REVIEW

Epithelial-like transport of mineral distinguishes bone formation from other connective tissues

Harry C. Blair[1,2] ⓘ | Quitterie C. Larrouture[2] | Irina L. Tourkova[1,2] |
Deborah J. Nelson[3] | Steven F. Dobrowolski[2] | Paul H. Schlesinger[4]

[1]Department of Research and Development, Veteran's Affairs Medical Center, Pittsburgh, Pennsylvania, USA

[2]Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[3]Department of Neurobiology, Pharmacology & Physiology, University of Chicago, Chicago, Illinois, USA

[4]Department of Cell Biology, Washington University, Saint Louis, Missouri, USA

Correspondence
Harry C. Blair, Department of Pathology, University of Pittsburgh, 720 Scaife Hall, Pittsburgh, PA 15261, USA.
Email: hcblair@imap.Pitt.edu

Abstract

We review unique properties of bone formation including current understanding of mechanisms of bone mineral transport. We focus on formation only; mechanism of bone degradation is a separate topic not considered. Bone matrix is compared to other connective tissues composed mainly of the same proteins, but without the specialized mechanism for continuous transport and deposition of mineral. Indeed other connective tissues add mechanisms to prevent mineral formation. We start with the epithelial-like surfaces that mediate transport of phosphate to be incorporated into hydroxyapatite in bone, or in its ancestral tissue, the tooth. These include several phosphate producing or phosphate transport-related proteins with special expression in large quantities in bone, particularly in the bone-surface osteoblasts. In all connective tissues including bone, the proteins that constitute the protein matrix are mainly type I collagen and γ−carboxylate-containing small proteins in similar molar quantities to collagen. Specialized proteins that regulate connective tissue structure and formation are surprisingly similar in mineralized and non-mineralized tissues. While serum calcium and phosphate are adequate to precipitate mineral, specialized mechanisms normally prevent mineral formation except in bone, where continuous transport and deposition of mineral occurs.

KEYWORDS
arterial wall, osteoblast, osteocalcin, phosphate transport, skin, tendon, type I collagen

---

Abbreviations: bgp, bone gla protein (also called bglap or osteocalcin); mgp, matrix gla protein.

Capitalized abbreviations are murine gene names.

- - - - - - - - - -

1 | THE ORIGIN OF MINERALIZED TISSUES AND AN OUTLINE OF BONE STRUCTURE

Mineral first appeared in teeth; it later was adapted for the vertebrate skeleton. Either skeletal or dental mineral is mainly a calcium phosphate salt, hydroxyapatite. The source of the phosphate is a key to understanding the physiology of mineralized tissues. In evolution, skin denticles, small mineralized structures, first appeared in the skin epithelium of jawless fishes and localized in an epithelial layer at the endoderm-ectoderm interface, preceding skeletal mineral in evolution.[1] Mineral in teeth and in the vertebrate skeleton is mainly a calcium phosphate salt, hydroxyapatite. Teeth developed within an epithelial layer adjacent to sea water that contains practically no phosphate. Calcium, in contrast, is generally present in quantities not requiring its concentration to form crystalized mineral. But phosphate is limiting and occurs in sea water at nanomolar concentrations.[2] The evolutionary solution was to make the tooth in an epithelium internal to the animal and capable of dramatic phosphate accumulation for tooth formation.[3] Teeth subsequently move to the ectoderm, and the dental epithelium is removed after mineralization; any new teeth require new epithelial structures.

2 | SKELETAL BONE IS SURROUNDED BY CELLS FORMING AN EPITHELIAL-LIKE SURFACE

The bone surface is composed of specialized cells **osteoblasts** (Figure 1A,B), except during degradation (by **osteoclasts**, is not reviewed here). Osteoblasts maintain the phosphate transport function, but in a more complex organ capable of regeneration and repair (Figure 1C). The epithelial-like surface is connected to underlying osteocytes. Osteocytes are derived from osteoblasts buried in matrix; the combination of connected surface cells and osteocytes is the **osteon**, the bone forming unit. Osteoblasts produce and secrete matrix structural proteins, type I collagen and osteocalcin, or bone gla protein, (bgp, gene BGLAP).[4] Extracellular collagen trimers and osteocalcin occur in layers at alternating right angles forming extracellular matrix; onto this mineral is deposited.[4] Extracellular collagen trimers and osteocalcin occur in layers at alternating right angles; onto this mineral is deposited (Figure 2).[4] The dense collagen matrix is mineralized by osteoblasts transporting phosphate and removing acid (protons).[5,6] Bone represents the large majority of the Ca^{2+}, HPO_4^{2-}
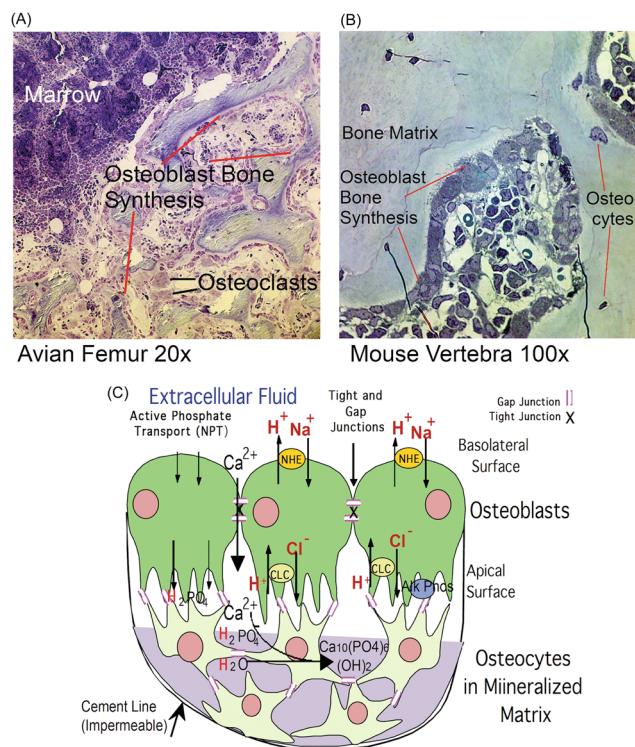

FIGURE 1 Rapidly fixed bone preserves the its cellular structure. A and B are unpublished frames of rapidly fixed bone as described.[9] (A) A semi-thin section of high-turnover avian bone. Methylene blue stained, photographed at ×20. The image is 400 μm wide. In the center is trabecular bone, light blue (collagen, acellular) containing interspersed round cells (osteocytes, not labeled). The bone is bounded by cuboidal osteoblasts and a few large multinucleated bone degrading cells, osteoclasts; this review does not consider osteoclast physiology, focusing on bone formation. At the top left, marrow is seen, with dark clusters of red blood cells. (B) A semi-thin section of normal mouse vertebral bone. Methylene blue stained; ×100 oil. The section is 80 μm wide. The osteoblasts have processes on their apical (bone forming) side. Two osteocytes in matrix are labeled. (C) Schematic diagram of an osteon, the bone forming unit. Surface osteoblasts are an epithelial-like layer. The major transported ions and relation of surface and deep cells are shown. Osteoblasts express NHE Na/H exchangers, important in mineralization. Also highly expressed are ClC family H^+/Cl^- antiporters, hypothesized to be important in acid uptake from matrix. This is reflected in the stoichiometry of bone formation: $6\ HPO_4^{2-} + 2\ H_2O + 10\ Ca^{2+} \leftrightarrow Ca_{10}(PO_4)_6(OH)_2 + 8\ H^+$. Recent work suggests that Ca^{2+} transport is largely paracellular. Phosphate transport involves the neutral phosphate transporter-2 and the liver-kidney-bone alkaline phosphatase, highly expressed on the apical surface, liberating free phosphate. Not all transporters described in osteoblasts are illustrated.

