Angiogenesis and Pericytes in the Initiation of Ectopic Calcification

G.D.M. Collett, A.E. Canfield

Abstract—Ectopic calcification of blood vessels, heart valves, and skeletal muscle is a major clinical problem. There is now good evidence that angiogenesis is associated with ectopic calcification in these tissues and that it is necessary, but not sufficient, for calcification to occur. Angiogenesis may regulate ectopic calcification in several ways. First, many angiogenic factors are now known to exert both direct and indirect effects on bone and cartilage formation. Second, cytokines released by endothelial cells can induce the differentiation of osteoprogenitor cells. Third, the new blood vessels provide oxygen and nutrients to support the growing bone. Finally, the new blood vessels can serve as a conduit for osteoprogenitor cells. These osteoprogenitor cells may be derived from the circulation or from pericytes that are present in the neovessels themselves. Indeed, there is now compelling evidence that pericytes can differentiate into osteoblasts and chondrocytes both in vitro and in vivo. Other vascular cells, including adventitial myofibroblasts, calcifying vascular cells, smooth muscle cells, and valvular interstitial cells, have also been shown to exhibit multilineage potential in vitro. Although these cells share many properties with pericytes, the precise relationship between them is not known. Furthermore, it still remains to be determined whether all or some of these cells contribute to the ectopic calcification observed in vivo. A better understanding of the underlying mechanisms that link angiogenesis, pericytes, and ectopic calcification should provide a basis for development of therapeutic strategies to treat or arrest this clinically significant condition. (Circ Res. 2005;96:930-938.)

Key Words: pericytes ■ angiogenesis ■ calcification ■ atherosclerosis ■ smooth muscle cells
gesting that pericytes may also be involved in ectopic calcification. Together, these data suggest that angiogenesis and ectopic calcification may be intimately associated and, moreover, that pericytes may be one of the common factors linking these processes. This review will critically evaluate the evidence supporting this suggestion. Particular emphasis will be placed on ectopic calcification of blood vessels; calcification at other sites will be mentioned briefly.

**Angiogenesis and Ectopic Calcification of Blood Vessels**

Calcification of blood vessels is a common complication of many diseases including atherosclerosis, diabetes, end stage renal disease, and calciphylaxis, and is generally considered to be a significant predictor of future adverse clinical events.\(^1\)\(^-\)\(^6\) Calcification can occur throughout the vasculature, from the large aorta through to dermal microvessels, and it can be identified in both the media and intima. Within arteries, calcification occurs as small dispersed crystals of hydroxyapatite, mineralized bone-like tissue containing mineralized cartilaginous tissue.\(^1\)\(^-\)\(^6\) Current evidence suggests that vascular calcification is a complex process reminiscent of endochondral bone formation and repair. Several factors involved in the regulation of vascular calcification have been identified, including bone morphogenetic protein-2 (BMP-2), transforming growth factor-\(\beta\) (TGF-\(\beta\)), fetuin-A, parathyroid hormone-related polypeptide, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), matrix Gla protein, Axl, osteopontin, osteoprotegerin, oxidized lipids, and deregulated calcium and phosphate metabolism.\(^1\)\(^,\)\(^2\)\(^,\)\(^5\)\(^,\)\(^7\) However, whether the mechanism of calcification is the same throughout the vasculature or is dependent on site-selective responses to specific modulators is still not known.

**Angiogenesis and Calcification in Atherosclerotic Lesions**

The association between angiogenesis and arterial disease was first suggested more than a century ago, when it was noted that atheromatous vessels contained new blood vessels within their intima. However, it is only in the last decade that the potential functional significance of this neovascularization to disease progression has come to be realized. The association between neovascularization and sites of calcification in atheromatous lesions is only just starting to be appreciated with, to date, only a few studies commenting on its potential significance.

