Vascular calcification—a passive process in need of inhibitors

Thorsten Schinke and Gerard Karsenty
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

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Introduction

Vascular calcification occurs frequently in atherosclerotic lesions. In addition, several inherited diseases have been described that are characterized by isolated medial calcification of arteries. The existence of such diseases, together with some recently developed mouse models, indicates that vascular calcification and atherosclerosis are different genetic entities that can be studied separately. Our understanding of the genetic basis of vascular calcification has significantly increased after mouse genetics became available. The generation of two mutant mouse strains, i.e. mice lacking matrix GLA protein or osteoprotegerin, has had the biggest impact on vascular calcification as both strains display isolated medial calcification of arteries. These phenotypes demonstrate that vascular calcification is mostly a passive process and that its inhibition is genetically controlled.

Calcification of atherosclerotic lesions

Atherosclerosis is the major cause of mortality in the Western hemisphere [1]. It is characterized by the presence of atherosclerotic lesions in the arterial intima leading to narrowing of the vessels. The genetic basis underlying the formation of atherosclerotic lesions has been uncovered recently, mostly through the use of mouse genetics [2]. It involves a defective metabolism of lipoprotein particles that initially leads to the formation of a fatty streak lesion characterized by the presence of lipid-filled macrophages (foam cells) in the subendothelium. This stage is followed by the accumulation of lipid and eventually by fibrosis and thrombosis. In humans, calcification is found in the majority of advanced atherosclerotic lesions. It is considered to contribute to the overall morbidity of atherosclerosis by decreasing the elasticity of the vessels [3].

For now it is unclear why atherosclerotic lesions have a tendency to calcify. The high frequency of atherosclerotic calcification has led to the hypothesis that it may be an active, even beneficial process [4]. Indeed, several groups have isolated subpopulations of vascular smooth muscle cells that behave, in vitro, like bone-forming osteoblasts [5]. However, it is not clear yet if these cells show the same behaviour in vivo. Another explanation for the property of an atherosclerotic lesion to calcify could be the accumulation of necrotic foam cells that would serve as a nidus for calcification, i.e. hydroxyapatite formation [6]. Indeed, necrotic cells generally release high concentrations of mitochondrial phosphate and also display crystalline phosphatidylinerine molecules that are usually not found on the cell surface [7,8]. Thus, the microenvironment of an atherosclerotic lesion may be a perfect setting for calcification to take place.

Vascular calcification in the absence of atherosclerosis

In humans several diseases have been described that are characterized by isolated medial calcification of arteries [9–11]. Although the genetic basis has not been defined for any of these diseases, their existence indicates that atherosclerosis and vascular calcification are separate genetic entities. This has also been confirmed by mouse genetics. First, the analysis of a genetic cross between several inbred and genetically engineered mouse strains showed that there is no co-segregation of arterial calcification and atherosclerotic lesion development [12]. Second, in mouse models of atherosclerosis, e.g. mice lacking apolipoprotein E or low-density lipoprotein receptor, calcification of atherosclerotic lesions is a rare and probably secondary event [13–16]. Third, at least two mutant mouse strains, e.g. mice deficient in osteoprotegerin or matrix GLA protein, display arterial calcification in the absence of atherosclerosis [17,18].

In addition to these types of medial calcification in mice and humans, there are rare cases of ectopic bone formation inside the vessel wall [5]. By definition, bone formation is not simply mineralization. It requires that cells of the vessel wall gain osteoblast-like characteristics and produce a bone matrix. This matrix needs to be subsequently resorbed by osteoclast-like cells leading to the formation of bone marrow. Cases of ectopic bone formation in human arteries are too rare to be understood genetically. However, one recent mouse model indicates that ectopic bone formation can occur in arteries and that it has a genetic basis. Mice lacking Smad6, an inhibitory molecule in the...
TGF-β signalling pathway, have various defects of the cardiovascular system including bone formation in the media of the outflow tracts of the heart [19]. The underlying molecular mechanisms and the physiological relevance of this finding need to be further investigated.