and base-equivalents in the vertebrate body.[4] This is important: hundreds of grams of Ca^{2+}, phosphate, and base equivalents, many moles, are deposited in bone. Specifically, bone is on average 2/3 hydroxyapatite mineral, $Ca_{10}(PO_4)_6(OH)_2$, molecular weight 500, so 750 g of bone
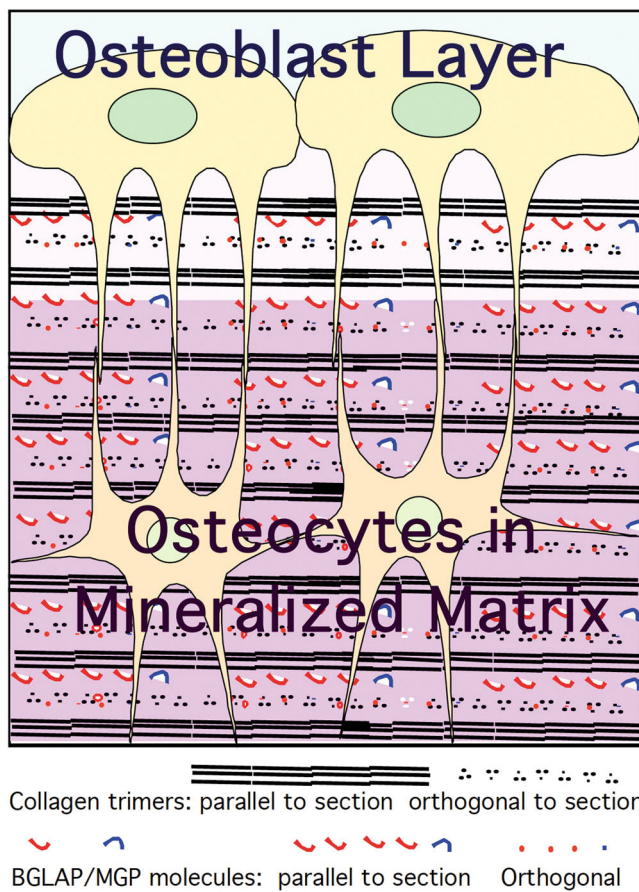
FIGURE 2 Schematic diagram of the major bone matrix structural proteins, collagen and gla proteins, bglap and mgp. The collagen strands in mature bone occur in layers aligned with the main axis of stress alternating with right angles to the main axis of stress. The collagen and gla proteins are not to scale; the gla proteins are <5% the size of collagen proteins (4–5 kDa).

contains ~1 mole of hydroxyapatite with 10 M of calcium, 6 M of PO_4^{3-}, and 2 M of OH^-. In contrast, human serum H^+ is ~40 nM (pH 7.4); total serum Ca^{2+} or phosphate are ~2.5 and ~1.3 mM, respectively. The functional significance and physiology of concentration of phosphate[7] and base equivalents in bone[4] by an epithelial-like layer is is often overlooked; it is important because bone mineral cannot form without transport of components including outward transport of H^+ (Figure 1C).

3 | OSTEOBLAST SUBTYPES AND DERIVATION

Osteoblasts make the dense collagen bone matrix, and mineralize it, or survive as flattened, inactive bone-lining cells. Bone modeling by active osteoblasts requires that the epithelial-like cells are integrated on a microscopic scale diagrammed in (Figure 1C).

Even among those who work with bone, many rarely see intact bone surfaces. This reflects loss of bone surface cells during fixation and sectioning. Briefly, active osteoblasts are very highly metabolically active, and thus do not survive more than seconds or a few minutes unfixed.[8,9] Not fixing the living cells shows denuded surfaces. In addition to speed of tissue processing, cell surfaces must be exposed directly to fixative, generally by cracking the bones open before fixation. Dropping a large piece of tissue typically does not show the full osteoblast layer because the fixative must penetrate to the active cells, which takes too much time. To avoid degradation of surface cells, small fresh bone fragments are rapidly fixed. Examples of well fixed avian and murine bone are shown (Figure 1A,B).

Osteoblasts develop from stromal stem cells which are abundant in marrow. The development mediated by bone-specific transcriptional promoters, particularly Runx2 and Osterix.[8] Osteoblasts are connected by gap and tight junctions, and bone synthesis is linked to the transport, particularly of phosphate, that enables mineralization of bone matrix (Figure 1C).[9] Efficient differentiation of osteoblasts in vitro is difficult; recent methods that assist in replicating osteoblast differentiation and function include differentiation of stromal stem cells on perforated polyethylene terephthalate membranes in Dubelco's Modified Essential Medium with 44 mM carbonate at pH ~7.8.[10] There are many other studies of differentiation of osteoblasts in vitro; the method is cited is efficient and reproducible.

4 | AFTER ITS SYNTHESIS, BONE STRUCTURE IS BOUNDED BY A TIGHT EPITHELIUM-LIKE CELL LAYER

Bone excludes most substances in circulation, including calcium-binding substances. There is an important exception: calcium-binding dyes are **transported** into bone by osteoblasts synthesizing bone, which we term **synthetic osteoblasts**, a feature allowing bone formation to be measured accurately.[11] In the opinion of the authors, in vivo labeling of bone formation by fluorescent dye markers[11,12] is the only known specific method that does not depend on preservation of surface osteoblasts. This reflects that living bone matrix is impermeable to diffusion. In contrast, dead bone, where surface cells die, is permeable and can be labeled with calcium-binding dyes such as tetracycline,[12] although tetracycline labels dead bone diffusely, rather than the discrete linear pattern labeling bone formation in vivo.[11,12] Mechanisms of transport of fluorescent compounds,

including calcein and tetracyclines, into bone during mineralization are not defined,[11] but probably include *ABCC1*, a multi-drug resistance protein that mediates transport of fluorescent calcium-binding dyes out of cancer cells.[13] It is possible to count cuboidal active osteoblasts and flat inactive osteoblasts, if preserved (called the **osteoblast number**).[14] We have done so, but it is often impractical and it is less accurate and reproducible than in vivo labeling of bone formation.

