Pathogenesis of Pulmonary Calcification and Homologies with Biomineralization in Other Tissues

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Lungs often present tissue calcifications and even ossifications, both in the context of high or normal serum calcium levels. Precise mechanisms governing lung calcifications have not been explored. Herein, we emphasize recent advances about calcification processes in other tissues (especially vascular and bone calcifications) and discuss potential sources of calcium precipitates in the lungs, involvement of mineralization promoters and crystallization inhibitors, as well as specific cytokine milieu and cellular phenotypes characteristic for lung diseases, which may be involved in pulmonary calcifications. Further studies are necessary to demonstrate the exact mechanisms underlying calciﬁcations in the lungs, document homologies in biomineralization processes between various tissues in physiological and pathologic conditions, and unravel any locally speciﬁc characteristics of mineralization processes that may be targeted to reduce or prevent functionally relevant lung calcifications without negatively affecting the skeleton. (Am J Pathol 2022, 192: 1496–1505; https://doi.org/10.1016/j.ajpath.2022.07.015)

Mineralization is one of the fundamental chemical processes in living organisms,1 and is involved in various physiological functions. Primarily, mineralization secures structural rigidity and strength, which is of high importance for bone and teeth.2 Indeed, calcified tissues withstand mechanical loads and protect vital organs in body cavities. Moreover, mineralized tissues are a reservoir for calcium, and some of them can be partially resorbed to provide sufﬁcient calcium in cases of low calcium concentrations in blood.3 There is continuous research interest in biomineralization processes in both physiological and pathologic conditions. In humans, physiological mineralization occurs in calcified tissues, such as bones, teeth, and calcified fibrocartilage, and is also present in otoliths in the inner ear.4 However, experimental and clinical reports indicate that pathologic mineralization may occur in various soft tissues,5 such as blood vessels, kidneys, lungs, thyroid gland, prostate, gastric mucosa, central nervous system, and eye. However, there is still insufﬁcient understanding of the mechanisms of soft tissue calcifications, including their similarities with and differences from bone mineralization and other mineralization processes in physiological and pathologic conditions. In particular, lung calcifications have rarely been investigated. Studies on the lung calcifications have typically focused on radiographic evaluation and differentiation of calcifications from other interstitial diseases, as well as on reports of individual cases with radiological and macroscopic/microscopic observation.6–8 However, comprehensive mechanistic overview and discussion of possible mechanisms of lung mineralization have been lacking. This review summarizes recent advances on biomineralization processes, with a particular emphasis on the potential mechanisms of lung mineralization. It also brings attention to homologies observed between different tissues as well as to some peculiarities of certain tissues with respect to biomineralization processes.

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**Lung Calcifications**

Lungs are one of the organs displaying frequent calcifications and even ossifications. They include calcifications of parenchyma, alveoli, blood vessels, pleura, and lymph nodes (Figure 1). Lung calcifications accompany several lung diseases and are not uncommon findings on the imaging or autopsy procedures. Among them, dystrophic calcifications occur in patients with damaged lung tissue (eg, granulomatous infections, tuberculosis, histoplasmosis, or hemorrhage), whereas metastatic calcifications are frequently seen in patients without primary lung disease, in the setting of end-stage kidney disease, primary or secondary hyperparathyroidism, sarcoidosis, intoxication with vitamin D, or metastatic deposits in bone. Lung calcifications may also be seen in some benign tumors (chondroma) or malignant tumors (adenocarcinoma), or in organizing pneumonia. Calcifications can be punctate or extensive, and may indicate severity and chronicity of disease. Apart from lung calcifications, there are also cases with real lung ossifications. Ossification in the lungs reflects metaplastic bone formation within the lung tissue, which almost exclusively develops on the ground of an underlying lung disease. For example, Bin Saedan et al have reported a case of cicatricial organizing pneumonia accompanied with dendriform pulmonary ossification. A recent case of accelerated lung ossification was deemed a consequence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia. Although dendriform pulmonary ossification is typically related to usual interstitial pneumonia, Gruden et al reported a case where the most likely risk factor was low-level, chronic aspiration of gastric acid due to gastroesophageal reflux disease.

