Stabilizing Lung Extracellular Matrix

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Abstract

Disruption of Pulmonary Extracellular matrix is a common finding in restrictive lung disease, chronic obstructive pulmonary disease and Pulmonary artery hypertension. These diseases are characterized by excessive elastin degradation, deposition of excessive collagen and dystrophic calcification. These changes in structural ECM proteins along with alteration in non-structural matricellular proteins alter mesenchymal cell phenotype to a more osteogenic lineage perpetuating the fibrosis, and further dystrophic calcification. Matrix-Gla protein (MGP) is a vitamin K dependent extracellular protein that has demonstrated an essential role in ECM stability by inhibiting dystrophic classification thus preventing elastin fiber breakdown and suppressing osteogenic differentiation of mesenchymal stem cells by suppressing BMP2/SMAD pathway. Vitamin K supplementation has been shown to increase activity of MGP and thus may offer benefits in pathologies involving the pulmonary Extracellular matrix. Vitamin K2 MK7 is a vitamer of vitamin K, which has better systemic availability as it is absorbed via the lacteal system bypassing the portal circulation, and thus serves as a better molecule to increase gamma-carboxylation of systemic proteins, such as MGP. Poor Vitamin K status has been associated with worse prognosis in pulmonary fibrosis and COPD. Also, warfarin, a commonly used vitamin K antagonist, has been shown to increase mortality in IPF and COPD

Keywords: COPD; vitamin K; Menaquinone-7 (MK-7)

Highlights

- COPD, PAH and Pulmonary fibrosis are among the most common irreversible pathologies seen in pulmonology.
- Common features of these pathologies include excessive elastin degradation, osteogenic differentiation of mesenchymal cells leading to fibrosis and Dystrophic calcification.
- Matrix Gla protein is a vitamin K dependent secreted matricellular protein that inhibits elastin degradation, inhibits Dystrophic calcification and suppresses osteogenic phenotype of mesenchymal cells, commonly seen in the above pathologies.
- Vitamin K2 in the form of Menaquinone-7 (MK-7), a more bioavailable form of vitamin K with greater systemic availability may offer therapeutic benefits by renormalizing/enhancing MGP activity

Introduction

The lungs are unique among other vital organs, in that the passive mechanical property of lung tissue is essential for its normal functioning. Disruption of normal passive mechanical properties, due to excessive deposition and calcification of collagen, is seen in fibrotic lung disease. On the other end of the spectrum, excessive elastin degradation and calcification is seen in emphysema. Pulmonary artery hypertension is characterized by smooth muscle cell proliferation, increased pulmonary artery calcification and osteogenic differentiation of vascular smooth muscle cells.

These pathologies are characterized by significant disruption of the lung extracellular matrix (ECM), leading to changes in tissue mechanical properties. Disruption in ECM also leads to changes in phenotype of cells in this niche, which further perpetuates tissue disruption.

Stabilization of the Extracellular matrix and preventing further degradation may offer potential benefits.

Biology of the Pulmonary ECM

Extracellular matrix refers to the complex of structural polymers, associated signaling protein, cell adhesion molecules and soluble components, collectively termed as the matrisome [1]. Anatomically, ECM can be divided into 2 compartments: 1. Basement membrane, which forms a thin sheet on the basal side of endothelium and alveolar epithelium and 2. Interstitial matrices, which is a meshwork of fibrillar proteins and glycoproteins that interconnects various cell types and provide 3-dimensional structure to the tissue. These 2 compartments provide different niches for cell phenotype.

