Noncollagenous Matrix Proteins
Controlling Mineralization: Possible Role in Pathologic Calcification of Vascular Tissue

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Biomineralization is a highly controlled process that is believed to be regulated by noncollagenous proteins found in the organic matrix of bone. Dystrophic calcification possesses several features of bone, including the presence of noncollagenous proteins, which are also thought to regulate pathologic calcification. Noncollagenous proteins have been demonstrated to be present in a wide variety of tissues. They are also believed to play a role in the pathogenesis of a number of disease processes, including atherosclerosis, restenosis, valvular stenosis, nephrolithiasis, glomerulonephritis, malignant transformation, and metastasis. This review discusses the structure, function, and possible roles of noncollagenous proteins in physiologic and pathologic processes. (Trends Cardiovasc Med 1998;8:199–206) © 1998, Elsevier Science Inc.
Biomineralization is a complex process involving cell differentiation, synthesis, and secretion of matrix in a highly controlled manner. In spite of extensive research, the precise mechanism of tissue calcification is still poorly understood. This process has been meticulously examined in skeletal tissues such as bone, where physiologic tissue mineralization occurs. Bone is composed of inorganic matrix, in the form of hydroxyapatite, and an organic phase consisting of type I collagen and several noncollagenous proteins (NCPs). Although NCPs comprise only 10% of the organic matrix, these proteins have been closely evaluated in order to elucidate the mechanism of calcification. Several observations have led researchers to believe that these proteins are important for matrix organization and regulation of mineralization. NCPs appear in newly forming bone in a precise temporal and spatial distribution (Ingram et al. 1993). Also, alterations in the concentrations of NCPs in metabolic bone diseases such as osteogenesis imperfecta and Paget’s disease suggest that normal bone structure and ossification depend on NCPs (Vetter et al. 1991, Ingram et al. 1996). Finally, type I collagen dissociated from NCP is unable to support mineralization alone (Takano-Yamamoto et al. 1994).

When calcification occurs in nonskeletal tissues, it is considered pathologic. Abnormal calcification of soft tissues in the presence of normal serum calcium levels is termed dystrophic calcification. It is often found in areas of necrosis or injury including atherosclerotic plaques, aging or damaged cardiac valves, or neoplasia. Dystrophic calcification has many similarities to physiologic tissue mineralization. Ectopic calcification is calcification found in any nonsosseous tissue, with or without associated necrosis or injury. Recent evidence demonstrates that calcifications within nonskeletal tissues such as atherosclerotic plaques have many features of bone. These areas of mineralization within nonskeletal tissues are composed of both hydroxyapatite (Anderson 1983) and organic matrix, including type I collagen and NCPs (Fitzpatrick et al. 1994). Also, both physiologic and pathologic calcification involve collagen-associated crystal deposition and initiation of mineralization within matrix vesicles (Kim 1976). This information has led to the concept that dystrophic calcification is an active, regulated process rather than a passive accumulation of mineral.

In addition to the proposed mechanism that NCPs play in mineralization, some of these proteins appear to play a role in other processes such as tumorigenesis, kidney function (including stone formation), immune response, and formation of metastases. This review discusses the possible role of NCPs in physiologic and dystrophic calcification and provides an overview of the proposed functions and the potential roles of these proteins clinically.

- **Noncollagenous Proteins: Structure, Localization, and Potential Functions**

Calcification is common in patients with known coronary artery disease (Figure 1), and the incidence of coronary artery calcification is positively correlated with age (McCarthy and Palmer 1974). The onset and progression of calcification in arterial plaques remains unknown, but the presence of hydroxyapatite, bone matrix vesicles (Anderson 1983), and bone morphogenetic protein-2a (Bostrom et al. 1993), a potent osteoblastic differentiation factor, implies that vascular calcification is analogous to bone ossification. This led researchers to hypothesize that NCPs, which are thought to regulate mineralization of bone, may also play a role in the development of arterial plaques.

