Expression of smooth muscle actin in osteoblasts in human bone

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Abstract

It is well known that certain connective tissue cells (viz., dermal fibroblasts) can express the gene for a muscle actin – α-smooth muscle actin – and can contract. This process contributes to skin wound closure and is responsible for Dupuytren's contracture. The objective of this study was to determine if human osteoblasts can also express the gene for α-smooth muscle actin. Immunohistochemistry using a monoclonal antibody for α-smooth muscle actin was performed on human cancellous bone samples obtained from 20 individuals at the time of total joint arthroplasty. The percentages of resting and active osteoblasts on the bone surfaces containing this muscle actin isoform were evaluated. Explants of human bone were also studied for the expression of α-smooth muscle actin in the tissue and in the outgrowing cells with time in culture. Western blot analysis was performed to quantify the α-smooth muscle actin content of the outgrowing cells relative to smooth muscle cell controls.

Nine ± 2% (mean ± SEM; n = 20) of the cells classified as inactive osteoblasts and 69 ± 3% (n = 19) of the cells identified as active osteoblasts on the bone surface contained α-smooth muscle actin. This difference was highly statistically significant (Student's t test, p < 0.0001). Similar profiles of α-smooth muscle actin-expressing cells were found in explants cultured for up to 12 weeks.

Cells forming a layer on the surface of the explants and growing out from them in monolayer also contained α-smooth muscle actin by immunohistochemistry and Western blot analysis.

Keywords: Osteoblasts; Bone; Smooth muscle actin; Contraction

Introduction

The contraction of fibroblasts in certain connective tissues (viz., dermis) plays a critical role in wound healing [20,21] and has been identified as the cause of pathological contractures (e.g., Dupuytren's contracture) [29,51]. This contractile behavior has been found to be enabled by the expression of a specific muscle actin, α-smooth muscle actin. Recently several musculoskeletal connective tissue cells including articular chondrocytes [30,55], meniscus [1,37] and intervertebral disc [25,48] fibroblasts and fibrochondrocytes, and anterior cruciate ligament fibroblasts [38] have been found to express this same contractile actin isoform in vivo. Moreover, these α-smooth muscle actin-containing cells have demonstrated the capacity to contract a collagen-glycosaminoglycan analog of extracellular matrix in vitro [33,35,37,48]. The roles of α-smooth muscle actin-expressing cells in the formation, remodeling, healing, and pathology of these musculoskeletal tissues have yet to be determined. It has been proposed that the contractile forces generated by α-smooth muscle actin-incorporated cytoplasmic apparatus may facilitate the cell manipulation of its extracellular matrix to impart tissue-specific architecture. These contractile forces may be disadvantageous when the cells are seeded or migrate into scaffolds employed for tissue engineering in that they may be responsible for distorting the matrix and collapsing the pores.

A recent preliminary report [36] has also described the presence of α-smooth muscle actin in osteoblasts lining trabeculae in human and canine bone samples. In related work most of the osteoblast-like cells of the MC3T3-E1 cell line were also found to contain...
α-smooth muscle actin by immunohistochemistry and Western blot analysis [36]. These α-smooth muscle actin-containing cells were found to be able to contract a collagen–glycosaminoglycan scaffold [36] in the same way as myofibroblasts. The roles of α-smooth muscle actin-enabled contraction of osteoblasts may include those outlined above. In addition, contraction may play a role in the “activation” stage of bone remodeling. It has been proposed [23] that retraction of cell processes of the normally contiguous osteoblasts on the surface of bone exposes the bone matrix making it accessible to osteoclasts and resorption. Prior work [23] has shown that the increased retraction and rounding of osteoblasts by the action of 1,25-dihydroxyvitamin D3 explained the influence of this hormone on bone remodeling. Those studies suggest a role for a contractile actin isoform in osteoblast behavior.

Other work that rationalizes the investigation of specific actin isoforms in osteoblasts is that describing the microfilament organization of the cells. Transmission electron microscope studies dating back 30 years revealed the microfilaments comprising the cytoskeleton of osteoblasts in vivo [4,27,59]. The microfilaments were found to occur in densely packed bundles running along the longitudinal axis of cell processes much like they do in smooth muscle cells. Moreover, it was proposed that the configuration and location resembled those of the contractile ring of dividing cells. Of interest was that the microfilaments in the osteoblasts were seen to encircle bundles of collagen fibers. This suggested to the authors [59] that, by contracting, the microfilaments provided a mechanism for cellular orientation of collagen fibers. Also of interest was the finding in other studies of differences in the distribution of microfilament bundles in resting and active osteoblasts [56]: microfilaments formed thick bundles around the cell membrane in formative osteoblasts but did not form bundles in resting osteoblasts. That the microfilaments may be contractile was supported by studies demonstrating that they bind to heavy meromyosin [31].