**Types of Calcifications in the Lungs**

Because calcium phosphate is considered metastable, passive calcifications in various tissues may easily occur in presence of hypercalcemia (metastatic calcifications). In the lungs, these calcifications present as fine, reticular deposition of calcium. However, calcium phosphate may also precipitate despite normal serum calcium (dystrophic calcifications), typically as aggregates of calcium on a background of fibrosis, chronic granulomatous disease, or repair reaction. Some mechanisms may involve local sensitization of tissues to calcium precipitation, including high phosphate, parathyroid hormone, vitamin D, more alkaline pH, uremia, as well as local tissue damage. However, precise mechanisms governing lung calcifications have not been explored.

**Possible Mechanisms of Calcifications**

In general, although it is tempting to believe that the source of calcium for calcification is primarily extracellular, the actual data from other tissues (bone, vascular media, and calcified cartilage) suggest an important role of intracellular calcium in initiating the calcification process. Namely, two main suggested mechanisms of active cellular involvement in the initiation of mineralization include extracellular vesicles, such as matrix vesicles and apoptotic bodies. Extracellular vesicles are a heterogeneous set of membrane-covered particles, which are released by almost all mammalian cell types. Interest in extracellular vesicles is continuously growing as the evidence of their importance for numerous biological processes has been becoming apparent. Indeed, they are currently considered crucial mediators of intercellular communication. Matrix vesicles are membrane-bound particles approximately 100 nm in size. They are released by numerous cells and contain various molecules, such as proteins, lipids, carbohydrates, and nucleic acids (DNA, RNA, and small RNAs, such as miRNA). The calcification potential of matrix vesicles depends on their origin and composition. Indeed, only matrix vesicles with a certain composition initiate the calcification process. The calcification-competent matrix vesicles contain annexins (such as annexin A5, annexin A2, and annexin A6), phosphatidylinerse, S100A9, phosphate transporters, and various phosphatases, and an amorphous calcium phosphate nucleus is formed within them. As demonstrated by Kirsch et al, influx of calcium ions into phosphatidylinerse-enriched liposomes originating from authentic matrix vesicles and intact authentic matrix vesicles is mediated by annexins A2, A5, and A6. The specific presence of phosphatidylinerse on the surface of liposomes or matrix vesicles has been shown to induce the formation of a hexameric annexin A5, its insertion into the lipid bilayer, and consequent calcium ion influx into matrix vesicles. As summarized by Anderson et al, phosphatases contained in calcium-competent matrix vesicles include alkaline phosphatase (10-fold concentrated on the surface), adenosine monophosphoesterase, ATPase, inorganic pyrophosphatase, and phosphoethanolamine/phosphocholine phosphatase 1 (Phospho1), which are able to hydrolyze organic or inorganic phosphates to provide phosphate for mineralization. Therefore, specific composition of matrix vesicles endows them with the ability to attract and accumulate calcium and concentrate inorganic phosphate, thereby generating clear nucleation sites for calcium phosphate. Studies of growth plate mineralization indicate that mineralization initiated by matrix vesicles consists of two phases. In the first phase, calcium phosphate nuclei are formed within matrix vesicles based on the above-mentioned calcium- and phosphorus-accumulating abilities of matrix vesicles and physical processes of calcium phosphate precipitation. In the second phase, the formed hydroxyapatite crystals penetrate the membrane of matrix vesicles, probably by the function of phospholipases, and are exposed to the extracellular fluid. Further mineral proliferation and propagation is then dependent on the extravesicular concentrations of calcium and phosphate ions, the pH, the presence of various molecules, and the availability of extravesicular.
substrates for phosphatases, such as ATP, AMP, and pyrophosphate (PPi), which are hydrolyzed for incorporation into the mineral. However, although there is general evidence that extracellular matrix vesicles serve as foci of nucleation to initiate microcalcification, no studies have specifically focused on lung calcifications. Recent reviews have emphasized the importance of extracellular vesicles as mediators involved in the processes of lung repair, remodeling, or regeneration. There is also growing interest in the importance of extracellular vesicles for chronic lung diseases, such as chronic obstructive pulmonary disease, asthma, lung cancer, pulmonary hypertension, and lung fibrosis. However, although extracellular vesicles have been implicated in the cross talk involved in pulmonary fibrosis and other lung diseases, there has been no direct research evaluating their mineralization competence and involvement in lung calcifications. Nevertheless, an animal study showed that transforming growth factor-β and Wnt signaling were promoted in lungs of mice undergoing pulmonary fibrosis. Extracellular vesicles isolated from fibrotic lungs promoted pulmonary fibrosis in mice by up-regulating transforming growth factor-β/Wnt signaling. Wnt5a level was also shown to be increased in extracellular vesicles in individuals with idiopathic pulmonary fibrosis. Considering the importance of Wnt pathway, especially Wnt5a, in calcification and osteogenic differentiation, as reported in other tissues, these pathways may be involved in at least some of the calcification processes in the lungs.

