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SHORT COMMUNICATION

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Matrix vesicles are carriers of bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), and noncollagenous matrix proteins

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Abstract Matrix vesicles (MVs) are well positioned in the growth plate to serve as a carrier of morphogenetic information to nearby chondrocytes and osteoblasts. Bone morphogenetic proteins (BMPs) carried in MVs could promote differentiation of these skeletal cells. Vascular endothelial growth factor (VEGF) in MVs could stimulate angiogenesis. Therefore, a study was undertaken to confirm the presence of bone morphogenetic protein (BMP)-1 through -7, VEGF, and the noncollagenous matrix proteins, bone sialoprotein (BSP), osteopontin (OPN), osteocalcin (OC), and osteonectin (ON) in isolated rat growth plate MVs. MVs were isolated from collagenase-digested rachitic rat tibial and femoral growth plates. The presence of BMP-1 through BMP-7, VEGF, BSP, ON, OPN, and OC was evaluated by Western blot, plus ELISA analyses for BMP-2 and -4 content. The alkaline phosphatase-raising ability of MV extracts on cultured rat growth plate chondrocytes was measured as a reflection of MV ability to promote chondro-osseous differentiation. BMP-1 through -7, VEGF, BSP, ON, OPN, and OC were all detected by Western blot analyses. Chondrocytes treated with MV extracts showed a two- to threefold increase in alkaline phosphatase activity over control, indicating increased differentiation. Significant amounts of BMP-2 and BMP-4 were detected in MVs by ELISA. Combined, these data suggest that MVs could play an important morphogenetic role in growth plate and endochondral bone formation.

Key words bone morphogenetic proteins · endochondral bone formation · growth plate · matrix vesicles · mineralization

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Introduction

Matrix vesicles (MVs) are extracellular, membrane-invested vesicles, ~50–200 nm in diameter, that are the initiation site of calcification in all skeletal tissue, including growth plate cartilage, embryonic and growing bone, tendons, and the predentin of teeth [1]. Matrix vesicles are released in a polarized fashion by budding from the lateral edges of growth plate chondrocytes and from the osteoid-facing surfaces of osteoblasts and the apical surfaces of odontoblasts [2,3].

In the growth plate of a growing animal, MVs are released from chondrocytes of the upper hypertrophic zone (HZ). The first mineral crystals arise within MVs of the midhypertrophic zone, and then apatite crystals are released from MVs into the lower hypertrophic zone matrix. Proliferating mineral spreads from matrix vesicles radially outward to infiltrate the adjacent matrix. At the base of the growth plate, small vessels penetrate the transverse matrix septa, leaving calcified longitudinal septa on which osteoblasts from the marrow deposit new bone [4].

In addition to their role in mineralization, MVs also may serve as carriers of morphogenetic molecules, e.g., the bone morphogenetic proteins (BMPs) [1,5]. BMP-2 through BMP-7 regulate the development and repair of skeletal cartilage and bone [6]. It was suggested several years ago by Slavkin et al. that MVs may function as carriers of morphogenetic information during tooth development [3]. Later, it was shown that vesicle-like structures containing BMP-2 were secreted by chondrocytes during the process of ectopic bone formation in the rat [7]. Matrix vesicles isolated from collagenase-digested growth plate cartilage of the rat were shown in preliminary reports to contain significant amounts of BMPs [5,8]. It is likely that MVs receive BMP proteins from cytoplasm of the upper hypertrophic chondrocytes of the growth plate, because MV budding appears to be maximal at this level [4] and upper hypertrophic zone chondrocytes contain the highest levels of immunostainable BMP-1, -2, -3, -4 and -6 [9].

Vascular endothelial growth factor (VEGF), an important promoter of vascularization, is also highly expressed in hypertrophic growth plate chondrocytes [10,11], at a level where MV budding is prominent [4]. Therefore, it is likely that VEGF is packaged into MVs before their release into the extracellular space and may thus promote vascular ingrowth at the base of the growth plate.

Noncollagenous matrix proteins, especially bone sialoprotein (BSP), osteopontin (OPN), osteonectin (ON), and osteocalcin (OC), probably play important roles in regulating the mineralization of growth plate and bone. BSP is known to actively promote bone mineralization in vitro [12]. BSP has also been shown to promote osteoblast differentiation in vitro [13,14], and to stimulate bone repair when implanted into calvarial bone defects in vivo [15]. Thus, the presence of a concentration of BSP in matrix vesicles could serve a dual function by promoting not only mineralization but also the differentiation of growth plate chondrocytes and ingrowing osteoblasts. Osteopontin (OPN), osteonectin (ON), and osteocalcin (OC) have all been shown to inhibit mineralization in vitro [16,17], and genetic deficiencies of OPN [18] or OC [19] have been shown to increase mineral content in bones in vivo.