Matrix GLA protein and osteoprotegerin are required to prevent calcification of mouse arteries

Matrix GLA protein (Mgp) is a γ-carboxylated mineral-binding extracellular matrix (ECM) protein. In mice, its expression is restricted to two cell types: vascular smooth muscle cells and chondrocytes [18]. As demonstrated by a classical knockout experiment, Mgp acts in vivo as an inhibitor of mineralization in arteries and cartilage [18]. Mice deficient in Mgp are normal at birth, but develop severe calcification of all their arteries within weeks. This calcification is lethal with a 100% penetrance in different genetic backgrounds, and all Mgp-deficient mice die at around 8 weeks of age, mostly due to rupture of the aorta [18]. Cartilage calcification also occurs in Mgp-deficient mice showing that not only in arteries mineralization of the ECM needs to be inhibited. Histologically, the arterial calcification in Mgp-deficient mice appears in the media and, once initiated, advances rapidly along elastin fibres. There is no indication of atherosclerotic lesions or ectopic bone formation in these mice [18].

The phenotype of the Mgp-deficient mice is very informative. It demonstrates that vascular calcification is a process that would occur spontaneously if it would not be actively inhibited. Clearly, in mice Mgp is the major molecule ensuring that arteries do not become calcified. Surprisingly however, this does not seem to be the case in humans. Humans lacking functional Mgp have a disease called Keutel syndrome [20]. These patients show calcification of cartilage, but their arteries are not affected. This finding suggests that human arteries do not only rely on the presence of Mgp in their ECM, but that other proteins acting in a similar fashion participate in the inhibition of mineralization. Indeed, several mineral-binding proteins have been localized to calcifying atherosclerotic lesions [21,22]. Their involvement in regulating arterial calcification in humans needs further investigation.

Another molecule preventing arterial calcification in mice is osteoprotegerin (Opg). Opg is a secreted TNF-receptor-like molecule that acts as an inhibitor of terminal differentiation of bone-resorbing osteoclasts. Thus, Opg-deficient mice develop severe osteoporosis caused by increased bone resorption. In addition to this phenotype, Opg-deficient mice display calcification of the aorta and renal arteries [17]. Compared to the Mgp-deficient mice, this calcification is rather mild and does not affect all arteries. Unlike Mgp, Opg does not contain domains that are likely to mediate mineral-binding properties. Thus, the mechanism of Opg action to prevent arterial calcification in mice, as well as its function in human arteries are for now unknown.

Inhibition of mineralization is genetically controlled

The finding that mineralization needs to be inhibited in arteries raises the question if the same is true for other tissues. Although ectopic calcification has been described in many tissues other than arteries, only one of them has been clearly demonstrated to require active inhibition of mineralization. This tissue is cartilage, and again it is the presence of Mgp that prevents spontaneous mineralization [18,20]. The ECM of cartilage is different from the ECM of the arterial media suggesting that inhibition of mineralization is a general mechanism, and that in other tissues, where Mgp is not expressed, other inhibitors of mineralization have to exist. In fact, this would not be surprising, given the fact that extracellular fluids are supersaturated with calcium and phosphate [23].

What does this mean for physiological mineralization? So far there is still no explanation for the fact that the skeletal matrix is the only ECM that mineralizes. In addition, it is not clear yet if bone mineralization is an active process, i.e. if osteoblasts produce bone-specific ECM proteins that are required to induce mineralization. The phenotype of the Mgp-deficient mice raises the hypothesis that bone mineralization may be a passive process, explained, at least in part, by the absence of inhibitors of mineralization from the bone matrix. In this respect it is important to state that Mgp is not expressed by osteoblasts [18]. Future experiments will determine if indeed the absence of Mgp and possibly of other inhibitors of mineralization in bone is a factor favouring bone mineralization.

Concluding remarks

Mouse genetic studies have significantly improved our understanding of several developmental and physiological processes. This includes vascular calcification. Although calcification is most common in atherosclerotic lesions, we now know that the genetic basis of vascular calcification is different from the one underlying the formation of atherosclerotic lesions. Thus, the calcification aspect of atherosclerosis can and should be studied separately and may one day also be treated separately.

The second and even more important information comes mostly from the knockout of Mgp: vascular calcification is a passive process that requires active inhibition. Mgp is the molecule that is responsible for this inhibition in mice. Although the importance of Mgp to prevent arterial calcification in humans has not been clearly established yet, theoretically it seems that up-regulating Mgp expression in human arteries could be beneficial to prevent atherosclerotic calcification. In addition to Mgp, it is likely that other inhibitors of mineralization act in human arteries. Once identified, these molecules will also be good targets for future therapeutic approaches.
References