Osteocalcin, or bone Gla protein, is a small protein secreted by osteoblasts that can undergo γ-carboxylation. The γ-carboxylated form binds hydroxyapatite and is abundant in bone extracellular matrix. In contrast, the undercarboxylated circulating form has been implicated as a novel hormone and positive regulator of glucose homeostasis. Importantly, osteocalcin expression has been described in calcifying vascular smooth muscle cells (VSMCs), although the physiological significance of this observation has remained unclear. In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Idelevich et al1 show that osteocalcin is potentially a novel regulator of osteochondrogenic differentiation of pathologically mineralizing VSMCs. They reveal that osteocalcin, via hypoxia-inducible factor 1α signaling, stimulates expression of osteochondrogenic transcription factors in VSMCs, as well as a shift in cellular metabolism toward glycolysis. This study provides the first evidence that osteocalcin may be an active player in vascular calcification, with its presence in the calcified vasculature, and potentially the circulation, activating novel signaling pathways that promote mineralization.


Pathological mineralization of the vasculature has a detrimental effect on cardiovascular function and is associated with increased mortality in patients with aging, atherosclerosis, type 2 diabetes, and chronic kidney disease.2 Vascular smooth muscle cells (VSMCs) orchestrate the mineralization process, which is mediated in part by their osteochondrocytic differentiation in the vessel wall. This phenotypic transition is characterized by expression of Runx2 and Sox9, master transcription factors that regulate bone and cartilage differentiation during developmental osteochondrogenesis, as well as other bone- and cartilage-specific proteins, many with undefined functions.3 One of these proteins, osteocalcin, is a small γ-carboxylated protein that is expressed by both osteoblasts and VSMCs and abundantly deposited in the extracellular matrix of bone and in the calcified vasculature.4–6 Osteocalcin avidly binds hydroxyapatite via 3 γ-carboxylated glutamic acid residues and was originally thought to play a role as a regulator of mineral nucleation.5,78 However, osteocalcin is also found in the circulation, and a breakthrough in our understanding of the function of its circulating form came in 2007 when Gerard Karsenty’s laboratory established that the undercarboxylated form of osteocalcin has a positive effect on glucose metabolism.9 This effect on energy metabolism is mediated by upregulation of insulin secretion by pancreatic β-cells and adiponectin production by adipose tissues, which stimulates whole-body glucose uptake and utilization. Activation of insulin signaling pathways induces cross-talk between osteoblasts, osteoclasts, and non-skeletal tissues, leading to activation of bone resorption further stimulating the release of undercarboxylated osteocalcin.9,10 Importantly, in the present issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Idelevich et al present data revealing a novel function for osteocalcin in both cartilage and the vasculature and show that osteocalcin fuels glucose utilization by VSMCs and promotes their osteochondrogenic differentiation (Figure).1

Idelevich et al used vitamin D treatment of rats to induce mineralization of the vasculature. They demonstrated that osteocalcin mRNA was upregulated concomitantly with the onset of VSMC osteochondrocytic differentiation and that the protein accumulated in the calcified vessel wall. They also examined the expression and secretion of osteocalcin in a mouse VSMC line (MOVAS) induced to calcify by β-glycerophosphate treatment. Similarly, they observed significant upregulation of osteocalcin concomitant with elevated expression of Runx2 and Sox9 and elevated mineralization. These observations led them to hypothesize that osteocalcin mediates osteochondrogenic differentiation of VSMCs. To test this notion, they used stable plasmid overexpression of osteocalcin and showed that this promoted osteochondrogenic differentiation of MOVAS cells in vitro and was accompanied by an increased rate of mineralization. However, during the course of this experiment, they noted an important change in the color of the culture medium that was indicative of an increase in the metabolic activity of the cells. This led them to test whether osteocalcin could exert metabolic effects on vascular cells. Additional in vitro studies revealed that osteocalcin overexpression activated glucose uptake and shifted energy production toward enhanced glycolytic breakdown of glucose, which was accompanied by VSMC proliferation. Dramatic changes in the expression of the metabolic enzymes phosphofructokinase and pyruvate dehydrogenase kinase 1, which are involved in glucose uptake and glycolysis, were observed in vitro and in vivo in the mineralized vasculature, suggesting that mineralization may be accompanied by metabolic changes in VSMCs.
The next step was to establish the factors that drive osteocalcin-induced metabolic changes in VSMCs. They reasoned that hypoxia-inducible factor 1α (HIF-1α), which is activated during endochondral bone development and is crucial for osteoblast differentiation,11 upregulation of glycolytic enzymes in chondrocytes (which promotes their survival in the hypoxic growth plate),12 and the stimulation of angiogenesis during osteogenesis13 may be a logical candidate to test. Accordingly, they showed that HIF-1α was upregulated in calcifying MOVAS cells and that this upregulation was increased by osteocalcin overexpression. Addition of exogenous osteocalcin to MOVAS cells induced a rapid activation of HIF-1α, enhanced expression of glucose transporters and glycolytic enzymes, and stimulated insulin-signaling pathways. Importantly, the effects of exogenous osteocalcin were abrogated by knockdown of HIF-1α expression using small interfering RNA. In vivo evidence further supported these in vitro findings. HIF-1α expression was shown to be elevated in the mineralizing aorta of rats treated with vitamin D. Moreover, activation of HIF-1α induced by hypoxic conditions also promoted osteochondrogenic differentiation of VSMCs in vitro. Finally, silencing of osteocalcin in vivo, using a novel whole animal small interfering RNA strategy, reduced HIF-1α stabilization, prevented an elevation of Sox9 and pyruvate dehydrogenase kinase 1, and inhibited vascular mineralization in response to vitamin D. However, other factors were unaffected, including Runx2 and glycolytic enzymes previously shown to be elevated in MOVAS cells.