Of note, recent data from cardiovascular calcifications suggest roles for vascular smooth muscle cells and macrophages in the release of calcium-loaded matrix vesicles. Given that the lungs are one of the organs with the highest vascular density in the human body, such a role of VMSCs should not be neglected.

Unlike matrix vesicles, which are usually considered to be released from living cells, apoptotic bodies are a product of a regulated process of cell death. However, there is increasing body of evidence for the presence of apoptotic exosomes and their roles in inflammation, indicating that apoptosis provides an active communication from dying cells to living cells rather than being just a silent cell death. Apoptotic bodies may also serve as nidi for calcification, based on their specific membrane composition and increased intracellular calcium as the initiator of apoptosis. Once calcification starts, they may serve as mineralization nidi for further addition of the mineral crystals, as well as crystal growth and coalescence, even in the context of normal serum calcium levels. A previous study showed apoptotic markers in bronchial and alveolar epithelial cells in idiopathic pulmonary fibrosis, and this could be one potential link for the observed calcifications in pulmonary fibrosis. Further research is needed to confirm such assumptions, but research from other tissues, such as
on vascular calcifications, provides sound basis for such an assumption.

**Insights from Bone**

Mineralization is an essential and physiological characteristic of bones that endows them with strength, hardness, and load-bearing capacity. Moreover, bone provides the largest storage of calcium in the body, from which calcium can be released to blood to ensure extracellular calcium homeostasis. However, mineralization is not exclusively a normal process in bone tissue, as it may also occur in relation to the local pathologic events in bone. Namely, recent research in bone tissue has highlighted a remarkable phenomenon of mineralization of osteocytic lacunae, representing a witness of previous osteocyte cell death.\(^{17,43}\) Impregnation of apoptotic osteocytes with calcium, which has also been recognized as in vivo fossilization of osteocytes,\(^ {17,48}\) may be an alternative way of disposing of the cellular remnants, considering that it is almost impossible for many of the apoptotic bodies to be taken up by the surrounding osteocytes, given the hard mineralized matrix around the cells.\(^ {17}\) Specifically, osteocyte lacunae are connected by canaliculi, which are of the order of 200 to 300 nm in diameter, whereas apoptotic bodies in osteocyte lacunae may even reach the size of 5 μm.\(^ {39}\) In this setting, most of the apoptotic bodies remain entrapped in the lacunae, and cannot be internalized by neighboring osteocytes, so an alternative way for their elimination or control is necessary, which is reflected in their impregnation with mineral (so-called micropetrosis). Although this mechanism is understandable in bone tissue, the biological justification for a similar process to occur even when apoptotic bodies can be easily phagocytosed by normal surrounding cells, such as in vascular walls and other tissues is unclear. In this context, one should think particularly about other promoters of crystallization process and/or relative lack of crystallization inhibitors, but also about contradictory roles of various types of macrophages in pathologic conditions.