In order to more definitively determine the prevalence of α-smooth muscle actin expression in human osteoblasts, this study evaluated immunohistochemically the percentage of cells on the bone surface containing the isoform. The cells were stratified based on their morphology as “resting” or “active” osteoblasts. Osteoblasts engaged in the process of bone formation are referred to as active osteoblasts [26]. They appear as plump cells on the surface of the osteoid that they have synthesized or on the surface of mineralized bone matrix: about to begin, in the process of, or just having completed matrix synthesis. Osteoblasts that are not engaged in the process of bone formation are often referred to as bone-lining cells, inactive osteoblasts, or resting osteoblasts [26]. These cells appear elongated and flattened on the bone surface which is absent of osteoid.

For the purpose of the current work an aspect ratio of 3 was used to distinguish the more flattened from the more rounded cells on the bone surface.

Explants cultured for selected time periods were also evaluated to determine the change in the distribution of α-smooth muscle actin-containing cells on the trabecular surfaces under this environmental condition. Cells growing out from these cultures, while representing a heterogeneous population, were isolated for determination of the α-smooth muscle actin content using Western blot analysis. These outgrowing cells may display properties reflective of those that might be expected of cells in certain clinical situations such as microfracture [44].

Material and methods

Human cancellous (metaphyseal) bone was obtained from 20 patients undergoing total joint replacement for osteoarthrosis (Table 1), under a protocol approved by the Institutional Review Board of the Brigham and Women’s Hospital. The mean age was 63 years (44–82 years). Bone specimens that were macroscopically judged to be sclerotic or noticeably osteopenic were excluded from the study. The bone specimens were cut into pieces 20 mm³, fixed in formalin, decalcified in EDTA, and embedded in paraffin. Five to 7-μm thick sections were microtomed and stained with hematoxylin and eosin and Masson trichrome stain. Other sections were stained for α-smooth muscle actin using the immunohistochemical procedure described below.

Explant culture

Bone samples from six of the patients were harvested under sterile conditions within 2 h of surgery. After intensive rinsing — phosphate buffered saline (PBS) supplemented with 1% antibiotic solution — specimens 10–20 mm² were prepared with a sharp blade and placed into individual 35-mm diameter wells in 6-well tissue culture plates (Corning #430343, Cambridge, MA). The specimens were incubated in tissue culture medium comprising α-minimal essential medium

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For α-smooth muscle actin immunohistochemistry, a monoclonal anti-α-smooth muscle actin antibody (product no. A-2547, clone 1A4, monoclonal anti-α-smooth muscle actin, Sigma Chemical, St. Louis, MO) was used [54]. Deparaffinized, hydrated micrometrotome sections that were digested with 0.1% trypsin for 1 h at room temperature were treated with 3% hydrogen peroxide to quench endogenous peroxidase. Non-specific binding was blocked with 5% fetal bovine serum (FBS) (MEM, Sigma Chemical) and the sections subsequently treated with the monoclonal antibody. This antibody binds to the amino terminal decapeptide of α-smooth muscle actin [54] and has been employed in musculoskeletal tissue studies [37,38,48] and in numerous investigations for immunolocalization of α-smooth muscle actin in a wide variety of other cell types [2,15,34,45]. After incubation with the primary antibody the slides were incubated with biotinylated goat/horse anti-mouse IgG antibody. ExtrAvidin-conjugated peroxidase was used before processing the slides either using the AEC chromogen kit (Sigma Chemical) or the ABC kit (Vectorstain, CA). Mayer's hematoxylin was used as a counterstain.