In this study, Western blot analyses and enzyme-linked immunosorbent assay (ELISA) confirmed the presence of a relative concentration of BMP-1 through BMP-7 in isolated MVs, and it was shown that MV extracts can stimulate the expression of alkaline phosphatase activity by growth plate chondrocytes as an indicator of differentiation. We also demonstrated the presence of VEGF (to our knowledge, for the first time) in isolated rat MVs, suggesting a role for MVs in promoting capillary invasion of the growth plate. Combined, these data indicate a possible role for MVs in regulating endochondral bone formation.

Materials and methods

Isolation of MVs and chondrocytes

After 4 weeks on a rachitogenic diet in a dark room, Sprague–Dawley rats were killed according to guidelines of the Institutional Animal Care and Use Committee (University of Kansas Medical Center), and their tibial and femoral growth plate cartilages were isolated. Matrix vesicles and chondrocytes were collected by collagenase digestion and differential ultracentrifugation as described previously [20–22]. The yield of MVs was estimated by measuring the relative specific activity of alkaline phosphatase in microvesicle fractions [20,22]. Protein concentrations of MVs and chondrocytes were measured by Bradford assay (Bio-Rad, Hercules, CA, USA).

Chondrocyte culture

The chondrocytes, released from collagenase-digested rat tibial and femoral growth plates, were seeded as monolayers, 3×10^5 cells per 35-mm plate, and grown in BGJ_b medium as previously described [23]. When cells were confluent at 7 to 8 days postseeding, extracts of MVs at a concentration of 50 ng protein/ml were added to the chondrocyte culture. Extract of MVs was prepared by solubilizing 10 µg MV protein in 1 ml sterile 4 mM HCl containing 0.1% bovine serum albumin. MV extracts with freshly prepared ascorbic acid (0.25 mM) were added every other day to the chondrocyte cultures. Control chondrocyte cultures were exposed to complete media without added MVs. Cultures were terminated after 4 days of treatment with MV extracts and assayed for alkaline phosphatase activity.

Western blot analysis

Isolated matrix vesicles were dissolved in 1 µg protein/µl in sodium dodecyl sulfate (SDS) containing sample buffer and heated at 95°C for 10 min to denature. Matrix vesicle protein (25–50 µg) samples were separated by electrophoresis in precast, 10%–20% Tris-HCl polyacrylamide gels (Bio-Rad) under reducing conditions. The proteins in the gels were transferred onto polyvinylidene fluoride (PVDF) membranes and blocked with 5% nonfat milk for 2 h. The membranes were then immunoblotted using anti-BMPs, anti-VEGF, or antibodies to noncollagenous matrix proteins diluted with 5% nonfat milk in TBS-T; 1:500 dilution for BMP-5; 1:200 dilution for BMP-2, -3, -6 and VEGF; 1:100 for BMP-7, BSP, and OC; 1:50 for BMP-1; 1:25 for BMP-4; 1:20 for ON; and 1:10 for OPN; and incubated at 4°C overnight. The primary antibody was washed with TBS-T and the membrane was incubated with secondary antibody (1:5000 to 1:7000) for 1 h at room temperature. After the membrane was washed with TBS-T, the immunostained bands were visualized by using the ECL chemiluminescence detection system (Amersham Biosciences). Anti-BMPs and anti-VEGF were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-BMP 4 was obtained from Novocastra (Newcastle upon Tyne, UK) and anti-BMP 5 from Biovision (Mountain View, CA, USA). Antibodies to BSP (WVIDI, 9C5), ON (AON-1), and OPN ($MPIIIB10_1$) were obtained from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA, USA) and anti-OC from Santa Cruz Biotechnology. Recombinant BMP-1 through BMP-7 and VEGF, used as standards to confirm the specificity of bands corresponding to BMPs and VEGF, were obtained from R&D Systems (Minneapolis, MN, USA).