5 │ THE COLLAGENOUS MATRIX IN BONE AND OTHER TISSUES

The densely produced structural proteins are collagen and the γ-carboxylate (gla) containing proteins, bone gla protein (gene BGP) and matrix gla protein (MGP), with vitamin K dependent carboxylation.[15] Over 90% of bone matrix protein by mass is type I collagen,[16] structurally identical to type I collagen in other organs, including skin and arteries. Type I collagen normally is formed from two *COL1A1* and one *COL1A2* gene products. The major structural proteins—collagen and gla proteins—are present in roughly molar equivalent amounts, although the collagen is ~20 times the mass of gla proteins. Regulatory proteins in much lower amounts that are selectively embedded in the matrix, mainly for surface cell binding, briefly discussed below. Collagen and gla proteins are expressed in bone and in other collagenous tissues. Dermal fibroblasts and differentiating stromal stem cells make similar amounts of osteocalcin[17]; insofar as it has been examined, matrix produced by other organs gives similar results,[18] suggesting that all dense collagen matrices might have similar gla protein content. A possible role of osteocalcin in localizing mineral deposition is widely hypothesized without a clear role emerging.[19] As far as the authors can determine, quantitative comparison of mgp and bgp in skin, tendon, and arteries has not been done. Bgp appeared by gene duplication from mgp or an ancestral gene and both bgp and mgp might regulate mineralization of type I collagen extracellular matrix.[20,21] Bgp is discussed as a serum protein with endocrine regulatory function; serum bgp is measurable and typically 20 ng/mL, ~40% uncarboxylated, while procollagen type I propeptide in serum has similar concentration, typically 40 ng/mL, in older adult humans.[22] Lack of major endocrine effects of bgp deficiency in mice indicate that endocrine functions of bgp may be species specific; mice but not humans have multiple bgp genes.[23] In humans, careful study showed that uncarboxylated osteocalcin is bioactive and correlates negatively with fasting glucose[24]; osteocalcin is measured as a variable in evaluating diabetes in some circumstances. As suggested here, there are inconsistencies in endocrine data and further study will be needed to address this.[24] Further, the ~4.5 kD protein is filtered in the kidney and thus a marker of bone metabolism.[25]

6 │ ECTOPIC MINERALIZATION

This is an important topic with several interesting findings. All of these reflect that serum phosphate and calcium are sufficient to precipitate amorphous mineral nonspecifically.[26] It is important to make the distinction of ectopic mineralization, where tissue dysfunction occurs such as in the aorta, and bone mineralization, where very dense mineral is continuously accumulated. Early work suggested the importance of pyrophosphate, present in micromolar concentrations in human serum.[27] Causes of pyrophosphate deficiency and arterial mineralization include defects in expression of ENPP1, the ectonucleotide pyrophosphatase/phosphodiesterase 1.[28] Another cause of ectopic mineralization is deficiency of mgp. In mice, bgp knockout has relatively mild,[23] but mice not expressing mgp develop to term and then die within 2 months from arterial and vessel calcification.[29,30] There are probably relationships between impaired carboxylation of matrix gla protein, reduced pyrophosphate, and reduced ENPP1,[31] although no general mechanism is known. Other tissues including skin are affected by mgp defects reflecting that mechanisms not related to bone mineralization are common to all other dense collagenous tissues.[32]

7 │ Bgp (OSTEOCALCIN) BINDS TO COLLAGEN IN BONE

Bgp binds to insoluble type I collagen, with a pH dependence from pH 6 to 10. Highest affinity binding was in 25 mM 2(N-morpholino)-ethane sulfonic acid with 1 mM calcium chloride at pH 6.[33] Calcium and phosphate did not affect association, and the saturation curves were consistent with a single binding site for bgp in collagen.[33] Work in human cortical bone localized bgp by antibody labeling after demineralization by acetic acid. The bgp associated with concentric layers of collagen in the osteons.[34] It is likely that losses during acid processing affected antibody labeling. Bgp is variably modified by carboxylation at three sites after translation,[35,36] Inhibiting carboxylation causes subtle, but detectable, differences in mineralization.[37] A schematic model of lamellar (mature) bone matrix with collagen and gla proteins is shown (Figure 2).

8 | COLLAGEN DEGRADATION

Bone type I collagen is degraded by multinucleated osteoclasts. This subject is not our focus, but it is briefly mentioned since it affects bone proteins important in matrix. Briefly, degradation is assessed by products include C-telopeptides, released with their crosslinks. These can be assayed in the serum as indicators of resorption.[38,39] Osteocalcin is also released into serum by osteoclasts,[40] where it may indicate bone turnover and to an extent is related to endocrine effects in diabetes.[24] Bone degradation mechanisms are outside of our topic; for a review, see Lo Iacono et al.[41]

9 | OTHER PROTEINS THAT REGULATE MINERALIZED AND NON-MINERALIZED CONNECTIVE TISSUES

This topic is interesting, in part, because almost all of the proteins are shared with other connective tissues including skin, tendons, and vascular walls.

Integrins and acidic proteins: Association of bone matrix with osteoblasts is mediated by specific binding proteins, integrins,[42] and proteins binding toll-like receptor or tyrosine kinase receptors, decorin and biglycan.[43] Osteoblasts express numerous integrins including fibronectin, binding the integrin receptor $\alpha 5\beta 1$ and vitronectin, binding the receptor $\alpha v\beta 3$.[42] In some cases bone sialoprotein, was reported to be specific to calcified tissues,[44] but in general osteoblast collagen-binding proteins are are widely expressed in all collagenous tissues. The collagen binding proteins are individually dispensable in bone and other organs with relatively minor defects, probably because gene duplication function to be supported by multiple proteins, although double knockouts may cause severe skin or bone defects (Figure 3).

Integrin binding matrix proteins in bone, skin, and arterial wall: The only integrin binding protein specific, as far as is known, for mineralized tissues, is bone sialoprotein.[44] Others, including osteopontin and periostin are widely expressed; expression is important in inflammatory or autoimmune diseases.[45] Vitronectin and fibronectin are widely distributed and found in the skin, particularly during repair by fibroblasts,[46] and in aorta in endothelial cells associated with repair.[47] Thus, in general only one mineral-associated integrin appears to be specific to mineral; others are widely expressed. In skin or arteries they appear mainly to act during repair or tissue differentiation.
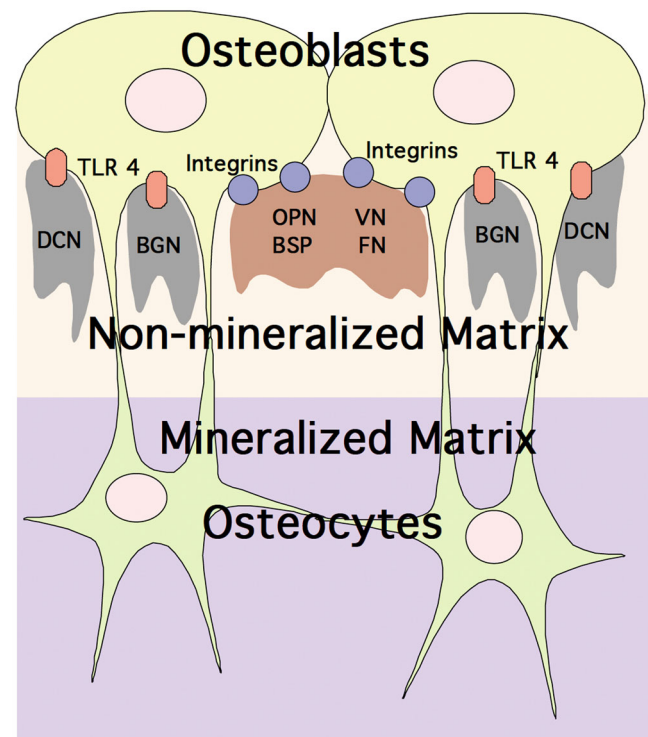

FIGURE 3 Key collagen binding proteins, other than gla proteins, in osteoblasts. The integrin binding proteins include osteopontin (OPN), vitronectin (VN), bone sialoprotein (IBSP) fibronectin (FN). Proteins binding to toll-like receptor-4 (TLR4) or tyrosine kinase receptors (not illustrated) expressed in bone and other connective tissues include decorin (DCN) and biglycan (BGN).