The first indication that angiogenesis and ectopic calcification may be associated came from the imaging studies conducted in the 1980s in which silicone polymers were injected into cleared fixed human hearts, and the pattern of vascular filling was recorded by cinematography.\(^5\)\(^,\)\(^9\) Detailed analysis of the cinematographic images demonstrated abundant neovascularization in association with sites of lesional calcification. Subsequent histological examination of these specimens revealed that the microvessels were localized in both the media and thickened intima of atheromatous vessels and that the extent of neovascularization correlated with disease severity.\(^9\) Furthermore, by comparing the histology of these lesions, the anatomical location of microvessels within the lesions and the cinematographic findings, it was shown that calcification was only detected in vessels with abundant neovascularization, although not all lesions with neovascularization were calcified.\(^9\) Using a complimentary approach, Zamir & Silver produced “casts” of the entire coronary network in human hearts and demonstrated the presence of “extensive mesh-like vascular networks” near the lumen of atheromatous vessels.\(^10\) Furthermore, they demonstrated that the networks were frequently associated with sites of calcification within the lesions and that some of the casts contained calcium deposits.

Since that time, the majority of evidence for the presence of angiogenesis in atheromatous plaques has been obtained from histological studies using markers of endothelial cells (eg, von Willebrand factor, CD31, and CD34) to identify new blood vessels within the artery wall. Using these techniques, it has been possible to demonstrate where the new blood vessels are localized, their origin, and at which stage of lesion development they appear. Nevertheless, there is still a dearth of information regarding the ultrastructure of these neovessels; in particular, it is still not known whether these new blood vessels contain an intact perivascular coat.

Immunohistochemical staining for endothelial cells has demonstrated that the majority of new blood vessels are localized in the shoulder and cap regions of late stage plaques (Figure 1) in an apparently asymmetrical distribution.\(^11\)\(^-\)\(^\text{14}\

The bases of plaques are also, in general, highly vascularized.\(^11\) The majority of new vessels have been shown to originate from the adventitial vasa vasora, with some new vessels originating from the lumen of the artery.\(^11\)\(^,\)\(^\text{14}\

In agreement with the earlier morphological studies, these studies have also confirmed that intimal neovascularization is present in early, advanced, and late-stage plaques and that the extent of neovascularization correlates with disease severi-
ty,11–14 Interestingly, Jeziorska and colleagues15 also noted that numerous blood vessels were localized around bony deposits in the atherosclerotic lesions. However, it has also been reported that vascular density decreases in extensively hyalinized or calcified intimas, although new vessels were still reported in these lesions.14 As angiogenesis may contribute to the initiation of ectopic calcification in atheromatous lesions, a study detailing the precise association between sites of angiogenesis and ectopic calcification of blood vessels is clearly warranted.

It is now evident that angiogenesis is a key event in both the initial development of atheromatous lesions and in their subsequent progression. This close association between angiogenesis and progression of atherosclerosis has been confirmed in recent studies conducted using animal models that have shown that treatment with angiogenic inhibitors inhibits disease progression, whereas angiogenic factors promote this process.16–18 In addition, the presence of microvessels in plaques is generally associated with adverse clinical events. First, expansion of the vasa vasora allows perfusion of the artery wall when thickening diminishes the extent to which oxygen can diffuse from the luminal blood.16 Second, the new blood vessels contribute to plaque formation by leaking albumin and fibrinogen into the artery wall.11 Third, intimal microvessels are associated with sites of hemorrhage in the plaques and with plaque rupture.11,19 Fourth, leukocytes, macrophages, and mast cells are localized around new blood vessels in lipid-rich plaques, suggesting that these neovessels are directly involved in inflammatory cell recruitment, and hence, lesion progression, calcification, and destabilization.12,13 Finally, as will be discussed in detail, the new blood vessels may directly contribute to the development of ectopic calcification in the lesions.