Alkaline phosphatase assay

Alkaline phosphatase assay of chondrocyte cultures previously exposed to MV extracts, or not exposed, was done as previously described [22]. Specific activity of alkaline phosphatase was expressed as units/mg of protein [22]. One unit of alkaline phosphatase activity is defined as the amount of enzyme required to hydrolyze 1 µmole *p*-nitrophenylphosphate (p-NPP) to 1 µmole p-nitrophenol at 37°C.

ELISA

Proteins were extracted from three batches of isolated MVs and chondrocytes of rachitic rat growth plate, according to instructions, using RIPA buffer [1 × phosphate-buffered saline (PBS), 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS] with freshly prepared protease inhibitors [phenylmethylsulfonyl fluoride (PMSF), aprotinin, and sodium orthovanadate], obtained from Santa-Cruz Biotechnology. All samples were preserved at –20°C until assay.

BMP-2 and -4 were detected and measured at room temperature using commercially available ELISA kits (Quantikine Immunoassay Kits) from R&D Systems. Standards and samples were assayed for BMP-2 and -4 according to the instructions. A standard curve was generated from the results of standard dilution, and concentration of BMP-2 and -4 was calculated using this standard curve. Results were expressed as nanograms of BMP-2 and 4 per gram of total protein in a sample.

Statistical analysis

Data are expressed as the mean ± SEM ($n = 3$) for alkaline phosphatase assays of both culture groups as well as for BMP-2 and -4 concentration in MVs and chondrocytes by ELISA. Statistical differences of alkaline phosphatase levels observed in the absence (control) or presence of MV extract-treated cultures were assessed by analysis of t test. $P < 0.05$ was considered to be significant.

Results

Western blot analyses

BMP-2, -4, -5, -6, and -7 were detected at expected, mature monomeric molecular weights, approximately 18–20 kDa (Fig. 1). BMP-3 was detected as expected at about 12.5 kDa with a higher band at about 70 kDa (Fig. 1), corresponding to a similar band we observed in rhBMP-3. The additional higher band might result from posttranslational modification. Other previous studies have mentioned higher bands of BMP-3 [24,25]. Multiple bands were also detected for BMP-5 and BMP-7 in both recombinant and MV samples, as seen in other studies [26–28]. For BMP-5, a dimer at 36 kDa and a homodimer at 52 kDa were detected (Fig. 1) as previously reported [28]. For BMP-7, multiple bands were present at 18, 40, 55, 70, and 110 kDa in both recombinant and MV samples, as shown in previous studies [26–28]. Immunoblots of BMP-1 showed a precursor form at the expected 111 kDa with two spliced variants at 79 and 34 kDa (Fig. 1) [29]. VEGF was detected at the expected 42 kDa level (Fig. 2). The noncollagenous matrix proteins BSP, ON, OPN, and OC were detected at 81, 40, 80, and 8 kDa, respectively, as expected (Fig. 2). Immunoblots of OC also showed other higher bands at 75 and 30 kDa; these could be high molecular weight Gla-containing polypeptides that share antigenic determinants with osteocalcin [30].

Alkaline phosphatase assay

Alkaline phosphatase activities in untreated (control) chondrocyte cultures versus in cultures exposed to MV extract, are shown in Fig. 3. Chondrocytes treated with MV extract showed a two- to three-fold increase in alkaline phosphatase activity above that seen in non-MV-exposed control cultures, which was statistically significant ($P < 0.01$).

ELISA

BMP-2 and -4 were detected by ELISA in each sample of MVs and chondrocytes (Table 1). Results indicate that BMP-2 and -4 concentrations in MVs are comparable to primary chondrocytes from which the MVs were budded. The BMP-2 and -4 content of MVs was at least two- to

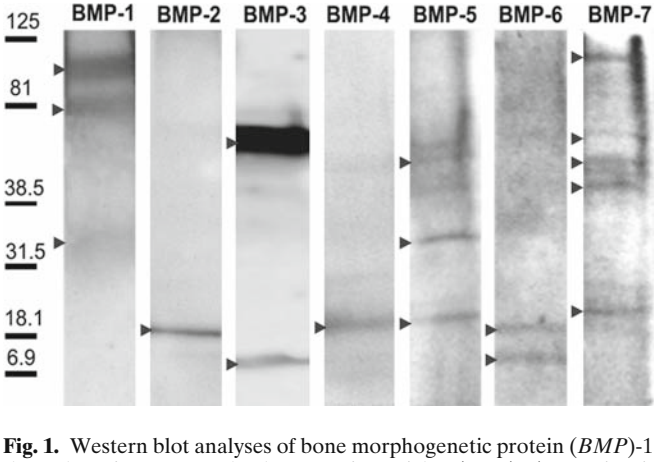

Fig. 1. Western blot analyses of bone morphogenetic protein (*BMP*)-1 through -7 in rat growth plate matrix vesicles (MVs). (*Arrowheads* indicate expected positions of standard and recombinant BMP-1 through BMP-7 proteins)