Bone sialoprotein and osteopontin: These are small integrin binding ligand N-linked glycoprotein (SIBLING) molecules[48]; they are is characterized by acidic amino acids and sugar residues.[49] These are much less abundant than osteocalcin, and accumulate at the border of calcified tissues.[50] Knockouts of bone sialoprotein or osteopontin, and double knockouts, do not have lethal phenotypes,[51] consistent with complementary effects of several bone accessory proteins. But absence of the proteins reduces stability of bone and increases bone turnover,[51] and double KO mice[51] exhibited intriguing dynamics in BSP and OPN response to mechanical stimuli. For a review including other SIBLING proteins see.[52]

Additional integrin binding proteins in bone and in many other tissues: Vitronectin is named for its useful property of binding to glass, vitronectin is a 54 kDa glycoprotein found in bone, in serum, and in many other tissues. It affects cell adhesion in contexts including clotting and wound healing.[53] Homozygous null mice deficient from the original vitronectin knockout showed

normal development, including skeleton, fertility, and survival.[54] As with many adhesion proteins, this suggests overlapping function and dispensability of individual proteins.

Fibronectin: A high-molecular weight glycoprotein dimer, ~550 kDa,[55] has been shown to regulate interaction of proteinases with developing bone matrix.[56] Fibronectin gene inactivation is embryonic lethal.[57] Conditional fibronectin knockout using a tamoxifen-induced cre-loxp system in adult mice has no major effect on major organs, suggesting that other proteins overlap in function limiting the effect of its absence.[58]

10 | MATRIX PROTEINS BINDING TOLL-LIKE RECEPTORS OR RECEPTOR TYROSINE KINASES

In this group decorin and biglycan, two closely related peri-cellular matrix proteoglycans, are key proteins present in all dense collagen-containing tissues. They bind to type I collagen and to osteoblasts or other cells via toll-like receptors and receptor tyrosine kinases.[43] Decorin and biglycan both regulate collagen fibrillogenesis.[59] In bone, these proteins are largely expressed at the border of bone formation where fibrillogenesis and regulation mainly occur.[60] Systemically, the proteins are widely expressed in connective tissues; decorin and biglycan double knockouts have severe bone and skin abnormalities, although defects occur if either protein is absent.[61]

Decorin: Gene DCN is abundant in bone, skin, and other connective tissue organs. It is a leucine-rich proteoglycan with molecular weight ~100–150 kDa. Decorin is characterized by high-affinity interactions with collagen and regulation of collagen fibrillogenesis.[62] It sequesters multiple growth factors including transforming growth factor (TGF)-β1, and inhibits tyrosine kinases receptors, including epidermal growth factor (EGF) receptor, the insulin-like growth factor receptor I, and the hepatocyte growth factor receptor.[63]

Biglycan: Gene BGN is probably related to decorin by gene duplication.[61] Lack of either protein results in reduced bone mass or regulatory defects centered on TGF-β1.[61] Biglycan is X-linked,[64] of interest to genetic defect analysis, with various functional defects causing spondyloepimetaphyseal dysplasia[65] and Meester-Loeys syndrome, thoracic aortic aneurysm. It is not primarily a bone defect.[66]

Tenascins: Several homologous extracellular matrix proteins, tenascins include tenascin-X, with EGF-like repeats, fibronectin like repeats, and a fibronectin-like domain.[67] Tenascins modify cell adhesion fibronectin interaction, and typically increase cell motility. Tenascin-X associates with type I collagen; its absence causes classic-Like Ehlers Danlos Syndrome, with defects in skin, joints, and blood vessels.[68]

Laminin: Laminins are high-molecular weight protein trimers of basement membranes. At least one laminin is expressed by osteoblasts and plays a major role in regulating bone turnover.[69] However, the localization of laminin in bone matrix is unclear.

Fibrillin-1: The fibrillin-1 gene (FBN1) deserves mention. Its expression in bone is minor; it occurs at many sites in other tissues including great vessels and lung. Fibrillin-1 is a large extracellular glycoprotein important in TGF-β signaling.[70] Its mutations cause autosomal dominant Marfan's Syndrome with increased long bone growth generating the characteristic "marfanoid" body habitus.[71]

11 | MECHANISMS REGULATING BONE INDEPENDENTLY OF OTHER CONNECTIVE TISSUES

Signals may be specific to bone or non-specific: Many signals that regulate bone are not, at least mainly, products of bone, including vitamin D, parathyroid hormone, and others. On the other hand, although vitamin D traditionally requires kidney 1α-hydroxylase, it was recently shown that osteoblasts express vitamin D 1α-hydroxylase: thus, the bone-modifying hormone 1,25-dihydroxyvitamin D is activated by osteoblasts as well as in the kidney.[72] A second example, its ramifications still uncertain, is that low concentrations of adrenocorticotropic hormone (ACTH) induce bone formation directly.[72] Another key cytokine in bone, but not specific to it, is TGF-β. TGF-β is expressed in osteoblasts (and in many organs other than bone).[73] Active TGF-β1 is sequestered in bone matrix. It is present in acellular bone matrix.[74] TGF-β is secreted in latent complexes in the matrix; in bone resorption, TGF-β is released from bone matrix and recruits osteoblasts to the site of bone resorption.[75]

FGF23 and Sclerostin are specific to bone turnover regulation (*Figure 4*): These are produced within bone by osteocytes.[76] An unresolved issue is how the proteins get out of the bone since they function in bone formation or as hormones. This might reflect osteocyte-osteoblast transport: these cells are connected by the haversian system. However, the gap junction system between the cells, particularly the connexin CX43, while playing a role in cellular control,[77,78] is too small to transport molecules over 1–2 kD, which is to say, FGF23 and sclerostin. Transport of FGF23 and sclerostin from osteocytes may depend, at least in part, on the FGF
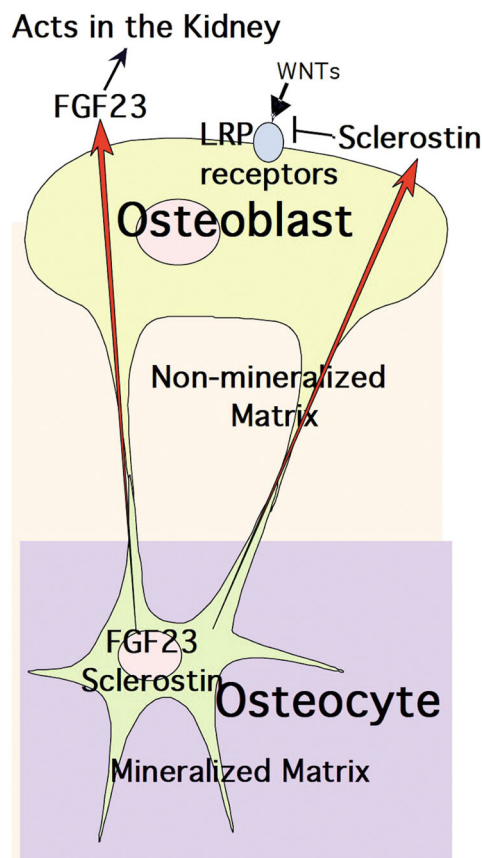
FIGURE 4 Translocation of osteocyte-produced hormones, sclerostin and Fgf23, to the osteoblast surface. While the pathway of secretion is unclear, a hypothetical pathway shown considers that the osteocytes are connected to the surface by processes running in haversian canals. The most likely mechanism (see text) involves secretion by osteocytes with re-uptake to translocate of sclerostin and Fgf23 to surface osteoblasts. In addition, under some conditions osteocytes may communicate directly with blood vessels, a major alternative mechanism (see text for references).