**Angiogenesis and Calcification in Transplant Arteriosclerosis, End Stage Renal Disease, and Diabetes**

Angiogenesis and calcification are common features of transplant arteriosclerosis.20,21 However, it is not clear whether the new blood vessels observed in the media and neointimal lesions of transplanted vessels are formed by the same mechanism as in native vessel atherosclerosis. For example, Hu et al21 have recently shown that when mouse aortas were allografted into the carotid arteries of chimeric mice with bone marrow derived from Tie2-LacZ mice, β-galactosidase activity was seen within the neointimal lesions of the allografts. This indicates that the endothelial cells within the microvessels were derived from bone marrow cells rather than from the vasa vasora as described for atheromatous lesions.

Interestingly, it has recently been shown that a large population of progenitor cells, expressing the Stem cell antigen Sca-1, are present in the adventitia of ApoE-deficient mice.22 These Sca-1+ cells differentiated into smooth muscle cells in response to incubation with PDGF-BB in vitro or, when incubated with VEGF, into endothelial cells. Furthermore, when Sca-1+ cells carrying the lacZ gene were transferred to the adventitial side of vein grafts in ApoE-deficient mice, β-galactosidase–positive cells were detected in the atherosclerotic lesions of the intima where they were found to contribute to lesion development and progression.22 It is tempting to speculate that these progenitor cells may be activated by the dyslipidemic stimuli that concomitantly promote angiogenic responses within atherosclerotic lesions, thereby contributing to lesion development and progression, including vascular calcification.

The occurrence of angiogenesis in vessels with medial calcification caused by end stage renal disease or diabetes has not been examined in detail. However, it has recently been demonstrated in animal models that diabetes increases oxidative stress and induces an inflammatory response in the adventitia23 and that removal of the adventitia can prevent medial artery calcification in rats fed an atherogenic calcification-inducing diet.24 Moreover, adventitial myofibroblasts (pericytes) have been shown to contribute to the medial calcification in diabetes (see below).19 Whether these cells are related to the Sca-1+ adventitial progenitor cells as mentioned in the previous paragraph remains to be determined.

**Association Between Angiogenesis and Ectopic Calcification at Other Sites**

**Cardiac Valves**

Calcification of native human heart valves and porcine xenograft bioprosthetic valves can lead to valve stenosis and, ultimately, death. Current evidence suggests that clinical risk factors associated with atherosclerosis may also be involved in valvular calcification and degeneration.25,26 Features related to bone formation and remodeling, such as the presence of cartilaginous nodules and mature lamellar bone with hemopoietic elements, have been demonstrated in surgically removed human heart valves.27,28 In addition, proteins associated with chondrogenesis and osteogenesis have been identified in these tissues, including osteopontin, bone sialoprotein, alkaline phosphatase, and BMP-2 and -4.27,29 Recent studies suggest that valvular calcification in calcific aortic stenosis is regulated by many factors, including TGF-β1, oxidized lipids, the renin-angiotensin system, and RANKL-OPG pathway.26,31,32

As with blood vessels, angiogenesis and ectopic calcification are colocalized in heart valves.27,30,33 Extensive neovascularization has been noted adjacent to regions of woven bone within calcified human heart valves.30 Furthermore, immunostaining of samples identified expression, by both smooth muscle actin–positive cells and macrophages, of proteins associated with osteogenesis. These cells were located along the calcifying front of mineralization zones, at the valve edge, zona fibrosa, and in the developing neovascular.30 More recently, Mohler et al27 reported neoangiogenesis within all heart valves undergoing ossification. These authors postulated that inflammatory cells and subendothelial microvascular pericyte–like cells (myofibroblasts) created a microenvironment that could support ectopic calcification and ossification of heart valves.27 Extensive angiogenesis has also been demonstrated in non-rheumatic aortic valves with moderate stenosis.33 Furthermore, VEGF and its receptors have been localized to activated endothelial cells, stromal
Skeletal Muscle
Much of our understanding of heterotypic ossification within skeletal muscle is derived from case reports that provide detailed pathological descriptions of the lesions. However, the identification and study of a hereditary form of the condition, fibrodysplasia ossificans progressiva (FOP), has provided a basis for understanding the molecular nature of heterotypic bone formation in skeletal muscle. FOP is an autosomal dominant disorder, which is recognized by congenital malformation of the great toes, heterotypic ossification of soft tissue, and its temporal progression along a characteristic anatomic pattern. Lesions can appear spontaneously or as a result of local trauma. This makes FOP difficult to study as taking biopsies from these patients is contraindicated, because the local trauma would stimulate further advancement of the disease. Pathological development of FOP lesions arises as severe inflammation of the connective tissue with intramuscular edema. These areas gradually form an angiogenic fibroproliferative mass that calcifies over time and is reminiscent of endochondral osteogenesis. Identification of a general underlying defective gene or family of genes responsible for FOP remains elusive.