The NCPs are a diverse group of proteins and can be classified into four groups according to their basic structures. These groups include proteoglycans (versican, decorin, biglycan, and hyaluronate), glycoproteins (osteonectin, osteopontin, bone sialoprotein, BAG-75, thrombospondin, fibronectin, and vitronectin), the γ carboxy glutamic acid (gla)-containing proteins (osteocalcin, matrix gla protein, and protein S), and serum-associated (albumin, α2-HS glycoprotein, and growth factors) (Gehron Robey 1996).

Proteoglycans are complex macromolecules composed of repeating disaccharides linked covalently to a protein core. Versican, a large proteoglycan, has a molecular weight of approximately 106 and is present during the initial stages of bone formation. Versican, when associated with hyaluronic acid, may function to demarcate the area to be mineralized. Biglycan (PGI) and decorin (PGII) are two smaller chondroitin sulfate proteoglycans. Biglycan is composed of a 45-kD core protein with two chondroitin sulfate moieties attached near the amino terminus of the protein. Decorin has a 38-kD core protein and one chondroitin sulfate chain attached. Both core proteins are composed almost exclusively of a leucine-rich sequence that may influence binding of these proteins to matrix constituents (Gehron Robey 1996). These proteins are expressed in both skeletal and nonskeletal tissues in a specific spatial orientation (Bianco et al. 1990, Ingram et al. 1993). Decorin is also a negative inhibitor of transforming growth factor (TGF)-β, an autocrine factor that stimulates cell growth and differentiation (Yamaguchi et al. 1990). TGF-β has also been implicated in the in vivo resistance of tumors to certain chemotherapeutic agents. Decorin binds to TGF-β via its core protein and restores tumor sensitivity to these agents.

![Figure 1. Calcified tissue within an atherosclerotic artery. This photomicrograph represents a section of human coronary artery obtained at autopsy. (A) Contact microradiograph of the tissue section. White areas designate calcified tissue. (B) von Kossa’s staining reveals dark calcified lesions within the atherosclerotic plaque.](image-url)
Tissue/cell | Noncollagenous matrix protein
---|---
Atherosclerotic plaques | Plaque Gla protein
 | Matrix Gla protein
 | Vitronectin
VSMC | OPN, BSP, ON, OC, decorin, biglycan
Foam cells | OPN
Macrophages | OPN, ON
Endothelial cells | OPN, ON
Cardiac valves | OC, OP, ON, vitronectin, fibronecrtin (monocyte-macrophages, T-lymphocytes, fibroblastlike mesenchymal cells)
Necrotic myocardium | OPN

OPN, osteopontin; ON, osteonectin; OC, osteocalcin; BSP, bone sialoprotein.

(Teicher et al. 1997). This finding suggests that decorin may prove to be an important agent in anticancer therapy. A rich source of TGF-β in the cardiovascular system are platelets. Deposition of TGF-β in association with thrombus formation may result in regulation of matrix formation during plaque formation.

Glycoproteins within bone matrix have the common feature of being highly modified posttranslationally by glycosylation, phosphorylation, and sulfation. Some glycoproteins contain the RGD-sequence similar to osteopontin, thrombospondin, matrix Gla protein, and decorin. The glycoproteins that contain the RGD-sequence include fibronectin, thomboospondin, vitronectin, BAG-75, osteopontin, and bone sialoprotein.

Fibronectin is a glycoprotein that is composed of two distinct disulfide bonded subunits of approximately 200 kD each and is widely distributed among connective tissues, including bone (Gehron Robey 1996). The RGD (Arg–Gly–Asp) sequence is a motif common to many matrix proteins and serves as a recognition site for diverse integrin receptors. The integrins interact with various cell types within the matrix milieu. In addition to its RGD-binding sequence, which mediates attachment to integrins, binding sites for cells, collagen, and glycosaminoglycans are also present. Owing to these interactions, fibronectin can mediate cell adhesion and migration within the vascular wall. Another RGD-containing protein, thomboospondin, is actually a family of related glycoproteins. It has been localized to osteoid early in osteogenesis and has subsequently been described as a component of various embryonic tissues, including bone, muscle, and cartilage (Corless et al. 1992). Thrombospondin has also been directly implicated in the induction of cell proliferation (Majack et al. 1988). Vitronectin, another glycoprotein in this group, has been localized to mineralized matrix but may not be synthesized locally (Gehron Robey 1996). BAG-75 is a 75-kD acidic glycoprotein with limited sequence homology to osteopontin. It contains polyacidic sequence repeats similar to those identified in osteopontin and bone sialoprotein. Unique to BAG-75 is the presence of 44 mol of organic phosphate per mol and an electronegative charge density similar to proteoglycans (Gorski and Shimizu 1988). Recent evidence indicates that BAG-75 self-associates in vivo to form large fibrillar complexes. Other acidic glycoproteins do not form similar complexes. In view of this property of self-association, it is hypothesized that BAG-75 may function in a supportive mechanical role or as an electronegative ionic barrier that concentrates phosphate ions within bone matrix (Gorski et al. 1996).