The authors concluded that HIF-1α is an important regulator of VSMC osteochondrogenic differentiation and metabolism that can be activated by osteocalcin signaling activity, which ultimately promotes vascular calcification.

This study has provided a number of intriguing possibilities suggesting that metabolic pathways are linked to the osteochondrocytic differentiation of VSMCs and that systemic metabolic changes induced by osteocalcin may affect vascular calcification. However, the study does have a number of limitations that need to be addressed before the full significance of these findings can be measured. First, the in vitro studies were performed in a transformed mouse cell line rather than primary human VSMCs, which have a limited proliferative capacity and behave very differently from rodent cells in vitro. Second, calcification was induced by vitamin D treatment of rats. Importantly, the osteocalcin promoter contains a vitamin D responsive element, suggesting that its upregulation may be due to vitamin D exposure rather than calcification. Whether the mechanisms outlined in this study, in particular HIF-1α activation, are also evident in alternate animal models of calcification now needs to be determined. As HIF-1α can be activated by other factors in addition to hypoxia, including insulin and cytokines,11 this signaling pathway may play a central role in vascular calcification.

Third, no definitive experiment was performed to determine how the carboxylation status of osteocalcin affected its role in driving VSMC metabolic changes and osteochondrogenic differentiation. Indeed, the overexpression studies suggested that only the carboxylated form of osteocalcin could drive metabolic changes, as a carboxylation defective mutant form had no effect. Importantly, previous studies have demonstrated that the metabolic activity of osteocalcin is restricted to the undercarboxylated form.9,10 This suggests that only locally produced carboxylated osteocalcin can affect VSMC phenotype and that circulating levels would have no effect. Unfortunately, circulating levels were not measured in the rat models used in the study by Idlevich et al, so this question remains to be answered. It would have been interesting to observe whether systemic knockdown of osteocalcin using small interfering RNA affected bone remodeling and circulating osteocalcin in rats, as both may impinge on vascular calcification. It is also important to note that to date, clinical studies have found no clear link between levels of circulating undercarboxylated osteocalcin and vascular calcification; in patients with type 2 diabetes, the serum levels of
Osteocalcin are inversely correlated with brachial-ankle pulse wave velocity however, no correlation between osteocalcin levels and abdominal aortic calcification was recently reported.

Finally, further interrogation of the mechanisms of vascular calcification in response to osteocalcin and HIF-1α needs to be performed. Although it is clear that in the study by Idelevich et al. HIF-1α signaling was promoting both metabolic changes and osteochondrocytic differentiation, whether the metabolic changes were required for osteochondrocytic differentiation was never directly tested. Indeed, these changes were induced by osteocalcin at very different rates: minutes for the metabolic changes in experiments with recombinant osteocalcin compared with 7 to 21 days for osteochondrogenic differentiation. As osteocalcin also promoted VSMC proliferation, it is plausible that HIF-1α-induced metabolic changes are an adaptive response aimed at promoting cell survival in a suboptimal environment, with osteochondrocytic differentiation being a secondary downstream consequence or indeed an independent event. Further careful examination of the causal links between osteocalcin, activation of HIF-1α, and metabolic alterations will help to resolve this issue.

In conclusion, osteocalcin can no longer be considered a passive marker of mineralization. It is an active modulator of VSMC cell signaling and osteochondrogenic differentiation, but additional studies are required to establish the form and source of osteocalcin that can exert these effects, the VSMC receptor that mediates downstream signaling, and the relationship between metabolism and osteochondrogenic differentiation. Given the important role of osteocalcin in the regulation of metabolism, it may be a new link between vascular calcification and bone and metabolic disorders that may help to explain some of the apparent association of bone loss with vascular calcification in some patient groups.

Disclosures

None.

References


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