Recent studies have suggested that osteoprogenitor cells in skeletal muscle may originate from the muscle tissue or associated microvasculature. Osteogenic progenitor cells isolated from skeletal muscle were shown to induce ectopic bone in vivo when transduced with BMP-2; however, the identity of these cells was not determined. Subsequently, primitive osteoprogenitor cells isolated from intramuscular connective tissue were identified as pericyte-like. These cells also expressed the osteogenic transcription factor Cbfa1 and were immunoreactive for osteocalcin and bone sialoprotein. Hegyi and colleagues postulated that these osteoprogenitor cells might originate from perivascular pericytes, medial vascular smooth muscle cells, adventitial fibroblasts, or from the bone marrow stroma, highlighting the strong association between angiogenesis and the heterotypic ossification of skeletal muscle.

Ectopic Calcification in Other Tissues
The association between angiogenesis and ectopic calcification has also been noted at other sites. For example, a statistically significant association between increased microvascular density and microcalcifications in ductal carcinoma in situ (DCIS) has been noted. Circumstantial evidence has also indicated a role for pericytes or pericyte-like cells in calcifying fibroblastic granuloma (CFG), a lesion of the gingiva characterized by recurrent calcified nodules within the vascular basement membrane, metaplastic bone formation, and dense neovascularization. Chloroidal osteoma, which is characterized by the presence of mature bone containing marrow spaces within the choroid, is usually associated with choroidal neovascularization. Calcifications of the retina, retinal vessels, and the presence of osseous metaplasia in the retina have also been noted in patients with renal failure, Coats’ disease, tuberous sclerosis, and retinocytomas. Whether calcification in these lesions is associated with angiogenesis has not been determined.

Placental calcification is associated with placental matura-
tion. Hydroxyapatite crystals have been localized within the basement membrane of chorionic villi, and membrane particles resembling matrix vesicles have been reported. It is still not clear whether the mechanism underlying placental calcification is similar to that described for physiological and ectopic calcification or is more reminiscent of metastatic calcification. Whether placental calcification is associated with angiogenesis is not known, but it is noteworthy that pericytes isolated from placental microvessels spontaneously form nodules that mineralize.

How Might Angiogenesis Initiate Ectopic Calcification?
Angiogenesis may initiate ectopic calcification in several ways. First, the invading blood vessels can act as a conduit for osteoprogenitor cells. The source of osteoprogenitor cells in blood vessels is unclear, but it may include circulating progenitor cells or pericytes present in the neovessels themselves. This latter possibility will be discussed in detail below. Second, cytokines (for example, BMP-2 and BMP-4) released by endothelial cells have been shown to induce both the differentiation of osteoprogenitor cells and calcification in vitro and in vivo, although this effect is context-dependent. Interestingly, the expression of these cytokines is markedly upregulated in response to inflammatory and mechanical stresses, which themselves can also promote vascular calcification in specific clinical contexts. Third, many angiogenic factors can also exert direct and indirect effects on osteogenic and chondrogenic cells. For example, FGF-2 induces angiogenesis, is mitogenic for mesenchymal cells and osteoblasts, enhances bone formation in vivo, and induces remodeling of bone by osteoclasts. In addition, it is now recognized that VEGF, long considered to be endothelial cell–specific, can act directly on osteoblasts, chondrocytes, and osteoclasts. Thus VEGF and its receptors are expressed by osteoblasts, and VEGF induces their migration and differentiation. VEGF also stimulates the formation, survival, and resorption activity of osteoclasts and is a chemoattractant for osteoclasts. Chondrocytes also express VEGF and its receptors and respond to stimulation by exogenous VEGF in vitro by activating VEGFR-2.