Two of the major NCPs are osteopontin and bone sialoprotein. Bone sialoprotein (BSP) has a highly conserved polyglutamic acid sequence. BSP is found in mineralized tissues such as bone, dentin, cementum, and hypertrophic cartilage (Chen et al. 1992, Bianco et al. 1990). The functions of BSP include: (a) promotion of attachment and spreading of various cells via RGD-dependent mechanisms (Oldberg et al. 1988), (b) potent nucleation of hydroxyapatite in vitro (Hunter et al. 1996), and (c) probable hydroxyapatite nucleation in vivo. Evidence to support the in vivo action of BSP is its presence at the mineralization front of developing bone in association with newly mineralized matrix (Chen et al. 1992, Roach 1994, and Ingram et al. 1993). In addition, BSP expression is regulated by steroid hormones that control mineralized tissue formation. 1,25-dihydroxyvitamin D₃ suppresses BSP expression while dexamethasone upregulates the glycoprotein (Sodek et al. 1995). Finally, BSP is capable of stimulating calcification in a cell line of osteoblast-like MC3T3-E1 cells in a dose-dependent manner (Zhou et al. 1995). The nucleation regions of BSP have recently been localized to its glutamic-acid sequences (Goldberg et al. 1996).

The final RGD-containing protein to be discussed is osteopontin (OPN); also known as bone sialoprotein I, 2ar, Spp-1, and Eta-1). OPN is a multifunctional protein with widespread expression in both skeletal and nonskeletal tissues. OPN appears in the following tissues: bone, kidney, brain, smooth muscle cells, activated T cells, macrophages, and many others. OPN contains an N-terminal signal sequence often seen in secreted molecules and a highly conserved RGD sequence that interacts with integrin receptors. This RGD sequence is required for its adhesive activity. Evidence supporting this requirement is shown by the introduction of a mutation in the RGD sequence of mouse OPN, resulting in the inability to support cell attachment and migration (Xuan et al. 1995).

OPN has been associated with multiple diverse functions in both physiologic and pathologic processes. OPN has been implicated in biomineralization owing to its ability to bind Ca²⁺ at high capacity and low affinity. In addition, OPN has been localized at the mineralization front, the lamina limitans, and cement lines (Ingram et al. 1993). OPN also plays a role in cell adhesion and, owing to its expression in tumor cells, demonstrates a positive correlation with metastatic ability (Craig et al. 1990). OPN is chemotactic for vascular smooth muscle cells, macrophages, and endothelial cells in vitro (Liaw et al. 1995a, O’Brien et al. 1994). Finally, OPN has multiple potential roles in disease and tissue injury including (a) calcification in atherosclerotic plaques (Fitzpatrick et al. 1994),...
(b) T-cell response to infection (Patarca et al. 1989), and (c) wound healing (Murry et al. 1994, McKee and Nanci 1996). The potential roles of OPN in these disorders is discussed in further detail later in this review.

Osteonecrotic (ON) or SPARC (secreted protein acidic and rich in cysteine, also termed BM40, and 43K protein) is a collagen-binding glycoprotein widely distributed in human tissues undergoing cell proliferation, migration, and developmental remodeling. SPARC was originally described as a major NCP in bone (Termine et al. 1981). SPARC has also been localized to other developing and mature human tissues (Mundlos et al. 1992). The precise role of ON is unknown, but it appears to regulate cell-matrix interactions.