**Signature of Promoters of Crystallization in the Lungs**

Mineralization process involves formation of insoluble forms of calcium salts, and the balance of crystallization and dissolution may be affected by several factors. For example, in terms of chemical laws, relatively alkaline environment is known to increase chances for calcium phosphate precipitation.\(^ {40}\) The lung apex has a relatively more alkaline environment because of a higher ventilation/perfusion ratio,\(^ {6}\) which may increase the risk of calcification in that area of the lungs. The fact that calcification does not occur there under normal conditions suggests that additional factors are necessary, including systemically or locally increased calcium level and other factors that sensitize pulmonary tissue to calcium levels (Figure 2).

Indeed, there are various molecules that may promote mineralization in the lungs. An important example is tissue-nonspecific alkaline phosphatase (TNAP), which is mainly expressed by type II alveolar cells and bronchiolar exocrine cells, but also by other cell types.\(^ {30}\) Although there is still a lot to learn about the roles of TNAP in biological mineralization processes, TNAP may be involved in a critical step of matrix vesicle-mediated mineralization. Namely, matrix vesicles accumulate phosphate, which is mediated by TNAP, annexin, ectonucleotide pyrophosphatase/phosphodiesterase (ENPP1), and sodium-dependent phosphate transporter 1 (Pit1). Pyrophosphate is generated from extracellular ATP by ENPP1; TNAP catalyzes hydrolysis of pyrophosphate into phosphate ions, and Pit1 transports them into the matrix vesicle, where they accumulate together with calcium ions to provide substrate for crystallization.\(^ {51}\)

In addition, the presence of 1α-hydroxylase has been documented in the lungs,\(^ {32,53}\) showing that vitamin D3 can also be produced locally in airway epithelium, alveolar macrophages, dendritic cells, and lymphocytes. These affect local calcium levels in the lung tissue, and subsequently, the chance for mineralization.

Collagen might also play a role in the process of lung calcification/ossification. Kirsch et al\(^ {25}\) showed that binding of collagens II and X to annexin A5 stimulated calcium influx into liposomes originating from lipids extracted from matrix vesicles in growth plate cartilage. Similarly, Genge et al\(^ {54}\) showed that collagens II and X markedly increased mineral formation by matrix vesicles in an annexin A5-dependent manner. However, these two types of collagen have not been reported in the lungs.\(^ {55}\) Whether collagen types present in organizing pneumonia or lung fibrosis (such as collagens I, III, and VI)\(^ {56}\) have a similar effect on calcium taken up by matrix vesicles is unclear.

Another molecule potentially involved in lung calcification/ossification is runt-related transcription factor 2 (Runx2). Individuals with pulmonary arterial hypertension have calcified lesions in the distal pulmonary arteries, which is accompanied by up-regulated Runx2 in the lungs and distal pulmonary arteries.\(^ {57}\) The expression of Runx2 is also up-regulated in animals with bleomycin-induced lung fibrosis as well as in lung homogenates from patients with idiopathic pulmonary fibrosis.\(^ {58}\) Of note, the increased Runx2 expression appears to favor worse outcomes, given that it is associated with worse survival in patients with idiopathic pulmonary arterial hypertension and with reduced diffusing capacity in idiopathic pulmonary fibrosis.\(^ {57,58}\) Hence, Runx2 has been clearly linked to profibrotic phenotype in the lungs.\(^ {38}\) Considering also that Runx2 has been implicated in vascular calcifications, and is considered one of the key drivers of osteogenic differentiation of mesenchymal stem cells, there may be a link between profibrotic and osteogenic phenotypes in the lungs. However, clinical reality does not support an extensive overlap
between the two phenotypes, so further studies should try to elucidate on what basis the same signaling pathways sometimes favor osteogenic differentiation.