Finally, several proteins (eg, bone sialoprotein and osteopontin) that were originally identified in bone and which have also been identified at sites of ectopic calcification in blood vessels, heart valves, and skeletal muscle are now known to exhibit angiogenic properties. Thus, evidence is accumulating for a proangiogenic role for bone sialoprotein through an RGD-mediated interaction with αvβ3 integrin.
In addition, bone sialoprotein is involved in the early stages of mineralization and bone resorption. Osteopontin has been implicated in several processes including angiogenesis, inflammation, biomineralization, and bone resorption. It is noteworthy that phosphorylated osteopontin is a potent inhibitor of ectopic calcification in blood vessels.

**Pericytes, Angiogenesis, and Ectopic Calcification**

Ebreth and Rouget first described the presence of perivascular cells closely associated with capillaries more than 100 years ago. Since then these cells have been given a variety of different names, including adventitial cells, Rouget cells, mural cells, periendothelial cells, perivascular cells, histiocytes, and deep cells. Zimmerman introduced the term “pericyte” (peri, around; cyto, cell or vessel) in 1923. Pericytes are present in precapillary arterioles, capillaries, and postcapillary venules. In addition, recent studies have suggested that pericytes, or cells resembling pericytes, have a more widespread distribution in the human body. Thus, using antibody 3G5, which recognizes a cell-surface ganglioside present on pericytes but not smooth muscle cells, fibroblasts, or endothelial cells, pericyte-like cells have been identified in the inner intimal layer, the outer layer of the media, and in vasa vasorum in the adventitia of large, medium, and small arteries and veins. Cells with pericyte-like characteristics have also been identified in the kidney (mesangial cells), liver (Ito cells), and bone marrow (reticular cells). Furthermore, pericytes share many similarities with myofibroblasts, and the possible distinction between these cells is not always clear.

The origin of pericytes is still not fully understood. Studies conducted using quail-chick chimeras have demonstrated that pericytes are derived from the neural crest, whereas endothelial cells are derived from the mesoderm. However, other studies have suggested that pericytes have a mesodermal origin. In addition, it has recently been shown that perivascular cells (i.e., pericytes or smooth muscle cells) and endothelial cells can be derived from a common progenitor cell (Figure 2). Incubation of these progenitor cells with PDGF-BB stimulates their differentiation into pericytes/smooth muscle cells, whereas VEGF stimulates their differentiation into endothelial cells.