ON contains four modular domains that can function independently to inhibit cell spreading and proliferation and selectively disrupt focal adhesions between cells. Therefore, it has been referred to as an antiadhesive (Lane and Sage 1994). In addition, ON also promotes cytoskeletal rearrangement, increases levels of matrix metalloproteinases and plasminogen activator inhibitor (PAI-1), and decreases fibronectin and thrombospondin and extracellular matrix production is decreased. It also increases endothelial cell permeability and inhibits the cell cycle. ON is also produced by platelets, smooth muscle cells, macrophages, fibroblasts, capillary endothelium, and malignant cells at sites of wound repair and tumor invasion (Lane and Sage 1994). Expression of ON in fibroblasts was shown to be maximal during the period when there is active angiogenesis in the wound bed (Reed and Sage 1996). Finally, ON has also been found to be significantly increased in injury-induced liver fibrosis compared with normal livers (Frizell et al. 1995). The possible role of ON in cardiovascular disease is originally discovered as a result of an upregulation of OPN mRNA levels in phenotypically distinct smooth muscle cells (Giachelli et al. 1991). These smooth muscle cells displayed a phenotype that appeared to be critical for arterial neointima formation, a lesion often seen in atherosclerosis and restenosis (Giachelli et al. 1991, Severson et al. 1995). OPN mRNA levels were found to be low in uninjured ves-
sels but markedly elevated in the injured vessel (Giachelli et al. 1993). OPN mRNA is also elevated in proliferating smooth muscle cells in vitro (Gadeau et al. 1993). Cell proliferation is thought to be brought about by OPN functions of adhesion, migration, and chemotactic activity. Not only is OPN produced by smooth muscle cells, but it is also produced by macrophages and endothelial cells (O’Brien et al. 1994) and is colocalized with calcification in arterial plaques (Fitzpatrick et al. 1994). Further evidence of the role of OPN in progression of atherosclerotic lesions includes OPN mRNA expression by smooth muscle–derived foam cells adjacent to sites of calcification (Ikeda et al. 1993). This suggests that OPN may contribute to smooth muscle proliferation, cellular accumulation, and mineralization of arterial plaques. Additionally, antibodies directed against OPN inhibits neointimal thickening after endothelial injury. These data provide convincing evidence of OPN’s role in the pathogenesis of atherosclerosis (Liauw et al. 1997).

Current research has concentrated on the role of integrin receptors in mediation of the cellular adhesive and migratory effects of OPN. Only one integrin type receptor, αvβ3, appears to mediate migration of smooth muscle cells to OPN (Giachelli et al. 1995). Absence of the αvβ3 receptor abolished migration in response to OPN and resulted in a 50% reduction in the number of cells able to adhere to OPN compared with αvβ3-containing cells (Liauw et al. 1995b). Moreover, OPN has recently been demonstrated to serve as a substrate for platelet adhesion. Agonist-stimulated platelets were found to adhere to OPN via a process mediated by αvβ3 binding (Bennett et al. 1997). This suggests that OPN may play an additional role in the progression of atherosclerosis by contributing to vascular occlusion.

In addition, ON was also detected in atherosclerotic plaques. In contrast to OPN, the expression of ON decreased with development and progression of atherosclerosis (Hirota et al. 1993). Its role in calcification of atherosclerotic plaques is not clear.