**Crystallization Inhibitors in the Lungs**

The lack of crystallization inhibitors (such as fetuin A, fibroblast growth factor 23, Klotho, pyrophosphate, and matrix Gla protein)\(^{16,40}\) may be another important factor contributing to the propagation of calcification in soft tissues. Although there is still scarce evidence for the lack of specific crystallization inhibitors in the lungs, TNAP, which is expressed in the lungs and presumably increased in interstitial lung diseases,\(^{50}\) would break down pyrophosphate (a crystallization inhibitor), thereby additionally facilitating calcification. Indeed, a recent study on an animal model has shown that TNAP inhibitor (SBI-425) is able to block medial arterial calcifications, which is not followed by any negative effects on the skeleton.\(^{59}\) It would be interesting to see whether similar effects could be achieved in other soft tissues, including the lungs. Fetuin A is another protein implicated in the inhibition of ectopic calcifications, which suggests that the relative or absolute lack of fetuin A would favor calcification of various soft tissues. Indeed, a recent animal study has shown that mice deficient in fetuin A had calcified lesions in various organs, such as the heart, lungs, spleen, pancreas, kidney, skin, and adipose tissue, as shown by live imaging by microcomputed tomography.\(^{60}\) Another suggestion of the study by Herrmann et al\(^{60}\) is that calcified lesions in fetuin A–deficient mice start in the lumen of microvessels, which suggests the importance of disturbed mineral ion handling in the extracellular fluids. Although that study clearly provided experimental support for the importance of fetuin A in promotion of lung calcifications,\(^{60}\) the relevance of this finding in a clinical context and in various lung diseases is unknown.

Research on rare genetic diseases, such as pseudoxanthoma elasticum, generalized arterial calcification of infancy, and calcification of joints and arteries, may provide further insights into the mechanisms of ectopic calcifications. These diseases are mostly related to the impairment of the extracellular ATP catabolic pathway. For example, generalized arterial calcification of infancy is characterized by loss-of-function mutations in \(ENPP1\), which encodes an extracellular enzyme known as ENPP1. Given that ENPP1 converts ATP into AMP and pyrophosphate, the loss of ENPP1 activity reduces the level of pyrophosphate both locally and systemically, which causes ectopic mineralization in various tissues.\(^{61}\) Pseudoxanthoma elasticum results from a loss-of-function mutation in \(ABCC6\), the gene that encodes a transporter that mediates a major part of ATP release from cells.\(^{62}\) Hence, the lack of ATP-binding cassette subfamily C member 6 (\(ABCC6\)) protein is responsible for the reduced level of pyrophosphate,\(^{62,63}\) which favors ectopic mineralization in various tissues, including the lungs. Recent data have also highlighted the role of plasma membrane protein ankylosis homologue in
preventing pathologic mineralization. By promoting the release of ATP out of the cells, which is then hydrolyzed extracellularly into pyrophosphate and AMP by ENPP1, ankylosis homologue controls extracellular levels of pyrophosphate and prevents excessive mineralization. Ankylosis homologue provides about 25% of plasma pyrophosphate, whereas 60% to 70% of plasma pyrophosphate is derived from the ATP extruded by ABCC6.

Another important inhibitor of calcification is α-Klotho, and its deficiency has been experimentally linked to lung calcifications. Namely, it has been shown that Klotho-hypomorphic mice (kl/kl) experience severe tissue calcifications, including calcification of trachea, lungs, kidneys, heart and vessels, and stomach. Moreover, these mice showed increased plasma levels of 1,25-dihydroxycholecalciferol and phosphate, and respiratory acidosis due to lung emphysema. However, treatment with ammonium nitrate was able to reduce calcification of the organs, including the trachea and the lungs, without effects on pH and plasma levels of vitamin D3, calcium, and phosphate. These findings suggest that these calcifications are not dependent on the plasma vitamin D and calcium levels. There is evidence that the lungs do not express native α-Klotho, but only receive full-length α-Klotho from circulation. This may explain why the lungs may be susceptible to calcification in chronic renal failure or other conditions with low circulating α-Klotho levels.