For each staining run, positive (human aorta) and negative controls were included. The negative control section was stained with mouse serum, digested to the same protein concentration as that in the monoclonal antibody solution, instead of primary antibody. Whenever possible one of multiple sections on each microscope slide was used as the negative control. Cells were identified as α-smooth muscle actin positive based on the following criteria: the negative control displayed no distinct chromogen, the intensity of the chromogen in the cells of interest was comparable to that in the smooth muscle cells of the vessel walls, and the chromogen was restricted to the cytoplasm of the cells. Cells on the bone surface were categorized as "resting" or "active" osteoblasts based on their morphology: resting osteoblasts were flat cells lining the trabecular surface, with an aspect ratio—length divided by width—greater than 3; and active osteoblasts were plump cells with ample cytoplasm and an aspect ratio of 1–3. Active osteoblasts were not further stratified on the basis of whether they were on the surface of osteoid or mineralized bone. The areas of bone that were evaluated were 7–25 mm², including 50–200 cells that were identifiable. The percentage of α-smooth muscle actin-containing cells in the bone as well as the explant specimens was determined by dividing the number of α-smooth muscle actin-staining cells in a defined area by the total number of cells. In two of the 28 explants there was an insufficient number of inactive osteoblasts for the analysis (therefore, n = 26 for the analysis of the percentage of resting osteoblasts containing α-smooth muscle actin).

Western blot analysis for α-smooth muscle actin in cells growing out from the explants

The cells growing out from the explants were released from the flasks by incubation with 5 ml of 0.05% trypsin/ethylene-diamine-tetra- aetic acid in PBS. The trypsin solution was aspirated into 15 ml centrifuge tubes and spun at 400g for 10 min. Cell pellets were washed with PBS and centrifuged. The supernatant was removed, and the cell lysate buffer (0.1% sodium dodecyl sulfate, 1 mM sodium orthovanadate, 1 mM phenyl methylsulfonyl fluoride, and 1% glycerol) was added. The cell pellet and lysis buffer were incubated at 4 °C for 10 min and then centrifuged at 14,000 rpm for 20 min in 4 °C. The supernatant containing the total protein was collected in a microcentrifuge tube and frozen at −20 °C for future assay.

Bovine serum albumin (BSA) stock solution 1.5 µg/µl was diluted at 1:10 ratio to prepare standard curve of protein using the Bio-rad protein assay dye. Twenty microliters of supernatant were added from each sample into a plastic cuvette containing 1580 µl distilled deionized water and 400 µl of dye and mixed well. The mixture was incubated at room temperature for 10 min and the cuvette placed into an LKB Biochrom Ultraspec 4050 spectrophotometer operating at 595 nm. The optical density reading was interpolated from the standard curve to obtain the concentration of protein of each sample.

Cell extracts normalized for total protein content were resolved using 8% sodium-polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of α-smooth muscle actin is 42 kDa, and accordingly a 10% polyacrylamide gel was used. Samples containing 5 µg of total protein were diluted with sample buffer (1 ml 0.5 M Tris at pH 7, 0.8 ml glycerol, 1.6 ml 10% SDS, 0.4 ml 2-mercaptoethanol, 0.2 ml 1% bromophenol blue dye) and loaded in the slots of a mini-gel apparatus. As positive control, a sample of 5 µg of a protein extract from smooth muscle cells (human aorta cell) was employed. The gel was run at 110 V for 90 min and then transferred to a PVDF membrane at 100 V for 60 min. After the transfer the membranes were washed with TBS for 5 min and placed in blocking agent (5% dry skim milk, Bio-rad) overnight. After blocking the membranes were incubated with the primary antibody (mouse anti-α-smooth muscle actin, Sigma, 1:400 in TBS-T) for 90 min and subsequently processed using a peroxidase-labeled secondary antibody and a luminol-based chemiluminescent detection system.

Films were digitized for densitometric analysis using NIH image processing and analysis software. Results were reported as percentage of the densitometric readings of the positive smooth muscle cell control.

Contraction assay

The contractile potential of the outgrowing cell population was evaluated as described previously [32]. Briefly, cells were expanded in monolayer through passage 2. Two million cells were loaded onto sponge-like type I collagen—glycosaminoglycan matrices (n = 12), 9 mm in diameter and 3 mm thick. The cell-seeded matrices were cultured in agarose-coated 12-well plates.

Matrices used in this contraction assay were prepared by blending type I bovine tendon collagen (Integra LifeScience, Plainsboro, NJ) with chondroitin 6-sulfate (Chondroitin sulfate, Sigma) following a previously published protocol [61]. After mixing, the coprecipitate was degassed and lyophilized. Subsequently the matrices, in the form of 3-mm-thick sheets, were dehydrothermally cross-linked for 24 h. The resulting highly porous matrices had interconnected pores, and a mean pore diameter of 85 µm as previously determined by quantitative light microscopy analysis of microtomed sections [39].