Another approach to determining the mechanism of arterial calcification involves examining the autocrine, paracrine, and endocrine factors involved in regulation of matrix proteins. One of these factors that is responsible for the induction of matrix proteins is angiotensin II. Angiotensin II, a vasopressor peptide, has been implicated in the rat arterial injury response and caused vascular smooth muscle proliferation with increased smooth muscle DNA replication (Daemen et al. 1991). Moreover, smooth muscle DNA replication is expressed differentially in the arterial media and intima. After balloon-induced injury, angiotensin II–induced DNA replication disappears in the media after approximately 9 weeks, whereas intimal angiotensin II–induced DNA replication was not only greater at all times after injury but also remained elevated for approximately 6 months. DNA replication levels corresponded with AT1 receptor levels in the intima but not the media (de Blois et al. 1996a). Angiotensin II also induces OPN expression. After administration of angiotensin II in injured rat arteries, intimal smooth muscle cells overexpressed OPN, indicating a possible link between angiotensin II–dependent progression and OPN expression (de Blois et al. 1996b). Other proteins implicated in the rat arterial injury response are basic fibroblast growth factor (bFGF) and TGF-β. These proteins increase OPN expression in smooth muscle cells in vitro (Giachelli et al. 1993).

The incidence of coronary artery disease (CAD) is related to estrogen status of the individual. Premenopausal women have a significant reduction in CAD versus age-matched males (Furman 1968). This suggests that estrogen exerts a cardioprotective effect. The effect of estrogen on the development of atherosclerosis is poorly understood. Despite the favorable effects of estrogen on serum lipids, this accounts for only 30% to 50% of the cardiovascular protection in women undergoing estrogen replacement therapy (Bush and Barrett-Conner 1985). Studies have shown that estrogen has a negative effect on coronary artery smooth muscle proliferation in a porcine model (Moraghan et al. 1996). It has also been postulated that estrogen may directly affect calcification. Preliminary evidence suggests that estrogen may have a negative effect on NCP production within arterial plaques (Fitzpatrick 1996). However, another study has shown that 17β estradiol induces osteoblast differentiation and calcification in bovine aortic vascular cells in vitro. This study suggests that estrogen exerts a stimulatory effect on vascular calcification similar to bone calcification (Balica et al. 1997). These results are contradictory to what would be expected based on the inverse relationship of estrogen use to the development of CAD.

The similarities between aortic valvular disease and atherosclerotic coronary artery disease has long been recognized (Mohler et al. 1997). Both diseases result in calcific deposition in the form of hydroxyapatite in areas of lipid accumulation (Sarig et al. 1994), and the accumulation of lipid and calcium progresses with age in both. Also, both atherosclerotic and valvular lesions demonstrate matrix vesicles (Kim 1976), matrix proteins, and inflammatory cells (Srivatsa et al. 1997).

Dystrophic calcification of both native and bioprosthetic valves remains to be a significant cause of patient morbidity and mortality (Schoen et al. 1992, O’Keefe et al. 1991). Degenerative calcific aortic stenosis is the most common valvular abnormality, and its incidence increases with age (Lombard and Selzer 1987, Selzer 1987). It may result in sudden death after becoming symptomatic. Furthermore, late calcific degeneration occurs commonly in bioprosthetic valves, resulting in valve failure (Schoen and Hobson 1985). The mechanism of calcification is not well characterized, but it is thought that NCPs may also play a role in mineralization of both native and bioprosthetic valves.

Multiple NCPs have been isolated in cardiac valves including OC, OP, and ON. Areas of calcification within both native and bioprosthetic valves are intimately associated with NCPs. OPN, ON, and OC have been localized to the calcification front in both native and bioprosthetic valve lesions in association with monocyte–macrophage cells, T-lymphocytes, and fibroblastlike mesenchymal cells. Because these NCPs are all expressed in platelets also, it has been suggested that platelet thrombus may provide a concentrated source of NCPs (Srivatsa et al. 1997). Previously, OPN has been localized in areas of calcification and macrophage accumulation in both early and late stages of aortic stenosis (O’Brien et al. 1995). Also, OC has been localized to areas of calcification within glutaraldehyde-preserved
porcine valves (Levy et al. 1983b). These data suggest that valvular calcification is an active, controlled event that is potentially modifiable.

