REVIEW ARTICLE

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Inducers and inhibitors of biomineralization: lessons from pathological calcification

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Structured Abstract
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Objectives – Ectopic calcification is a common response to soft tissue injury and systemic mineral imbalance and can lead to devastating clinical consequences when present in joints, heart valves and blood vessels. We have hypothesized that mineralization of matrices in any tissue is normally controlled by a balance between procalcific and anticalcific regulatory proteins such that abnormal deposition of apatite is avoided. Alterations in this balance induced by injury, disease or genetic deficiency are postulated to induce ectopic mineral deposition. Over the past several years, we have developed *in vitro* and *in vivo* models of ectopic calcification to investigate potential inducers and inhibitors of this process.
Results – Osteopontin, a secreted phosphoprotein, has emerged as a major inhibitor of ectopic mineralization. Osteopontin is a potent inhibitor of vascular cell calcification *in vitro* and mice lacking osteopontin are highly susceptible to ectopic calcification. Furthermore, osteopontin treatment of biomaterials protected against ectopic mineralization. Our studies indicate that in addition to inhibiting apatite crystal initiation and growth, osteopontin stimulates resorption of ectopic calcification via peripheral macrophages and giant cells. In contrast, inorganic phosphate has emerged as a major inducer of mineralization in these systems. Elevated inorganic phosphate (Pi) was shown to induce smooth muscle cell matrix calcification with morphological properties similar to those observed in calcified human valves and atherosclerotic plaques. Furthermore, mineralization induced by inorganic phosphate was dependent on the activity of the sodium-dependent phosphate cotransporter, Pit-1.
Conclusions – These studies implicate controlled, transcellular transport of Pi as a major requirement for matrix calcification.

Key words: osteopontin; phosphate; sodium-dependent phosphate cotransporter; vascular calcification

Introduction

Under normal conditions, soft tissues do not mineralize. However, under certain pathological conditions, some organs, in particular blood vessels, are prone to calcification. For example, atherosclerosis, diabetes and end-stage renal failure, predispose arteries to calcification. Calcification of the blood vessels leads to stiffening and altered hemodynamics and may contribute to vessel rupture leading to important clinical sequelae. Growing evidence suggests that vascular calcification, like bone formation, is a highly regulated process, involving both inducers and inhibitors (1). For example, apatite, bone-related non-collagenous proteins and matrix vesicles have all been observed in calcified vascular lesions. Furthermore, outright cartilage and bone formation have also been observed. Finally, cells derived from the arterial media can undergo matrix mineralization under appropriate conditions *in vitro* (2, 3). These studies suggest that cell-mediated processes maintain a balance between the procalcific and anticalcific processes in the artery, such that, the ectopic calcification is normally avoided. Under pathological conditions, this balance is upset and leads to ectopic mineralization. Our group has identified both inductive and inhibitory mechanisms controlling vascular calcification and these are discussed in the following sections.

Inhibitors of vascular calcification: role of osteopontin

Osteopontin is a highly phosphorylated and glycosylated secreted protein originally discovered in bone, but more recently identified in calcified vascular lesions (4). The role of osteopontin in vascular calcification has been investigated using *in vitro* and *in vivo* approaches. Smooth muscle cells can be induced to mineralize in response to elevated phosphate in culture (2). The addition of exogenous osteopontin potently inhibits the calcification of smooth muscle cells in a dose-dependent fashion. Osteopontin was intimately associated with growing apatite crystals, suggesting physical inhibition of the crystal growth as one of the mechanisms for inhibition. Phosphorylation of osteopontin was required for mineral inhibitory effects (5). Finally, preliminary experiments show that smooth muscle cells derived from osteopontin null arteries are more susceptible to mineralization compared to wildtype cells (M.Y. Speer and C.M. Giachelli, unpublished observations).

In vivo, two separate approaches were used to determine the role of osteopontin in ectopic mineralization. In the first, osteopontin null mice were crossed to matrix gla protein null mice. Matrix gla protein null mice undergo spontaneous vascular calcification by 2 weeks of age and the mineralization of vessels was associated with highly induced osteopontin levels (6). In osteopontin/matrix gla protein double null mice, mineralization of vessels occurred earlier and was more severe leading to earlier death compared to mice deficient in matrix gla protein alone (7). Finally, glutaraldehyde-fixed porcine aortic valves implanted into osteopontin null mice calcified to a much greater extent than those implanted into wildtype mice (8). Furthermore, osteopontin not only inhibited but also caused regression of the mineral. The regression of calcification induced by osteopontin was correlated with the carbonic anhydrase II expression in the macrophages surrounding the implants and led to acidification of the implants. The calcification of implanted valves could be inhibited by coating with purified, phosphorylated osteopontin (R. Ohri and C.M. Giachelli, unpublished observations). Thus, osteopontin may function not only as a physical inhibitor of apatite crystal growth, but may also promote mineral regressive mechanisms by controlling the cellular gene expression patterns that favor mineral resorption.

Inducers of vascular calcification: role of inorganic phosphate

As previously mentioned, culture of smooth muscle cells in elevated phosphate levels similar to those

observed in hyperphosphatemic patients (>2 mM) leads to matrix mineralization. In addition, the cells undergo a dramatic phenotypic transition characterized by the loss of smooth muscle cell lineage marker expression and upregulation of genes related to osteoblast/chondrocyte differentiation, including osteopontin, osteocalcin and Runx2 (6). We have determined that the effects of inorganic phosphate on smooth muscle cell differentiation and mineralization are in part regulated by a sodium-dependent phosphate cotransporter (3). Phosphonoformic acid, an inhibitor of sodium-dependent phosphate cotransporters, blocked smooth muscle cell mineralization and phenotype transition in response to elevated phosphate treatment (3). Recent studies using RNA interference indicate that the specific sodium-dependent phosphate cotransporter involved is Pit-1 (GLVR-1) (Li and Giachelli, unpublished observations). Thus, elevated inorganic phosphate levels contribute not only to the elevation of the calcium phosphate product, but also signal smooth muscle cells to undergo phenotypic transition that may promote matrix mineralization. Such a pathway may also be relevant in bone, as effects of inorganic phosphate on gene expression in osteoblasts in culture have been recently reported (9–10). Further studies on the signaling mechanisms involved in this inorganic phosphate-induced signaling pathway in smooth muscle cells are currently underway.

Clinical utility and implications

Vascular calcification leads to the loss of compliance, elevated pulse pressure and left ventricular hypertrophy and is highly associated with mortality in patients with cardiovascular disease (1). Ectopic mineralization may also occur in bones and joints and may be a problem in bone and craniofacial tissue engineering. A better understanding of the cell-mediated mechanisms regulating matrix mineralization will aid in the development of novel therapeutics and strategies to address these problems. In addition, these mechanisms may also be useful in developing new strategies to promote healthy mineralization where required.

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