receptor-1.[79] An alternative is demonstration of connection, under some circumstances, of communication of the lacuno-canalicular network with the haversian canal directly to blood vessels.[80] Bone blood vessels do not occur within osteocytic bone, however, experimental work suggests that canalicular-to-vascular communication occurs.[80]

FGF23 function: FGF23 is a fibroblast growth factor protein that regulates phosphate in plasma in response to 1,25 dihydroxyvitamin D3. It is produced by osteocytes.[76] *FGF23* gain-of-function mutations cause autosomal dominant hypophosphatemic rickets; FGF23 loss-of-function mutations cause familial hyperphosphatemic tumoral calcinosis.[81] Regarding FGF23 transport, endosome to cytoplasmic translocation of other FGF family proteins is established,[82] so another reasonable hypothesis (without direct data) is that a chain of uptake and

transport of FGF23 is independent of signaling.[83] Osteocyte knockout of FGF23 causes hyperphosphatemia.[79] FGF23 is activated by the alpha isoform of the enzyme Klotho.[84]

Sclerostin function: Sclerostin, gene SOST, is a glycoprotein of the bone morphogenetic protein family that negatively regulates bone formation and bone mass. Sclerostin is expressed in osteocytes, and similarly to FGF23 is hypothesized to be transported to surface osteoblasts for release,[85] although a mechanism is not known. Sclerostin binds LRP5/6 receptors, inhibiting Wnt signaling.[86,87] Mutations of sclerostin or defective expression cause disorders with high bone mass, sclerosteosis and van Buchem disease. The van Buchem disease is an autosomal recessive skeletal disease characterized by bone overgrowth.[88] Functionally, sclerostin reduces bone formation[86,87]; providing a potential mechanism for therapeutic increase in bone mass.

Endocrine effects of sclerostin and FGF23: As introduced above, effects of bgp occur on response to reproductive hormones and glucose secretion under some conditions in some species.[89,90] Interestingly, there are also unexpected effects of sclerostin defects on adipocytes,[91] and, in parallel to effects of bgp on glucose, there are inter-relationships of FGF23 secretion and glucose/insulin.[92]

12 | MAJOR CHANGES IN BONE EXTENDING BEYOND DEFECTS IN INDIVIDUAL PROTEINS

Aging and several diseases change bone matrix amount or quantity, without clear relationship to individual matrix proteins, although individual diseases are caused by specific defects result in more widespread changes. Examples include phenylketonuria (PKU) and fibrous dysplasia. Effect of PKU and fibrous dysplasia on bone are briefly summarized.

Significant changes in bone matrix occur with aging: In general this is reflected as a loss of bone mass, in humans after age 30.[93] This is not a simple linear effect, and in particular fragility of the skeleton to damage increases in porosity but not mineral content.[94] The mechanism involved in bone loss with aging, at least in mice, involves reduced bone transport proteins.[95] In humans, bone mass generally peaks in early adulthood and declines in age by complex mechanisms, in women being dramatically increased in the menopause, frequently causing problems with fractures requiring surveillance and treatment.[96]

Phenylalanine hydroxylase-deficient phenylketonuria: PKU is shown in recent work to cause decreased bone

mass despite maintaining blood phenylalanine in the therapeutic range. Recent studies suggest that this involves a developmental defect in the osteoblast lineage with reduced oxidative ATP production necessary for bone synthesis.[97] It is not fully studied, but may be related to other types of osteopenia and osteoporosis, including with aging.

Fibrous dysplasia: Fibrous dysplasia is a genetic disease related to activating mutations of the *GNAS* complex, a gene locus producing Gs-α, essential to G protein-coupled receptor-regulated adenylyl cyclase signal transduction. it affects bone stem cell differentiation and formation of other cells including adipocytes.[98] Although there have been advances placing stem cells at the center of fibrous dysplasia mechanisms,[98] much remains unclear.

13 | SUMMARY

The unique nature of bone mainly reflects its production surrounded by an epithelial-like cell surface, enabling dense mineralization and regulation that is independent of other dense connective tissues. On the other hand, mineralized and non-mineralized tissues with dense collagen for structural support express, with a few exceptions, the same proteins.

Mineralization is mediated by transport of ions, especially of phosphates, in osteoblasts. There are several proteins involved, as summarized in Figure 1. There is sometimes difficulty with ectopic mineralization, which is regulated by pyrophosphate, matrix gla protein, and the ectonucleotide pyrophosphatase/phosphodiesterase 1.

Many regulatory proteins are expressed in the mineral-protein composite; these also occur in almost all cases in other connective tissues. These include proteins that bind Arg-Gly-Asp motifs, the integrins, and proteins associated with the the toll-like receptor 4, decorin and and biglycan and related proteins. There are many interesting, typically rare, defects in bone and in non-mineralized tissues that are caused by specific defects in regulatory proteins.

Specialized regulatory proteins for the turnover of mineralized tissue only, FGF23 and sclerostin, allow bone to function independently of turnover in other collagenous tissues. There are also hundreds of genetic defects primarily involving bone, the repository of the greatest amount of type I collagen, including over 100 varieties of osteogenesis imperfecta including collagen sequence defects, collagen processing and transport, reviewed elsewhere.[99]

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Harry C. Blair http://orcid.org/0000-0002-1152-3718

REFERENCES

1. McCollum M, Sharpe PT. Evolution and development of teeth. *J Anat*. 2001;199:153-159. doi:10.1046/j.1469-7580.2001.19910153.x
2. Menzel DW, Corwin N. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation1. *Limnol Oceanogr*. 1965;10:280-282. doi:10.4319/lo.1965.10.2.0280
3. Hasselgren G, Franzén A, Hammarström LE. Histochemical characterization of alkaline phosphatase in developing rat teeth and bone. *Eur J Oral Sci*. 1978;86:325-336. doi:10.1111/j.1600-0722.1978.tb00635.x
4. Blair HC, Larrouture QC, Tourkova IL, et al. Support of bone mineral deposition by regulation of pH. *Am J Physiol Cell Physiol*. 2018;315:C587-C597. doi:10.1152/ajpcell.00056.2018
5. Larrouture QC, Nelson DJ, Robinson LJ, et al. Chloride-hydrogen antiporters ClC-3 and ClC-5 drive osteoblast mineralization and regulate fine-structure bone patterning in vitro. *Physiol Rep*. 2015;3:e12607. doi:10.14814/phy2.12607
6. Liu L, Schlesinger PH, Slack NM, Friedman PA, Blair HC. High capacity Na+/H+ exchange activity in mineralizing osteoblasts. *J Cell Physiol*. 2011;226:1702-1712. doi:10.1002/jcp.22501
7. Wang B, Yang Y, Liu L, Blair HC, Friedman PA. NHERF1 regulation of PTH-dependent bimodal Pi transport in osteoblasts. *Bone*. 2013;52:268-277. doi:10.1016/j.bone.2012.10.001
8. Komori T. Regulation of bone development and maintenance by Runx2. *Front Biosci*. 2008;13:898-903. doi:10.2741/2730
9. Blair HC, Larrouture QC, Li Y, et al. Osteoblast differentiation and bone matrix formation in vivo and in vitro. *Tissue Eng Part B Rev*. 2017;23:268-280. doi:10.1089/ten.TEB.2016.0454
10. Larrouture QC, Tourkova IL, Stolz DB, et al. Growth and mineralization of osteoblasts from mesenchymal stem cells on microporous membranes: epithelial-like growth with

transmembrane resistance and pH gradient. *Biochem Biophys Res Commun*. 2021;580:14-19. doi:10.1016/j.bbrc.2021.09.075