Biochemically, ECM can be simplified as having a core protein meshwork of high tensile strength low elasticity fibers, mostly collagen type I and a meshwork of low tensile strength high elasticity fibers made of elastin and fibrillin. Within this protein/glycoprotein matrix, proteoglycans are found. These consist of a core protein component covalently linked to sulfated polysaccharides and glycosaminoglycans, making them hydrophilic, allowing for hydrogel formation. This further attributes to the viscoelastic properties of the tissue. Several adhesion molecules and microfibril associated proteins are bound to the protein meshwork, which allow cell-matrix communication and signal transduction. Also within the matrix are found fibril assembly proteins like the cross linking enzyme, Lysyl Oxidase, proteases like Matrix metalloproteinase which break down these molecules and anti-proteases which inactivate proteases. ECM is a dynamic structure, layed down and maintained by various mesenchymal derived cells, and their secreted enzymes.

Collagen is secreted in the form of soluble pro-collagen by fibroblast in the interstitium. It undergoes cleavage via collagen peptidase to form tropo-collagen, which is then assembled by cross linking with the help

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of the enzyme Lysyl-oxidase. Total tissue collagen is in equilibrium due to deposition and breakdown, and remodels under stress. Elastin is synthesized by vascular smooth muscle cells and endothelial cells. They is secreted as soluble tropoelastin which undergo spontaneous aggregation in the extracellular space. The elastin aggregates undergo elastin fiber assembly [2], where a core of amorphous elastin aggregates is sheathed with a scaffold of microfibrils, which are composed mostly of fibrillin [3]. Unlike collagen, most of elastic fiber depositions occur in the developmental stage, with very poor synthesis seen in adults.

Pathology of the Pulmonary ECM

Even though the underlying pathogenesis of pulmonary fibrosis, emphysema and pulmonary artery hypertension are distinct and diverse, they share a triad of self-reinforcing pathologies: Elastolysis, fibrosis, and calcification Figure 1. Chronic inflammation triggers a change in the extracellular matrix, and is considered to be the driving mechanism behind these lung diseases. Matrix-Metalloproteinase (MMPs), secreted by macrophages, along with several other proteases, increase breakdown of the deposited fibers. Most MMPs are broad spectrum and have significant elastolytic activity along with collagen degradation. The breakdown of these fibers function as signaling molecules to further induce inflammation and trigger initially myo-fibroblastic differentiation of fibroblast, leading to fibrosis by deposition of collagen and later osteo-fibroblastic differentiation, associated with dystrophic calcification [4]. This pattern of mesenchymal cell differentiation to a more osteoblastic phenotype regulated via BMP/SMAD pathway and is also seen in PAH, where vascular smooth muscle cells undergo similar changes [5]. Dystrophic calcification ossifies the fibrotic sites in the lung leading to further fibrotic activity. Since elastin is slow to renew, collagen deposition greatly reduces tissue elasticity. Calcification of elastin fibrils greatly increases the rate of elastin degradation, further perpetuating the triad. Microscopic calcific foci are sites of elastolysis in the initial phase of disease.

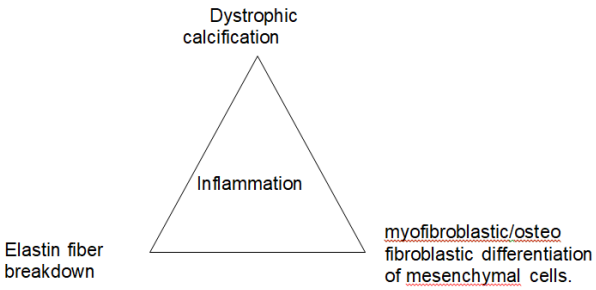

Figure 1: Chronic inflammation triggers

Emphysema Triggered by chronic inflammation, most commonly from cigarette smoke exposure. Inflammation leads to protease dependent loss of elastin and excessive deposition of collagen in the interalveolar space is a key feature of emphysema [6]. Desmosine and isodesmosine (DES) are two amino acids that are only present in crosslinked elastin fibers, and plasma DES levels have been directly correlated with worsening disease progression and prognosis, indicating higher elastin degradation rates. Net collagen content of the lung is increased in an emphysematous lung, pointing out that collagen remodeling rather than collagen loss is a feature of emphysema [7].