Finally, depending on the primary lung disease, specific cytokine milieu and cellular activation may have bearing on the risk of calcification. For example, although M2 macrophages are considered a profibrotic adaptation, there are also data on the role of M1 macrophages in lung fibrosis. Whether the presence of calcifications or even osteogenesis in interstitial lung diseases is associated with a higher proportion of M1 macrophages, a macrophage subpopulation mainly reported to release procalcific matrix vesicles, reduce pyrophosphate level via ectonucleoside triphosphate diphosphohydrolase 1 (eNTPD1), and favor osteogenic differentiation of cells through bone morphogenetic protein 2 (BMP-2) signaling needs to be analyzed. It has also been shown that M1 macrophages release oncostatin M to stimulate osteoblastic differentiation of vascular smooth muscle cells via the Janus kinase 3 (JAK3)-STAT3 signaling pathway. Predominance of M2 macrophages is related to worse prognosis in interstitial lung diseases. However, whether there is any clear relationship between presence of calcifications due to hypothetical M1 macrophage response and disease prognosis is not known.

Ectopic Mineralization of Other Tissues: Important Lessons to Drive Research of Lung Calcifications

Most of the data on ectopic mineralization derive from vascular calcifications, considering that they are related to the important processes of atherosclerosis and poor cardiovascular outcomes. Vascular calcification often affects the aging population as well as individuals with metabolic diseases, such as diabetes and dyslipidemia. In the process of vascular calcification, vascular walls can even acquire some functional characteristics of bone, which suggests that the mechanisms of calcification may resemble those in bone development. Despite the long belief that vascular calcification is a passive calcium precipitation process, several studies have established that it is rather an active cell-mediated process of hydroxyapatite crystal deposition in the vascular wall. This process heavily depends on vascular smooth muscle cells and macrophages and the changes in their phenotypes. Research on vascular calcification has been reviewed recently and extensively. In brief, previous studies have identified several factors involved in the pathogenesis of vascular calcification; some of them include alterations in systemic or local calcium and phosphorus levels, differentiation of vascular wall cells in a chondrogenic/osteogenic direction, reduced levels of mineralization inhibitors, inflammation, apoptosis, and release of calcified vesicles. Although M1 macrophages may favor calcification by promoting the expression of proinflammatory cytokines and releasing calcifying matrix vesicles, M2 macrophages may have the opposite effect. Nevertheless, recent research has suggested phenotypic diversity of macrophages beyond the usual distinction in only two types, and under certain conditions, most of these subpopulations can convert to each other. Clearly, most of the reported mechanisms, such as inflammation, apoptosis, osteogenic differentiation, and release of vesicles, may be shared with other tissues. Therefore, research on vascular calcifications (as well as research on bone development) may provide inspiration for research of mineralization phenomena in other tissues.

Nephrocalcinosis shares some features and pathogenetic mechanisms with vascular calcification. It is compatible with metastatic calcification of the renal interstitial tissue. In addition to several genetic disorders, diseases such as primary hyperparathyroidism, distal renal tubular acidosis, and medullary sponge kidney have been most often linked to nephrocalcinosis in a large clinical series from the United Kingdom. It has been suggested that local and/or systemic dysregulation of calcium homeostasis may be involved in the pathogenesis of nephrocalcinosis. Chronic kidney disease of various causes alters the homeostasis of calcium and phosphorus, but also reduces the bioavailability of some crystallization inhibitors (eg, α-Klotho) in the lungs and other tissues, thereby favoring calcification processes.

Considerable knowledge about mineralization processes derives from studies on growth plate cartilage ossification. Such studies have clearly shown that chondrocytes release matrix vesicles especially rich in alkaline phosphatase and annexin A5 to initiate mineral formation, and eventually die by apoptosis. Moreover, it has been shown that chondrocytes in the pathogenesis of osteoarthritis adopt the
phenotype similar to chondrocytes in growth plate cartilage, which promotes cartilage destruction.