**Figure 2.** Pericytes can be derived from a variety of progenitor cells including mesodermal cells and neural crest cells (collectively termed ‘smooth muscle progenitors’) or from common vascular progenitors which can be induced to differentiate into pericytes/smooth muscle cells by PDGF-BB or into endothelial cells by VEGF. These common progenitor cells include embryonic stem cells, circulating stem cells and Sca-1 progenitor cells present in the adventitia. Endothelial cells, fibroblasts, smooth muscle cells and bone marrow cells have also been reported to give rise to pericytes. Pericytes, adventitial myofibroblasts and smooth muscle cells share many properties including the ability to give rise to osteoblasts, chondrocytes and adipocytes. It is not known whether pericytes and adventitial myofibroblasts are the same entity. Calcifying vascular cells (CVCs) appear to have a more restricted differentiation capacity and may, therefore, represent a cell that is further along the differentiation pathway than pericytes.
Several markers for pericytes have been described, including α-smooth muscle actin (α-sm actin), non-muscle actin, muscle and non-muscle myosin, desmin, high molecular weight melanoma-associated antigen (HMW-MAA or NG2 proteoglycan), aminopeptidase-N, aminopeptidase-A, the regulator of G-protein signaling-5 (RGS5), and a cell-surface ganglioside (3G5). However, none of these markers are absolutely specific for pericytes nor will any of them recognize all pericytes in vitro and in vivo. For example, some antibodies (eg, α-sm actin and HMW-MAA) recognize pericytes in vitro and “activated” pericytes in vivo but do not recognize pericytes present in normal “resting” capillaries. Furthermore, pericyte identification is complicated by their marked heterogeneity and by their ability to differentiate into other cell types.

Pericytes serve a variety of functions in the normal vessel wall. They are contractile cells capable of regulating vessel caliber and permeability and, as such, are often likened to the smooth muscle cells of larger blood vessels. However, these cells are not identical. Pericytes also provide mechanical support and stability to the vessel wall as a result of their direct interactions with endothelial cells and by the synthesis of basement membrane proteins. In the brain, pericytes also appear to possess phagocytic activity and may therefore be important regulators of the blood–brain barrier. These functions have been reviewed extensively elsewhere and will not be discussed further herein.80,81,85,93,94

Pericytes and Angiogenesis

The involvement of endothelial cells in angiogenesis has been the subject of intense investigations for several decades. In contrast, our understanding of the extent of the involvement of pericytes in angiogenesis is only just beginning to be appreciated as new technologies become available.95,97 It is well established that the investment of newly developing capillaries with pericytes during the later stages of angiogenesis is essential for the production of stable, non-leaky vessels. However, how pericytes are recruited to newly forming blood vessels is still not fully understood, although several factors, including PDGF-BB, VEGF, and GTPase-activating proteins (eg, Rgs5), are clearly involved. In addition, it is now becoming accepted that pericytes are also involved in the early stages of angiogenesis, although their precise role may depend on where the angiogenesis occurs.95 Thus, pericytes have been localized to the growing tips (or angiogenic “sprouts”) of new vessels.96,98 In this location, they are thought to be involved in (1) the initial patterning of the vascular networks, particularly in the retina, (2) regulating endothelial cell proliferation, differentiation, and junction formation, (3) regulating capillary diameter, and (4) synthesizing matrix proteins. Further clarification of the involvement of pericytes in angiogenesis associated with atherosclerosis is eagerly awaited.

Pericytes and Ectopic Calcification

Maximow and Ehrlich first suggested that perivascular cells may be “resting wandering cells” or “primitive mesenchymal cells” more than 50 years ago, as reviewed by Tilton. Since then, it has been shown that pericytes can differentiate into many different cell types including osteoblasts, chondrocytes, adipocytes, smooth muscle cells, fibroblasts, and Leydig cells (Figure 2), suggesting that they may serve as a source of adult progenitor cells in situations of repair, inflammation, and disease.53,100,101 The finding that pericytes can differentiate into osteoblasts and chondrocytes suggests that these cells may play a role in mediating ectopic calcification.

Perhaps the first evidence that pericytes could differentiate into osteoprogenitor cells came from Urist’s group in the 1980s. Using an experimental model of wound repair in the skull, these workers demonstrated that BMP induced the osteogenic differentiation of pericytes resulting in the formation of chondroid and woven bone. Subsequently, by following the fate of Monastral blue–labeled vascular cells during experimentally induced osteogenesis and chondrogenesis, Diaz-Flores et al demonstrated that these cells could differentiate into osteoblasts and chondrocytes. In addition, recombinant endothelial cell growth factor was found to induce both angiogenesis and osteogenesis in an orthopaedically expanded suture in the rat. As the osteoprogenitor cells were located in close proximity to blood vessels, these workers concluded that activated pericytes were the source of osteoprogenitor cells. Definitive proof that pericytes could differentiate into osteoblasts and chondrocytes was obtained when pericytes were loaded in diffusion chambers and implanted into athymic mice. Areas resembling bone, cartilage, mineralized cartilage, fibrocartilage, and non-mineralized cartilage with lacunae containing chondrocytes were formed in these chambers.