Pulmonary fibrosis-Chronic inflammation has been implicated in the pathogenesis of pulmonary fibrosis. Characterized by decrease in lung elasticity due to excessive deposition of collagen and formation of fibrotic foci. Collagen deposition is promoted due to myofibroblast proliferation [8]. Chronic inflammation also leads to dystrophic calcification. Calcific foci and even bone tissue has been found in cases of IPF. Dendriform pulmonary ossification is also a consequence of Idiopathic pulmonary fibrosis, seen in advanced disease [9]. However, microscopic calcification seems to be a common finding in pulmonary fibrosis [10].

Pulmonary artery hypertension The pathogenesis of Pulmonary artery hypertension is diverse. They are classified into Type 1 to 4, based on the likely etiology. Irrespective of the etiology PAH is characterized by increased stiffness of the pulmonary artery and luminal narrowing, due to increased vascular smooth cell (VSMCs) proliferation and collagen deposition. Osteogenic differentiation of VSMCs and formation of calcific foci, is also seen in PAH, similar to those seen systemic hypertension, and may potentially play an important pathophysiological role in PAH [11].

Mechanism of Dystrophic Calcification

Dystrophic calcification is a common occurrence around regions of inflammation and cell injury. During cell injury, intracellular calcium is released into the extracellular space. This is accompanied by release of phospholipids from the dying cells. This leads to saponification, forming FFA-ca2+ soaps. Once these saponified loci are formed, they serve as sites for further calcium precipitation. Further, high collagen densities increase the rate of growth and stability of calcium salt crystals [12]. Therefore, it can be seen that inflammation and fibrosis makes tissue prone to dystrophic calcification and these calcific foci further perpetuate inflammation and fibrosis.

Matrix-Gla Protein and Tissue Calcification

Dystrophic calcification is a term given to pathological deposition of mineral salts of calcium, in the extracellular matrix. Dystrophic calcification is increased in chronic inflammatory states and plays a crucial role in setting up perpetual cycles of fibrosis and further calcification. Calcification of elastin and collagen increases local MMPs activity, and significantly increases degradation of these fibrils. This phenomenon is more important for elastin, as they are particularly prone to calcification given their amorphous nature. Preventing dystrophic calcification is vital for normal tissue functioning, and as such several mechanisms have evolved. Plasma calcium is closely regulated via the interplay of Parathyroid hormone, vitamin D and calcitonin. The Matrix GLA protein-fetuin complex in serum appears to prevent propagation of calcium phosphate precipitation [13] Tissue calcification has been extensively studied in the context of vascular calcification and the crucial role of matrix-gla protein in preventing and reversing such calcification has been made apparent [14].

Matrix GLA protein (MGP) is a small protein secreted by the endothelium and vascular smooth muscle cells. A vitamin K dependent protein, it possesses 4 sites for gamma carboxylation of glutamate residue [15]. This is an essential posttranslational modification for the calcium chelating properties of MGP. MGP undergoes the crucial gamma-carboxylation step in the endoplasmic reticulum of these cells, before secretion

via the Golgi pathway. However, the extent of gamma-carboxylation depends on the availability of a reduced form of Vitamin K, vitamin k hydroquinone (KH2). Under scenarios of low vitamin K status such as nutritional deficiency [16] or use of warfarin [17], uncarboxylated MGP (uc-MGP) is secreted and accumulates in the tissue [18]. These can be detected via histo-chemical tests, and uncarboxylated MGP may serve as a marker for vitamin K status [19]. As stated above, gamma carboxylation is essential for the calcium chelating properties of MGP, with uc-MGP showing minimal calcium binding property. MGP also plays a role in the inhibition of BMP2 pathway, by directly binding to BMP2, over activation of which is seen in fibrotic/osteoblastic differentiation of vascular smooth muscle cells and fibroblasts [20,21].