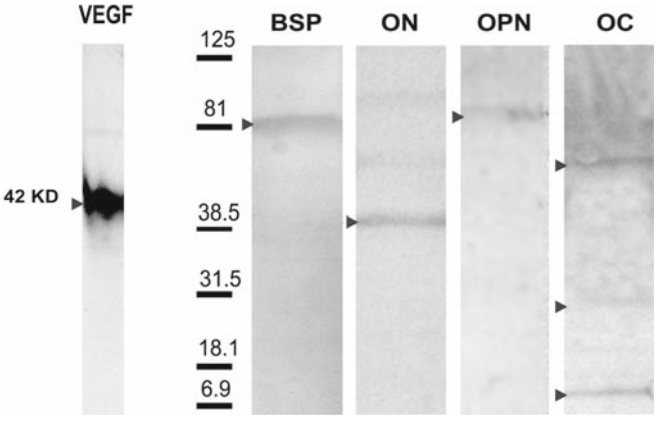

Fig. 2. Western blot analyses of vascular endothelial growth factor (*VEGF*) and noncollagenous matrix proteins of MVs, i.e., bone sialoprotein (*BSP*), osteonectin (*ON*), osteopontin (*OPN*), and osteocalcin (*OC*). *Arrowheads* indicate expected molecular weights of VEGF and noncollagenous proteins

Table 1. Comparison of bone morphogenetic protein (BMP)-2 content [by enzyme-linked immunosorbent assay (ELISA)] in matrix vesicles (MVs) and chondrocytes versus in demineralized bone matrix (DBM) in cited literature

Samples	BMP-2 (ng/g)	BMP-4 (ng/g)	References
MVs	121 ± 16.45 ng/g (n = 3)	17.21 ± 1.07 ng/g (n = 3)	Present study
Chondrocytes	137 ± 8.72 ng/g (n = 3)	15.27 ± 2.55 ng/g (n = 3)	Present study
DBM	33.8–65.8 ng/g	Undetectable	Kanim et al. [31]
	2.11 ± 1.26 ng/g	0.202 ± 0.071 ng/g	Blum et al. [32]
	21.4 ± 12.0 ng/g	5.45 ± 2.04 ng/g	Pietrzak et al. [33]

Results are expressed as mean ± SEM
DBM, demineralized bone matrix

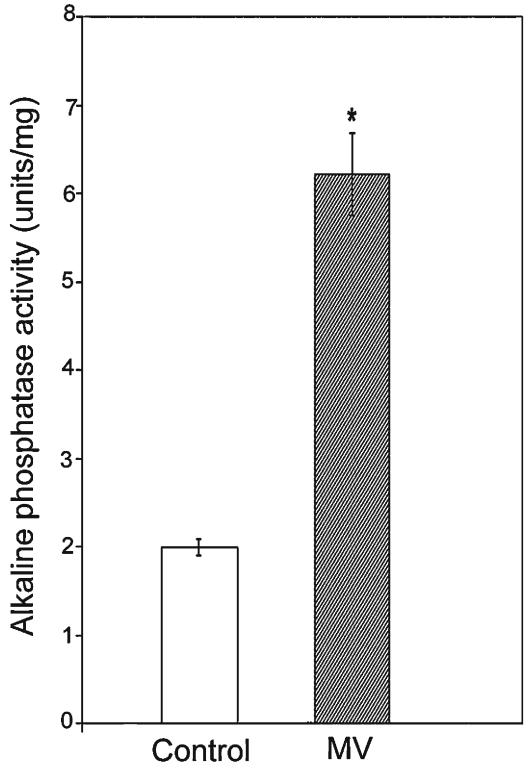

Fig. 3. Bar diagram comparing specific activity of alkaline phosphatase of cultured chondrocytes in the absence of MV extracts (*Control*) versus chondrocytes treated with *MV* extracts (50 ng/ml) for 4 days. One unit of alkaline phosphatase activity is defined as the amount of enzyme required to hydrolyze 1 μmole *p*-nitrophenylphosphate (p-NPP) to 1 μmole *p*-nitrophenol at 37°C. Values are mean ± SEM from three samples of MVs showing statistically significant differences ($P < 0.01$)

fourfold higher than levels previously reported in demineralized bone matrix (DBM) [31–33] (Table 1).