One additional pathologic condition of the cardiovascular system to be associated with NCPs is myocardial necrosis. Owing to its functions in cellular adhesion, chemotaxis, and migration, OPN has been implicated in the response to tissue injury in myocardial injury and infarction. After myocardial infarction, necrotic muscle is infiltrated with inflammatory cells, phagocytosed, and replaced by granulation tissue during tissue repair. Thus, osteopontin expression was examined following cardiac injury in rats in order to determine whether it played a role in the healing process. Macrophages infiltrating the necrotic myocardium expressed high levels of OPN and OPN mRNA during the first 48 hours after injury. Double labeling with the macrophage marker ED1 demonstrated, however, that only some of the macrophages expressed OPN. On day 4, OPN expression was somewhat diminished despite the presence of numerous macrophages in the myocardium during the formation of granulation tissue, and OPN was significantly downregulated at 1 and 4 weeks after injury. In addition, a human heart with an 8-day-old infarct displayed abundant expression of OPN protein and OPN mRNA in macrophages within necrotic and granulation tissue (Murry et al. 1994). OPN expression by macrophages is also seen in mineralized tissue injury (McKee and Nanci 1996). The production of OPN by macrophages after tissue injury indicates that OPN may be important in the healing response, possibly on actions on cellular adhesion, migration, and phagocytosis.

Recently, insights into the role of matrix gla protein (MGP) in vascular tissue calcification were gained through the use of knockout transgenic technology. Luo et al. (1997) reported findings in a mouse strain lacking MGP. These animals develop normally in utero but die within several months as a result of vessel rupture from arterial calcification. Chondrocytes, a common source of MGP, were located within vessel walls. This model is similar to Mönckeberg’s medial calcific sclerosis and suggests an important role for MGP in the inhibition of calcification (Luo et al. 1997).

### Noncollagenous Proteins and Kidney Disorders

Noncollagenous proteins have important roles in physiologic and pathologic regulation of kidney function [for review, see Furness (1996)]. Osteopontin was originally named uropontin because it was localized to the specific size within normal kidney parenchyma. Osteopontin is associated with embryogenesis of the kidney and antibodies directed against osteopontin will result in the development of abnormal kidney tubules. Osteopontin is a potent inhibitor of calcium oxalate crystals in vitro and may be associated with the inhibition of nephrolithiasis. Last, inflammatory diseases of the kidney have been associated with upregulation of osteopontin mRNA expression in experimental models. Osteopontin may mediate nitric oxide synthesis in the proximal tubules and protect proximate tubule epithelial cells against oxidative injury.

### Association of Noncollagenous Proteins With Metastatic Disease

Many tumors contain foci of calcification. NCPs have been found in association with the calcification process and are markers for neoplastic transformation [for review, see Bernstein and Liotta (1994), Ruoslahti (1994)]. The NCPs are found in a variety of tumors including breast, colon, and prostate cancers and melanoma. Metastatic prostate cancer and breast cancer, for example, are associated with microcalcifications. In other cell types, osteopontin expression is increased in association with neoplastic transformation. One hypothesis suggests that osteopontin may assert its effects by stimulation of signal transduction in cancer cells through the αvβ3 integrin receptor. Finally, tumor cells producing osteopontin are capable of suppressing NO synthase production in inflammatory cells and may be a mechanism by which host cells can protect themselves from immune destruction by the host.

### Future Directions

NCPs are present in multiple pathologic conditions. Most of these disease processes are prevalent within the general population and cause significant morbidity and mortality. For example, atherosclerosis and valvular stenosis are both positively correlated with age. As the average life expectancy of the population continues to rise, we can also expect to observe an increase in the incidence of these two disorders.

Many questions remain to be answered regarding the role of these NCPs in physiologic and pathologic conditions. The mechanism(s) that regulates the expression of these proteins needs to be determined. For example, the process by which estrogen exerts its cardioprotective function and its positive effects on bone mineral density would give us valuable insight into the prevention of both coronary artery disease and osteoporosis. In addition, NCP expression in tumor cells may be controlled by the same signal that causes metastases. These functions of NCPs may be demonstrated by the use of pharmacologic blocking agents, receptor blockade, and induction of mutations.

In conclusion, NCPs may play an integral role in the pathogenesis of multiple pathologic conditions and in the development of normal tissue. By determining the function of NCPs in both physiologic and pathologic processes, we may be able to intervene in the development of several diseases and make a significant impact on medical treatment, prevention, and outcome.

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