Warfarin

Warfarin use serves as a model to study the effect of chronic vitamin k deficiency and its effect on calcification. Warfarin acts as a non-selective Vitamin K epoxide reductase inhibitor (VKOR), and as such will impair recycling of Vitamin K. This leads to accumulation of vitamin K epoxide and a deficiency in Vitamin K hydroquinone (KH2), the active form of this vitamin. By depleting KH2, warfarin prevents gamma-carboxylation of vitamin K dependent proteins. Inhibition of gamma-carboxylation of clotting factors, suppresses the coagulation cascade. But warfarin will also inhibit gamma carboxylation of other VKDP including protein S, protein C and matrix-gla protein [22]. Warfarin has in fact shown to increase arterial calcification [23]. Warfarin use was also associated with worse outcome and increased mortality in the ACE-IPF trial [24], to the point that the trial had to be terminated early. Mortality rates were 14/72 in the warfarin group vs 3/73 in the placebo group. Retrospective studies have also seen this correlation [25]. Other Authors have suggested prohibiting the use of warfarin in IPF [26]. Warfarin induced pulmonary metastatic calcification has been reported in the setting of end stage renal disease [27]. Warfarin use has also been associated with increased mortality in COPD patients [28]. Low nutritional vitamin K status in patients with COPD demonstrate worse prognosis and increased elastin degradation as assessed by plasma desmosine and isodesmosine levels [29,30] Vascular calcification is also a known consequence of prolonged warfarin use [31] and inhibition of MGP carboxylation has been proposed as one of the possible mechanisms.

Discussion

Vitamin K2-MK7

Vitamin K is a term given to a group of molecules that serve as an essential cofactor for the enzyme Gamma-glutamyl carboxylase. Located in the Endoplasmic reticulum, this enzyme is vital for gamma-carboxylation of glutamate residue of various vitamin K dependent proteins. Clotting factors are much more well-known vitamin K dependent proteins (VKDP), but certain important systemic VKDP may play important physiological roles. Matrix-Gla protein has been discussed above. Other important protein in the context of lung disease is, protein S, a VKDP, majority of which is synthesized in vascular endothelium and plays important role in inactivation of factor Va and VIIIa and also serves to improve phagocytosis of apoptotic cells by macrophages which prevents uncontrolled inflammation [32].

Vitamin K is an essential molecule, required for the function of the above mentioned gamma carboxylation process. It comes in two natural forms. Both forms have a central menadione ring, with different side chains. Vitamin K1 has a phytyl side chain attached to it. Phytyl chains are a common occurrence in chlorophyll, and vitamin K1 is the major form of vitamin K we get from plants. Vitamin K2 has varying length side chains of isoprenoid groups. Most common being 4 or 7 carbon chains, termed MK-4 and MK-7 respectively [33]. Vitamin K2 is the dominant form for storage of vitamin K in mammals as these are more fat soluble. Another interesting consequence of this increased fat solubility is the vitamin k2 has greater systemic penetrance as compared to K1, Being more fat soluble it is absorbed into the lacteal system and bypasses the liver, and thus demonstrate better systemic availability and a longer half-life as compared to K1.MK7 has been shown to better increase gamma carboxylation of MGP in blood vessels and tissues [34].

Conclusion

MGP is a VKDP that plays a crucial role in maintaining the ECM. By chelating calcium salts, it prevents formation of dystrophic calcific foci, thus preventing initiation of elastolysis. MGP blocks the action of BMP2 preventing fibrotic/osteogenic differentiation of fibroblast and vascular smooth muscle cells, thus normalizing cell phenotypes. It serves to act on all three crucial aspects of ECM pathology, namely preventing elastin degradation, inhibiting tissue calcification and preventing osteogenic differentiation of mesenchymal cells. Vitamin K is essential for the activation of MGP and vitamin K2 being a more systematically available form of Vitamin K may serve a role in improving activity of MGP, and in theory slowing the progression of pulmonary diseases.

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