Discussion

The strong immunoreactivity for BMP-1 through -7 that we observed in MVs from rat growth plate by Western blots (see Fig. 1) and ELISA (see Table 1) suggests an osteogenesis-stimulating capability of MVs. This hypothesis is further supported by our finding that chondrocytes treated with MV extracts showed a two- to threefold increase in alkaline phosphatase activity, above that of non-MV-exposed control cultures (see Fig. 3). Presumably, MVs carrying BMPs are released into the matrix of the lower hypertrophic zone of the growth plate during the process of MV biogenesis. Later, after mineral initiation, MV membranes break down [4], and BMPs are released into the matrix of the growth plate. BMPs from MVs could signal differentiation of adjacent chondrocytes, plus osteoblasts and uncommitted osteoprogenitor cells arising in the subjacent metaphysis [34–36].

BMPs released from MVs at the base of the growth plate also may promote the resorption of cartilage matrix during conversion of growth plate cartilage into trabecular bone at the metaphysis. Phenotypic multinucleated osteoclasts resorb the mineralized cartilage matrix septa that project down into the metaphysis, and this creates a scaffold upon which osteoblasts will deposit new bone of the primary spongiosa. Earlier studies indicated a role for BMPs in promoting the differentiation of osteoclasts and their bone resorptive activity [37]. Recently, it was demonstrated that osteoclasts also express BMPs [9,38] as well as BMP receptors [37].

To our knowledge, our data are the first to indicate the presence of a significant concentration of VEGF in isolated rat growth plate MVs. The main action of VEGF is to promote the ingrowth of capillaries during neovascularization, which it does in the growth plate [10,11] and in a variety of malignant tumors [39]. VEGF released from MVs could thus play an important role in stimulating angiogenesis during the replacement of hypertrophic cartilage of the growth plate by ingrowing metaphyseal bone.

Our study also revealed that isolated rat growth plate MVs contain a significant amount of the noncollagenous bone matrix proteins BSP, OPN, ON, and OC. Western blot analyses showed that MVs contain higher levels of BSP and OC with less intense immunostaining for OPN and ON. Of these four calcium-binding noncollagenous proteins, only BSP has been shown to strongly promote hydroxyapatite (HA) deposition [12]. Interestingly, OPN recently was found to facilitate angiogenesis and the accumulation of osteoclasts during bone resorption in vivo [40]. Osteonectin apparently lacks strong mineral-stimulating or mineral-inhibiting ability [41]. Osteocalcin, on the other hand, strongly inhibits HA deposition in vitro [41] and in vivo [19]. Only BSP appears to promote both bone formation

and repair [13–15]. Nevertheless, BSP plus OPN, ON, and OC probably interact in regulating MV-initiated cartilage mineralization.

Very recently, a partial proteomic analysis of extracellular matrix vesicles from osteoblast culture indicated the presence of ON [42]. It will be interesting to confirm, in future proteomic studies of isolated MVs, the presence of BMPs, VEGF, and noncollagenous matrix proteins BSP, OPN, and OC. Also, extensive further study is necessary to help determine whether these MV proteins do have stimulatory or inhibitory functions in endochondral ossification in vivo.