11. Wilde CD, Jaworski ZF, Villanueva AR, Frost HM. Quantitative histological measurements of bone turnover in primary hyperparathyroidism. *Calcif Tissue Res*. 1973;12:137-142. doi:10.1007/BF02013729

12. Eberhardt AW, Yeager-Jones A, Blair HC. Regional trabecular bone matrix degeneration and osteocyte death in femora of glucocorticoid-treated rabbits. *Endocrinology*. 2001;142:1333-1340. doi:10.1210/endo.142.3.8048

13. Sampson A, Peterson BG, Tan KW, Iram SH. Doxorubicin as a fluorescent reporter identifies novel MRP1 (ABCC1) inhibitors missed by calcein-based high content screening of anticancer agents. *Biomed Pharmacother*. 2019;118:109289. doi:10.1016/j.biopha.2019.109289.

14. Dempster DW, Compston JE, Drezner MK, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR histomorphometry nomenclature committee. *J Bone Miner Res*. 2013;28:2-17. doi:10.1002/jbmr.1805.

15. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K- dependent proteins in bone. *Physiol Rev*. 1989;69:990-1047. doi:10.1152/physrev.1989.69.3.990

16. Weiner S, Traub W. Bone structure: from ångstroms to microns. *FASEB J*. 1992;6:879-885. doi:10.1096/fasebj.6.3.1740237

17. Zanata F, Curley L, Martin E, et al. Comparative analysis of human adipose-derived stromal/stem cells and dermal fibroblasts. *Stem Cells Dev*. 2021;30:1171-1178. doi:10.1089/scd.2021.0164

18. Severson AR, Ingram RT, Fitzpatrick LA. Matrix proteins associated with bone calcification are present in human vascular smooth muscle cells grown in vitro. *In Vitro Cell Dev Biol Anim*. 1995;31:853-857. doi:10.1007/BF02634569

19. Chen L, Jacquet R, Lowder E, Landis WJ. Refinement of collagen-mineral interaction: a possible role for osteocalcin in apatite crystal nucleation, growth and development. *Bone*. 2015;71:7-16. doi:10.1016/j.bone.2014.09.021

20. Cancela ML, Laizé V, Conceição N. Matrix Gla protein and osteocalcin: from gene duplication to neofunctionalization. *Arch Biochem Biophys*. 2014;561:56-63. doi:10.1016/j.abb.2014.07.020

21. Price PA, Toroian D, Lim JE. Mineralization by inhibitor exclusion. *J Biol Chem*. 2009;284:17092-17101. doi:10.1074/jbc.M109.007013

22. Smith C, Hiam D, Tacey A, et al. Higher bone remodeling biomarkers are related to a higher muscle function in older adults: effects of acute exercise. *Bone*. 2022;165:116545. doi:10.1016/j.bone.2022.116545

23. Komori T. Functions of osteocalcin in bone, pancreas, testis, and muscle. *Int J Mol Sci*. 2020;21:7513. doi:10.3390/ijms21207513

24. Lacombe J, Al Rifai O, Loter L, et al. Measurement of bioactive osteocalcin in humans using a novel immunoassay reveals association with glucose metabolism and β-cell function. *Am J Physiol Endocrinol Metab*. 2020;318:E381-E391. doi:10.1152/ajpendo.00321.2019

25. Ivaska KK, Käkönen SM, Gerdhem P, Obrant KJ, Pettersson K, Väänänen HK. Urinary osteocalcin as a marker of bone metabolism. *Clin Chem*. 2005;51:618-628. doi:10.1373/clinchem.2004.043901

26. Sandin K, Kloo L, Odselius R, Olsson LF. The observation of nano-crystalline calcium phosphate precipitate in a simple supersaturated inorganic blood serum model - composition and morphology. *J Appl Biomater Biomech*. 2009;7:13-22. doi:10.1177/228080000900700

27. Russell RGG, Bisaz S, Donath A, Morgan DB, Fleisch H. Inorganic pyrophosphate in plasma in normal persons and in patients with hypophosphatasia, osteogenesis imperfecta, and other disorders of bone. *J Clin Invest*. 1971;50:961-969. doi:10.1172/JCI106589

28. Li Q, Kingman J, Sundberg JP, Levine MA, Uitto J. Dual effects of bisphosphonates on ectopic skin and vascular soft tissue mineralization versus bone microarchitecture in a mouse model of generalized arterial calcification of infancy. *J Invest Dermatol*. 2016;136:275-283. doi:10.1038/JID.2015.377

29. Luo G, Ducy P, McKee MD, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix gla protein. *Nature*. 1997;386:78-81. doi:10.1038/386078a0

30. Schlesinger PH, Braddock DT, Larrouture QC, et al. Phylogeny and chemistry of biological mineral transport. *Bone*. 2020;141:115621. doi:10.1016/j.bone.2020.115621

31. Boraldi F, Annovi G, Bartolomeo A, Quaglino D. Fibroblasts from patients affected by pseudoxanthoma elasticum exhibit an altered PPi metabolism and are more responsive to pro-calcifying stimuli. *J Dermatol Sci*. 2014;74:72-80. doi:10.1016/j.jdermsci.2013.12.008

32. Gheduzzi D, Boraldi F, Annovi G, et al. Matrix Gla protein is involved in elastic fiber calcification in the dermis of pseudoxanthoma elasticum patients. *Lab Invest*. 2007;87:998-1008. doi:10.1038/labinvest.3700667

33. Prigodich RV, Vesely MR. Characterization of the complex between bovine osteocalcin and type I collagen. *Arch Biochem Biophys*. 1997;345:339-341. doi:10.1006/abbi.1997.0254

34. Ingram RT, Park YK, Clarke BL, Fitzpatrick LA. Age- and gender-related changes in the distribution of osteocalcin in the extracellular matrix of normal male and female bone. Possible involvement of osteocalcin in bone remodeling. *J Clin Invest*. 1994;93:989-997. doi:10.1172/JCI117106

35. Cairns JR, Price PA. Direct demonstration that the vitamin k-dependent bone gla protein is incompletely γ-carboxylated in humans. *J Bone Miner Res*. 1994;9:1989-1997. doi:10.1002/jbmr.5650091220