**Molecular Composition of Calcifications**

The question of molecular composition of soft tissue calcifications has not received sufficient research, in part because of technical challenges. In terms of bone mineral, there is a general consensus that it is composed of hydroxyapatite, but there is already sufficient evidence that it does not match the stoichiometric hydroxyapatite. Bone mineral may rather be a spatial and temporal mosaic of slightly different calcium phosphate phases, and contains several impurities and deviations from pure hydroxyapatite. Indeed, abundant research in bone mineral has also shown that the composition of bone mineral is a dynamic category, as it changes with age and in various diseases, which affects its mechanical competence and the risk of fracture. In that context, it is important to examine the exact chemical nature of calcifications in soft tissues as well. In terms of lung calcification, the early research has suggested that they have >50 times higher calcium content than normal, but it was also interesting that magnesium content was five times higher than normal; however, there has not been profound molecular characterization of lung calcifications. The original report of the increased magnesium content in lung calcifications has some similarities with mineralization of osteocyte lacunae and vascular calcifications, but further research is necessary to explore whether there may be any lung-specific variations in composition of calcifications depending on the disease and conditions. For example, multiple studies have shown differential composition of breast calcifications associated with benign and malignant lesions; one of the most consistent findings is a reduction in carbonate content in malignant lesions. Such evaluations require the use of spectroscopic techniques, such as Raman and Fourier-transform infrared spectroscopy, but to the best of our knowledge, these analyses have not yet been conducted on the lung calcifications.

**Perspectives**

Further studies are necessary to demonstrate the exact mechanisms underlying calcifications in the lungs as well as in other tissues. It will be particularly interesting to document homologies or differences in biomineralization processes between various tissues in physiological and pathologic conditions. For example, recent high-resolution and in-depth assessments have shown that magnesium-rich calcified spherites are involved both in osteocyte lacunar mineralization in bone and cardiovascular calcification of valves and arterial walls. However, any attempt for tissue-specific therapeutic targeting of calcifications requires unraveling any locally specific characteristics of mineralization processes. For example, it has been shown that VMSC-mediated calcification differs from osteoblast-mediated process (independence of TNAP, induction of their own apoptosis, and inability to form collagen). Moreover, osteocyte lacunar mineralization following osteocyte cell death exhibits features that substantially deviate from the regular bone matrix mineralization (difference in the amount of calcium and magnesium and difference in collagen content). In this context, although we are still in an early phase regarding the treatment of interstitial or other lung diseases accompanied with calcification, further experimental studies should evaluate whether lung cells follow any specific calcification routes that may be targeted for changing the course of lung diseases and potentially preventing negative health outcomes. Limited data from the lungs suggest that some of the molecules or pathways involved in the pathogenesis of lung diseases (such as matrix vesicles, apoptosis, Runx2, and Wnt signaling) are also involved in calcification, meaning that targeting these pathways or molecules could prevent lung calcification by targeting the earlier events in the disease pathogenesis. There are different types of lung calcifications and even ossifications, and it is likely that not all of them are governed by the same mechanisms. This area of research offers many opportunities for discoveries that will span more than one organ or tissue, given that certain homologies definitely exist between different tissues. Nevertheless, clinical treatment of local calcified lesions requires approaches that will not negatively affect the skeleton. Research on biomineralization phenomena should further try to distinguish between local and systemic effects. Recent work on pseudoxanthoma elasticum has shown that selective inhibition of TNAP prevents calcification in Abcc6-mutant cells in vitro and reduces calcification in Abcc6-/- mice in vivo, without the deleterious effects on bone, which suggests that such approaches may be possible.

**Author Contributions**

I.S. and P.M. conceived the study and wrote the article; and C.F. revised the article. All authors approved the final version.

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