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References

1. Anderson HC, Garimella R, Tague SE (2005) The role of matrix vesicles in growth plate development and biomineralization. Front Biosci 10:822–837
2. Anderson HC, Reynolds JJ (1973) Pyrophosphate stimulation of calcium uptake into cultured embryonic bones. Fine structure of matrix vesicles and their role in calcification. Dev Biol 34:211–227
3 Slavkin HC, Croissant R, Bringas P Jr (1972) Epithelial-mesenchymal interactions during odontogenesis. 3. A simple method for the isolation of matrix vesicles. J Cell Biol 53: 841–849
4. Anderson HC (1969) Vesicles associated with calcification in the matrix of epiphyseal cartilage. J Cell Biol 41:59–72
5. Missana LR, Aguilera XM, Hsu HHT, Anderson HC (1998) Bone morphogenetic proteins (BMPs) and non-collagenous proteins of bone identified in calcifying matrix vesicles of growth plate. Bone (NY) 23(suppl):S241
6. Nakase T, Yoshikawa H (2006) Potential roles of bone morphogenetic proteins (BMPs) in skeletal repair and regeration. J Bone Miner Metab 24(6):425–433
7. Nakagawa T, Sugiyama T, Kamei T, Murata T, Tagawa T (2001) An immuno-light- and electron-microscopic study of the expression of bone morphogenetic protein-2 during the process of ectopic bone formation in the rat. Arch Oral Biol 46:403–411
8. Nahar NN, Missana LR, Garimella R, Tague S, Anderson HC (2005) Matrix vesicles are carriers of bone morphogenetic proteins (BMPs) and non-collagenous matrix proteins J Bone Miner Res 20(suppl 1):S321
9. Anderson HC, Hodges PT, Aguilera XM, Missana L, Moylan PE (2000) Bone morphogenetic protein (BMP) localization in developing human and rat growth plate, metaphysis, epiphysis, and articular cartilage. J Histochem Cytochem 48:1493–1502
10. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N (1999) VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med 5:623–628
11. Horner A, Bishop NJ, Bord S, Beeton C, Kelsall AW, Coleman N, Compston JE (1999) Immunolocalisation of vascular endothelial growth factor (VEGF) in human neonatal growth plate cartilage. J Anat 194(pt 4):519–524
12. Hunter GK, Goldberg HA (1993) Nucleation of hydroxyapatite by bone sialoprotein. Proc Natl Acad Sci U S A 90:8562–8565
13. Cooper LF, Yliheikkila PK, Felton DA, Whitson SW (1998) Spatiotemporal assessment of fetal bovine osteoblast culture differentiation indicates a role for BSP in promoting differentiation. J Bone Miner Res 13:620–632
14. Gordon JAR, Tye CE, Sapaio AV, Underhill TM, Hunter GK, Goldberg HA (2007) Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization in Vitro. Bone (NY) 41:462–473
15. Wang J, Zhou HY, Salih E, Xu L, Wunderlich L, Gu X, Hofstaetter JG, Torres M, Glimcher MJ (2006) Site-specific in vivo calcification and osteogenesis stimulated by bone sialoprotein. Calcif Tissue Int 79:179–189
16. Menanteau J, Neuman WF, Newman MW (1982) A study of bone proteins which can prevent hydroxyapatite formation. Metab Bone Dis Relat Res 1982;4(2):157–162
17. Boskey AL, Maresca M, Ullrich W, Doty SB, Butler WT, Prince CW (1993) Osteopontin-hydroxyapatite interactions in vitro: inhibition of hydroxyapatite formation and growth in a gelatin-gel. Bone Miner 22:147–159
18. Boskey AL, Spevak L, Paschalis E, Doty SB, McKee MD (2002) Osteopontin deficiency increases mineral content and mineral crystallinity in mouse bone. Calcif Tissue Int 71:145–154
19. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G (1996) Increased bone formation in osteocalcin-deficient mice. Nature (Lond) 382:448–452
20. Ali SY, Sajdera SW, Anderson HC (1970) Isolation and characterization of calcifying matrix vesicles from epiphyseal cartilage. Proc Natl Acad Sci U S A 67:1513–1520
21. Anderson HC, Stechschulte DJ Jr, Collins DE, Jacobs DH, Morris DC, Hsu HH, Redford PA, Zeiger S (1990) Matrix vesicle biogenesis in vitro by rachitic and normal rat chondrocytes. Am J Pathol 136:391–398
22. Garimella R, Sipe JB, Anderson HC (2004) A simple and non-radioactive technique to study the effect of monophosphoesters on matrix vesicle-mediated calcification. Biol Proc Online 6:263–267
23. Garimella R, Bi X, Camacho N, Sipe JB, Anderson HC (2004) Primary culture of rat growth plate chondrocytes: an in vitro model of growth plate histotype, matrix vesicle biogenesis and mineralization. Bone (NY) 34:961–970
24. Anderson HC, Reynolds PR, Hsu HH, Missana L, Masuhara K, Moylan PE, Roach HI (2002) Selective synthesis of bone morphogenetic proteins-1, -3, -4 and bone sialoprotein may be important for osteoinduction by Saos-2 cells. J Bone Miner Metab 20:73–82
25. Daluiski A, Engstrand T, Bahamonde ME, Gamer LW, Agius E, Stevenson SL, Cox K, Rosen V, Lyons KM (2001) Bone morphogenetic protein-3 is a negative regulator of bone density. Nat Genet 27:84–88
26. Chubinskaya S, Merrihew C, Cs-Szabo G, Mollenhauer J, McCartney J, Rueger DC, Kuettner KE (2000) Human articular chondrocytes express osteogenic protein-1. J Histochem Cytochem 48:239–250
27. Jones WK, Richmond EA, White K, Sasak H, Kusmik W, Smart J, Oppermann H, Rueger DC, Tucker RF (1994) Osteogenic protein-1 (OP-1) expression and processing in Chinese hamster ovary cells: isolation of a soluble complex containing the mature and pro-domains of OP-1. Growth Factors 11:215–225
28. Wordinger RJ, Agarwal R, Talati M, Fuller J, Lambert W, Clark AF (2002) Expression of bone morphogenetic proteins (BMP), BMP receptors, and BMP associated proteins in human trabecular meshwork and optic nerve head cells and tissues. Mol Vis 8:241–250
29. Janitz M, Heiser V, Bottcher U, Landt O, Lauster R (1998) Three alternatively spliced variants of the gene coding for the human bone morphogenetic protein-1. J Mol Med 76:141–146
30. Hauschka PV, Frenkel J, DeMuth R, Gundberg CM (1983) Presence of osteocalcin and related higher molecular weight 4-carboxyglutamic acid-containing proteins in developing bone. J Biol Chem 258:176–182
31. Kanim LEA, Bae H, Zhao L, et al (2003) Inter and intravariability of BMPs in commercially available demineralized bone matrices. In: Proceedings of the North American Spine Society 18th Annual Meeting. Spine J 32:81S

