Ectopic calcifications under the microscope

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Inappropriate biomineralization of soft connective tissues may frequently occur in several cardiovascular complications (atherosclerotic lesions, arteriosclerosis, degenerative aortic stenosis), in diabetes, in renal disorders and with aging. The severity of athero- and arterio-sclerosis, for instance, is the key determinant of cardiovascular morbidity and mortality and uremic patients suffer from a dramatically increased cardiovascular risk of death, which is directly associated with the magnitude of vascular calcification [1]. Several studies indicate that ectopic calcification is an actively regulated process rising from different, non mutually exclusive, mechanisms and that bone-related molecules as well as mineralization inhibitory compounds may actively participate in regulating mineral deposition in soft connective tissues, where calcifications could be extremely dangerous [2].

Within soft connective tissues, elastic fibers are the most susceptible to calcification, because they are constituted by a stabilized polymer crosslinked by desmosines, with a very low turnover. Moreover, consequences of pathologic calcifications are at the basis of impaired elastic fiber deformability upon mechanical stretching, mainly affecting the cardiovascular system, the lungs, the dermis and the Bruch membrane of the retina. Since elastic fiber mineralization is also frequently observed as an age-related phenomenon, the comprehension of its pathogenesis is becoming even more important due to the increased life expectancy [3].

In order to shed light on the pathogenetic mechanisms responsible for ectopic calcification of soft connective tissue, a very intriguing model is represented by Pseudoxanthoma elasticum (PXE), a genetic disease associated to mutations in the ABCC6 gene and characterized by progressive mineralization of elastic fibers in several organs and tissues responsible for retinal haemorrhages with progressive visual loss up to legal blindness; yellowish dermal papules with laxity and redundant skin especially in the flexural areas associated to esthetical and psychological problems; anaemia due to recurrent haemorrhages of the gastrointestinal apparatus; cardiovascular complications such as claudicatio intermittens, atherosclerosis and premature heart and brain infarction [4]. The pathogenesis of elastic fiber mineralization in PXE is still elusive, but we have hypothesized that a crucial pathogenetic role could be exerted by mesenchymal cells, as dermal fibroblasts, the cells that, controlling synthesis and degradation of extracellular matrix components, are actively and directly responsible for connective tissue alterations.

Mineralized elastic fibers can be clearly visualized on histological sections after Von Kossa staining, by transmission electron microscopy and also by wet-SEM [5]. With X-ray microanalysis and immunocytochemical techniques, we have demonstrated that mineralized elastic fibers accumulate Ca and P ions, as well as proteins with high affinity for Ca ions, as vitronectin, alkaline phosphatase, bone sialoprotein and osteonectin [5,6]. By contrast, osteopontin, that is an acidic matrix protein, mainly expressed in mineralized tissues, kidney and atherosclerotic vessels, is actually present in normal elastic fibers, and in calcified areas

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characterized by fine needle precipitates, but absent from heavily deformed mineralized regions [6]. Furthermore, we have recently demonstrated that PXE fibroblasts are unable to fully carboxylate (through a vitamin K−dependent process) the Matrix Gla Protein (MGP) [7], a small protein produced by mesenchymal cells, which, when efficiently carboxylated (Gla-MGP), is capable to avoid calcification in soft connective tissues. In patients' calcified elastic fibers, non-carboxylated-MGP (Glu-MGP) intensively colocalized with mineral precipitates, whereas Gla-MGP is present at the mineralization front, suggesting that impaired maturation of MGP would be part of the metabolic alterations of PXE fibroblasts and could play a relevant role in time-dependent stability of elastic fibers. Consistently with this hypothesis, we have data indicating that in PXE fibroblasts other proteins, within the endoplasmic reticulum and related to vitamin K recycling (i.e. calumenin and protein disulfide isomerase), are differentially expressed compared to normal cells. Since, the same results were obtained also in patients affected by beta-thalassemia with PXE-like manifestations, data highlight the importance of vitamin-k dependent MGP carboxylation in the pathogenesis of elastic fiber mineralization in PXE and PXE-like disorders, thus contributing to understand the process of ectopic calcifications in the perspective of therapeutic approaches.

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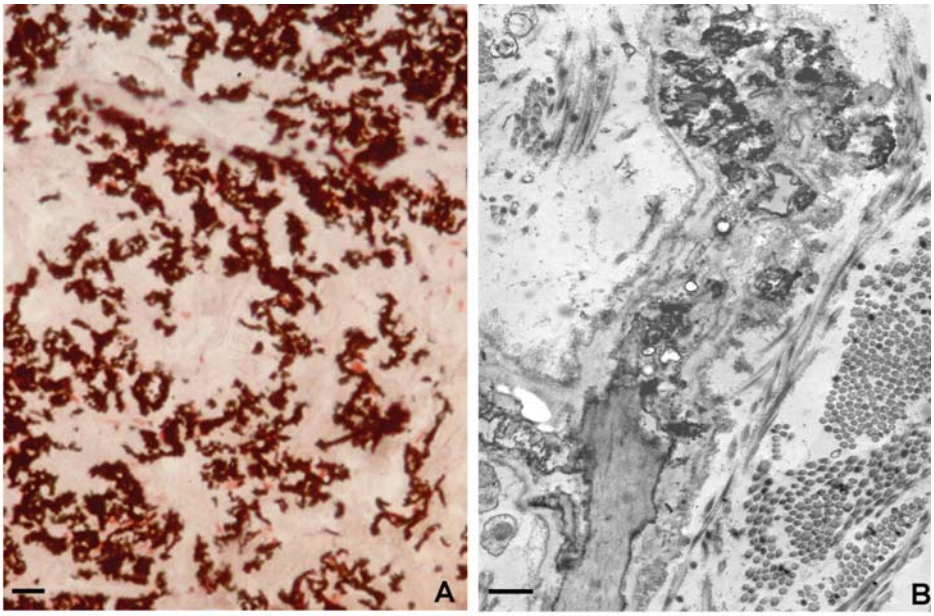

Figure 1. Mineralized elastic fibers in the dermis of a patient affected by Pseudoxanthoma elasticum (PXE) observed by light microscopy of histological sections stained with the Von Kossa method (A) and by transmission electron microscopy (B). Bar= 1μm