36. Ivaska KK, Hentunen TA, Vääräniemi J, Ylipahkala H, Pettersson K, Väänänen HK. Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro. *J Biol Chem*. 2004;279:18361-18369. doi:10.1074/jbc.M314324200

37. Amizuka N, Li M, Hara K, et al. Warfarin administration disrupts the assembly of mineralized nodules in the osteoid. *J Electron Microsc*. 2009;58:55-65. doi:10.1093/jmicro/dfp008

38. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. *The Lancet Diabetes & Endocrinology*. 2017;5:908-923. doi:10.1016/S2213-8587(17)30184-5

39. Chubb SAP. Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. *Clin Biochem*. 2012;45:928-935. doi:10.1016/j.clinbiochem.2012.03.035

40. Blair HC, Schlesinger PH. Survival of the glycosylated. *eLife*. 2021;10:e65719. doi:10.7554/eLife.65719

41. Lo Iacono N, Pangrazio A, Abinun M, et al. RANKL cytokine: from pioneer of the osteoimmunology era to cure for a rare disease. *Clin Dev Immunol*. 2013;2013:1-9. doi:10.1155/2013/412768

42. Bennett JH, Moffatt S, Horton M. Cell adhesion molecules in human osteoblasts: structure and function. *Histol. Histopathol*. 2001;16:603-611. doi:10.14670/HH-16.603

43. Neill T, Schaefer L, Iozzo RV. Decorin. *Am J Pathol*. 2012;181:380-387. doi:10.1016/j.ajpath.2012.04.029

44. Foster BL, Soenjaya Y, Nociti, Jr. FH, et al. Deficiency in acellular cementum and periodontal attachment in bsp null mice. *J Dent Res*. 2013;92:166-172. doi:10.1177/0022034512469026

45. Buback F, Renkl AC, Schulz G, Weiss JM. Osteopontin and the skin: multiple emerging roles in cutaneous biology and pathology. *Exp Dermatol*. 2009;18:750-759. doi:10.1111/j.1600-0625.2009.00926.x

46. Sethi KK, Yannas IV, Mudera V, Eastwood M, McFarland C, Brown RA. Evidence for sequential utilization of fibronectin, vitronectin, and collagen during fibroblast-mediated collagen contraction. *Wound Repair Regen*. 2002;10:397-408. doi:10.1046/j.1524-475x.2002.10609.x

47. Kappert K, Blaschke F, Meehan WP, et al. Integrins α v β 3 and α v β 5 mediate VSMC migration and are elevated during neointima formation in the rat aorta. *Basic Res Cardiol*. 2001;96:42-49. doi:10.1007/s003950170076

48. Bayless KJ, Meininger GA, Scholtz JM, Davis GE. Osteopontin is a ligand for the α4β1 integrin. *J Cell Sci*. 1998;111:1165-1174. doi:10.1242/jcs.111.9.1165

49. Kruger TE, Miller AH, Wang J. Collagen scaffolds in bone sialoprotein-mediated bone regeneration. *Scientific World Journal*. 2013;2013:1-6. doi:10.1155/2013/812718

50. Bosshardt DD, Degen T, Lang NP. Sequence of protein expression of bone sialoprotein and osteopontin at the developing interface between repair cementum and dentin in human deciduous teeth. *Cell Tissue Res*. 2005;320:399-407. doi:10.1007/s00441-005-1106-8

51. Bouleftour W, Juignet L, Verdière L, et al. Deletion of OPN in BSP knockout mice does not correct bone hypomineralization but results in high bone turnover. *Bone*. 2019;120:411-422. doi:10.1016/j.bone.2018.12.001

52. Boskey AL, Villarreal-Ramirez E. Intrinsically disordered proteins and biomineralization. *Matrix Biol*. 2016;52-54:43-59. doi:10.1016/j.matbio.2016.01.007

53. Leavesley DI, Kashyap AS, Croll T, et al. Vitronectin—master controller or micromanager? *IUBMB Life*. 2013;65:807-818. doi:10.1002/iub.1203

54. Zheng X, Saunders TL, Camper SA, Samuelson LC, Ginsburg D. Vitronectin is not essential for normal mammalian development and fertility. *Proc Natl Acad Sci USA*. 1995;92:12426-12430. doi:10.1073/pnas.92.26.12426

55. Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci*. 2002;115:3861-3863. doi:10.1242/jcs.00059

56. Saunders JT, Schwarzbauer JE. Fibronectin matrix as a scaffold for procollagen proteinase binding and collagen processing. *Mol Biol Cell*. 2019;30:2218-2226. doi:10.1091/mbc.E19-03-0140

57. George EL, Georges-Labouesse EN, Patel-King RS, Rayburn H, Hynes RO. Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development*. 1993;119:1079-1091. doi:10.1242/dev.119.4.1079

58. Yuan X, Yang S, Li W, et al. Construction of fibronectin conditional gene knock-out mice and the effect of fibronectin gene knockout on hematopoietic, biochemical and immune parameters in mice. *PeerJ*. 2020;8:e10224. doi:10.7717/peerj.10224

59. Zhang G, Chen S, Goldoni S, et al. Genetic evidence for the coordinated regulation of collagen fibrillogenesis in the cornea by decorin and biglycan. *J Biol Chem*. 2009;284:8888-8897. doi:10.1074/jbc.M806590200

60. Bianco P, Fisher LW, Young MF, Termine JD, Robey PG. Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. *J Histochem Cytochem*. 1990;38:1549-1563. doi:10.1177/38.11.2212616

61. Young MF, Bi Y, Ameye L, Chen XD. Biglycan knockout mice: new models for musculoskeletal diseases. *Glycoconj J*. 2002;19:257-262. doi:10.1023/A:1025336114352

62. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J Cell Biol*. 1997;136:729-743. doi:10.1083/jcb.136.3.729

63. Iozzo RV, Sanderson RD. Proteoglycans in cancer biology, tumour microenvironment and angiogenesis. *J Cell Mol Med*. 2011;15:1013-1031. doi:10.1111/j.1582-4934.2010.01236.x

64. Traupe H, van den Ouweland AMW, van Oost BA, et al. Fine mapping of the human biglycan (BGN) gene within the Xq28 region employing a hybrid cell panel. *Genomics*. 1992;13:481-483. doi:10.1016/0888-7543(92)90279-2

65. Cho SY, Bae JS, Kim NKD, et al. BGN mutations in X-linked spondyloepimetaphyseal dysplasia. *Am J Hum Genet*. 2016;98:1243-1248. doi:10.1016/j.ajhg.2016.04.004

66. Meester JAN, De Kinderen P, Verstraeten A, Loeys B. Meester-Loeys syndrome. *Adv Exp Med Biol*. 2021;1348:265-272. doi:10.1007/978-3-030-80614-9_12

67. Valcourt U, Alcaraz LB, Exposito JY, Lethias C, Bartholin L. Tenascin-X: beyond the architectural function. *Cell Adh Migr*. 2015;9:154-165. doi:10.4161/19336918.2014.994893

68. Chiquet-Ehrismann R, Tucker RP. Tenascins and the importance of adhesion modulation. *Cold Spring Harbor Perspect Biol*. 2011;3:a004960. doi:10.1101/cshperspect.a004960