32. Blum B, Moseley J, Miller L, Richelsoph K, Haggard W (2004) Measurement of bone morphogenetic proteins and other growth factors in demineralized bone matrix. Orthopedics 27:s161–s165
33. Pietrzak WS, Woodell-May J, McDonald N (2006) Assay of bone morphogenetic protein-2, -4, and -7 in human demineralized bone matrix. J Craniofac Surg 17:84–90
34. Enomoto-Iwamoto M, Iwamoto M, Mukudai Y, Kawakami Y, Nohno T, Higuchi Y, Takemoto S, Ohuchi H, Noji S, Kurisu K (1998) Bone morphogenetic protein signaling is required for maintenance of differentiated phenotype, control of proliferation, and hypertrophy in chondrocytes. J Cell Biol 140:409–418
35. Grimsrud CD, Romano PR, D'Souza M, Puzas JE, Schwarz EM, Reynolds PR, Roiser RN, O'Keefe RJ (2001) BMP signaling stimulates chondrocyte maturation and the expression of Indian hedgehog. J Orthop Res 19:18–25
36. Leboy PS, Sullivan TA, Nooreyazdan M, Venezian RA (1997) Rapid chondrocyte maturation by serum-free culture with BMP-2 and ascorbic acid. J Cell Biochem 66:394–403
37. Kaneko H, Arakawa T, Mano H, Kaneda T, Ogasawara A, Nakagawa M, Toyama Y, Yabe Y, Kumegawa M, Hakeda Y (2000) Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. Bone (NY) 27:479–486
38. Garimella R, Tague SE, Zhang J, Belibi F, Nahan N, Sun BH, Insogna K, Wang J, Anderson HC (2008) Expression and synthesis of bone morphogenetic proteins by osteoclasts: a possible path to anabolic bone remodeling. J Histochem Cytochem 56:569–577
39. Dvorak HF (2002) Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol 20:4368–4380
40. Asou Y, Rittling SR, Yoshitake H, Tsuji K, Shinomiya K, Nifuji A, Denhardt DT, Noda M (2001) Osteopontin facilitates angiogenesis, accumulation of osteoclasts, and resorption in ectopic bone. Endocrinology 142:1325–1332
41. Hunter GK, Hauschka PV, Poole AR, Rosenberg LC, Goldberg HA (1996) Nucleation and inhibition of hydroxyapatite formation by mineralized tissue proteins. Biochem J 317(pt 1):59–64
42. Xiao Z, Camalier CE, Nagashima K, Chan KC, Lucas DA, de la Cruz MJ, Gignac M, Lockett S, Issaq HJ, Veenstra TD, Conrads TP, Beck GR Jr (2007) Analysis of the extracellular matrix vesicle proteome in mineralizing osteoblasts. J Cell Physiol 210:325–335