When cultured in standard growth medium in vitro, pericytes display a reproducible pattern of growth and differentiation, culminating in the formation of large multicellular nodules containing a mineralized matrix after 8 weeks. The nodules formed by pericytes contain an extracellular matrix comprising type I collagen, osteopontin, matrix Gla protein and osteocalcin, strongly resembling the matrix found in calcified vessels. In addition, hydroxyapatite crystals have been identified within the nodules and the ratio of calcium to phosphate is similar to that found in bone. This pattern of growth and differentiation can be modulated by culturing the cells on collagen gels, in the presence of β-glycerophosphate, decreased oxygen tension, antibodies to MGP, advanced glycation end-products, or by inhibiting Axl-Gas6 signaling. By culturing the cells as pellets in the presence of a defined chondrogenic medium containing TGF-β3, pericytes can be induced to undergo chondrogenic differentiation in vitro. The chondrocyte markers type II collagen, aggrecan, and sox9 were identified in these pellets; these molecules have also been identified in calcified vessels. Together, these data demonstrate that pericytes can differentiate along the osteogenic and chondrogenic lineages in vitro and in vivo and deposit a matrix resembling that found in calcified blood vessels. Interestingly, cells resembling pericytes, namely calcifying vascular cells (CVCs), adventitial myofibroblasts, vascular smooth muscle cells, and interstitial cells isolated from heart valves have also been found to form mineralized nodules and to exhibit multilineage potential in vitro. In addition, CVCs stain positively with the 3G5 anti-
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or where the cell is located along the differentiation pathway. The reasons for these differences are not clear, but may reflect either the extensive heterogeneity that exists within the vasculature, the origin of the cells, or where the cell is located along the differentiation pathway. Furthermore, it is not yet known whether all or some of these cells contribute to the ectopic calcification observed in vivo.

Summary

The extensive vasculature associated with sites of ectopic calcification caused by active angiogenesis occurring in atheromatous vessels, cardiac valves, and skeletal muscle could act as a conduit for the delivery of osteoprogenitor cells to the lesions. In conjunction with growth factors and cytokines released by endothelial cells and localized inflammatory cells, the microenvironment would be permissive for these cells to commit to an osteogenic differentiation path and to deposit a calcified matrix. There is now compelling evidence that these osteoprogenitor cells may be the pericytes that are present in the new blood vessels, suggesting that these cells may provide the cellular link between ectopic calcification and angiogenesis. Several key questions still remain to be addressed. For example: What is the relationship between pericytes, adventitial myofibroblasts, calciﬁcing vascular cells, vascular smooth muscle cells, adventitial progenitor cells, and valvular interstitial cells? How are pericytes maintained as pericytes in normal vessels? How is pericyte differentiation along speciﬁc lineages regulated? Is pericyte differentiation reversible? What contribution do these cells make to ectopic calcification in vivo? A further understanding of the underlying mechanisms that link angiogenesis, pericytes, and ectopic calcification should provide a basis for development of novel therapeutic strategies to treat or arrest this clinically signiﬁcant process.

Acknowledgments

The financial support of the British Heart Foundation UK, Arthritis Research Campaign UK, Biotechnology and Biological Sciences Research Council (BBSRC), Engineering and Physical Sciences Research Council (EPSRC), and Medical Research Council (MRC) are gratefully acknowledged.

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*Circ Res.* 2005;96:930-938
doi: 10.1161/01.RES.0000163634.51301.0d
*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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