69. Uehara N, Kukita A, Kyumoto-Nakamura Y, Yamaza T, Yasuda H, Kukita T. Osteoblast-derived Laminin-332 is a novel negative regulator of osteoclastogenesis in bone micro-environments. *Lab Invest*. 2017;97:1235-1244. doi:10.1038/labinvest.2017.55

70. Coelho SG, Almeida AG. Síndrome de Marfan revisitada – da genética à clínica. *Rev Port Cardiol*. 2020;39:215-226. doi:10.1016/j.repc.2019.09.008

71. Judge DP, Dietz HC. Marfan's syndrome. *The Lancet*. 2005;366(9501):1965-1976. doi:10.1016/S0140-6736(05)67789-6

72. Tourkova IL, Liu L, Sutjarit N, et al. Adrenocorticotropic hormone and 1,25-dihydroxyvitamin D3 enhance human osteogenesis in vitro by synergistically accelerating the expression of bone-specific genes. *Lab Invest*. 2017;97:1072-1083. doi:10.1038/labinvest.2017.62

73. Kassem M, Kveiborg M, Eriksen EF. Production and action of transforming growth factor-β in human osteoblast cultures: dependence on cell differentiation and modulation by calcitriol: effects of TGF-β on human osteoblasts in vitro. *Eur J Clin Invest*. 2000;30:429-437. doi:10.1046/j.1365-2362.2000.00645.x

74. Panahipour L, Omerbasic A, Nasirzade J, Gruber R. TGF-β activity of a demineralized bone matrix. *Int J Mol Sci*. 2021;22:664. doi:10.3390/ijms22020664

75. Crane JL, Xian L, Cao X. Role of TGF-β signaling in coupling bone remodeling. *Meth Mol Biol*. 2016;1344:287-300. doi:10.1007/978-1-4939-2966-5_18

76. Tresguerres FGF, Torres J, López-Quiles J, Hernández G, Vega JA, Tresguerres IF. The osteocyte: a multifunctional cell within the bone. *Ann Anat*. 2020;227:151422. doi:10.1016/j.aanat.2019.151422

77. Riquelme MA, Cardenas ER, Xu H, Jiang JX. The role of connexin channels in the response of mechanical loading and unloading of bone. *Int J Mol Sci*. 2020;21:1146. doi:10.3390/ijms21031146

78. Moorer MC, Stains JP. Connexin43 and the intercellular signaling network regulating skeletal remodeling. *Curr Osteoporos Rep*. 2017;15:24-31. doi:10.1007/s11914-017-0345-4

79. Xiao Z, Huang J, Cao L, Liang Y, Han X, Quarles LD. Osteocyte-specific deletion of Fgfr1 suppresses FGF23. *PLoS One*. 2014;9:e104154. doi:10.1371/journal.pone.0104154

80. Dallas SL, Moore DS. Using confocal imaging approaches to understand the structure and function of osteocytes and the lacunocanalicular network. *Bone*. 2020;138:115463. doi:10.1016/j.bone.2020.115463

81. Garringer HJ, Malekpour M, Esteghamat F, et al. Molecular genetic and biochemical analyses of FGF23 mutations in familial tumoral calcinosis. *Am J Physiol Endocrinol Metab*. 2008;295:E929-E937. doi:10.1152/ajpendo.90456.2008

82. Wesche J, Małecki J, Więdłocha A, Skjerpen CS, Claus P, Olsnes S. FGF-1 and FGF-2 require the cytosolic chaperone Hsp90 for translocation into the cytosol and the cell nucleus. *J Biol Chem*. 2006;281:11405-11412. doi:10.1074/jbc.M600477200

83. Zakrzewska M, Sørensen V, Jin Y, Wiedlocha A, Olsnes S. Translocation of exogenous FGF1 into cytosol and nucleus is a periodic event independent of receptor kinase activity. *Exp Cell Res*. 2011;317:1005-1015. doi:10.1016/j.yexcr.2011.01.003

84. Lu X, Hu MC. Klotho/FGF23 axis in chronic kidney disease and cardiovascular disease. *Kidney Dis*. 2017;3:15-23. doi:10.1159/000452880

85. van Bezooijen RL, Roelen BAJ, Visser A, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. *J Exp Med*. 2004;199:805-814. doi:10.1084/jem.20031454

86. Li X, Zhang Y, Kang H, et al. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J Biol Chem*. 2005;280:19883-19887. doi:10.1074/jbc.M413274200

87. Maeda K, Kobayashi Y, Koide M, et al. The regulation of bone metabolism and disorders by Wnt signaling. *Int J Mol Sci*. 2019;20:5525. doi:10.3390/ijms20225525

88. Sebastian A, Loots GG. Genetics of Sost/SOST in sclerosteosis and van Buchem disease animal models. *Metabolism*. 2018;80:38-47. doi:10.1016/j.metabol.2017.10.005

89. Oury F, Ferron M, Huizhen W, et al. Osteocalcin regulates murine and human fertility through a pancreas-bone-testis axis. *J Clin Invest*. 2013;123:2421-2433. doi:10.1172/JCI65952

90. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007;130:456-469. doi:10.1016/j.cell.2007.05.047

91. Kim SP, Frey JL, Li Z, et al. Sclerostin influences body composition by regulating catabolic and anabolic metabolism in adipocytes. *Proc Natl Acad Sci USA*. 2017;114:E11238-E11247. doi:10.1073/pnas.1707876115

92. Bär L, Feger M, Fajol A, et al. Insulin suppresses the production of fibroblast growth factor 23 (FGF23). *Proc Natl Acad Sci USA*. 2018;115:5804-5809. doi:10.1073/pnas.1800160115

93. Burr DB. Changes in bone matrix properties with aging. *Bone*. 2019;120:85-93. doi:10.1016/j.bone.2018.10.010

94. McCalden RW, McGeough JA, Barker MB, Court-Brown CM. Age-related changes in the tensile properties of cortical bone. The relative importance of changes in porosity, mineralization, and microstructure. *J Bone Joint Surg Am*. 1993;75:1193-1205. doi:10.2106/00004623-199308000-00009

95. Tourkova IL, Larrouture QC, Onwuka KM, et al. Age-related decline in bone mineral transport and bone matrix proteins in osteoblasts from stromal stem cells. *Am J Physiol Cell Physiol*. 2023;325:C613-C622. doi:10.1152/ajpcell.00227.2023.

96. Lupsa BC, Insogna K. Bone health and osteoporosis. *Endocrinol Metab Clin North Am*. 2015;44:517-530. doi:10.1016/j.ecl.2015.05.002.

97. Dobrowolski SF, Sudano C, Phua YL, et al. Mesenchymal stem cell energy deficit and oxidative stress contribute to osteopenia in the Pahenu2 classical PKU mouse. *Mol Gen Metab*. 2021;132:173-179. doi:10.1016/j.ymgme.2021.01.014

98. Riminucci M, Gehron Robey P, Saggio I, Bianco P. Skeletal progenitors and the GNAS gene: fibrous dysplasia of bone read through stem cells. *J Mol Endocrinol*. 2010;45:355-364. doi:10.1677/JME-10-0097

99. Jovanovic M, Guterman-Ram G, Marini JC. Osteogenesis imperfecta: mechanisms and signaling pathways connecting classical and rare OI types. *Endocr Rev*. 2022;43:61-90. doi:10.1210/endrev/bnab017