Matrix diameters were measured on days 0, 1, 4, 6 and 7. Non-seeded matrices served as controls. On day 7, the matrices were allocated for RNA analysis (see below). The value of the matrix diameter for each day that it was measured was subtracted from that of day 0 value for the respective matrix, and then divided by the initial size (on day 0) in order to yield the percentage contraction. The average diameter for the non-seeded matrices for each day was subtracted from each of the cell-seeded matrix measurements to provide a measure of the "cell-mediated contraction." In order to correct for cell number, the cell-mediated contraction on day 7 was divided by the average value of the DNA content, reflecting the number of cells responsible for the contraction.

For the DNA analysis, the matrices were lyophilized overnight. One milliliter of papain buffer (6 µg papain in 0.1 M sodium phosphate, 5 mM Na₃EDTA, and 10 mM cysteine—HCl) was added to each matrix and then the samples were placed in a 65 °C water bath overnight. The Hoochst dye method was utilized in determining the DNA content of each sponge. A standard curve of DNA content per fluorometric reading (TC, in nM, collagen—glycosaminoglycan matrix) was generated using cell lysate DNA. Then 100 µl of each papain digest sample were added to 2 ml of DNA dye solution (Hoechst 33258 dye solution in TEB buffer consisting of 10 mM Tris, 1 mM Na₃EDTA and 1 M NaCl, pH 7.4) for fluorometric reading. The DNA content of each sample was extrapolated from the standard curve. The DNA content for the non-seeded sponge was subtracted from that of...
the cell-seeded sample in order to correct for the background fluorescence of the matrix.

Results

Analyses of bone specimens

Histologically, the bone specimens were found to be free of abnormalities. The bone marrow displayed a typical appearance, without fibrotic or other changes. The bony trabeculae, while varying in thickness displayed normal characteristics of osteoid when present – i.e., there were no signs of osteomalacia. There were no signs of abnormal remodeling as may have been signaled by excessive numbers of osteoclasts. Active and inactive osteoblasts could generally be clearly distinguished based on the classical morphological features that they present in light microscopy [26]. Histological analysis revealed that about 9% (range 0–30%) of the cells on the trabecular surface were active osteoblasts.

Positive α-smooth muscle actin staining of osteoblasts was a constant finding in all of the specimens (Fig. 1(a)–(c)). Nine ±2% (mean ± standard error of the mean (SEM); n = 20) of the cells classified as resting osteoblasts contained α-smooth muscle actin. In contrast 69 ± 3% (n = 19) of the cells identified as active were α-smooth muscle actin-positive (Figs. 1(a)–(c) and 2). This difference was highly statistically significant (Student’s t test, p < 0.0001). α-Smooth muscle actin-containing active, i.e., rounded, cells could be found on the surface of osteoid and also on mature bone surfaces (Figs. 1(b) and (c)). Occasionally osteocytes were found to label for α-smooth muscle actin. Quantitative analysis was not performed because of the uncertain preservation of osteocytes in the histological sections. There was no no-

Fig. 1. Immunohistochemical micrographs of sections of human trabecular bone stained with the antibody for α-smooth muscle actin. (a) Vascular cells lining a vessel (open arrow) staining positive for α-smooth muscle actin. Positive staining can also be seen in cells on the bone surface (arrowhead). Inset: negative control section. (b) Active osteoblasts on a mineralized bone surface containing α-smooth muscle actin (open arrows). Flattened, inactive osteoblasts (arrowheads) on a nearby bone surface did not demonstrate expression of α-smooth muscle actin. Inset: negative control. (c) α-Smooth muscle actin expression in cells on a mineralized bone surface. The osteocytes did not stain with the anti-α-smooth muscle actin antibody. (d) Osteoclasts without signs of the chromogen for α-smooth muscle actin.

Fig. 2. Graph showing the percentages of active and resting osteoblasts containing α-smooth muscle actin versus patient age.
table α-smooth muscle actin labeling of osteoclasts (Fig. 1(d)). While an analysis was not performed to stratify osteoblasts on the basis of whether they were on the surface of osteoid or mineralized bone, qualitatively, there was no noticeable difference in the actin expression of active osteoblasts lining what appeared to be osteoid or previously mineralized matrix.

Examination of the bone specimens revealed that, as expected, vascular smooth muscle cells also stained positive for α-smooth muscle actin (Fig. 1(a)). Marrow stromal cells were not readily identifiable so their staining for α-smooth muscle actin could not be determined definitively.

Primary cell-outgrowth could be observed after 7 ± 1 (mean ± SEM) days. Confluence was reached after 23 ± 2 days for the initial culture of the explant. After transferring the explant to another culture dish, the time to confluence decreased significantly (15 ± 2 days; Fig. 6). ANOVA revealed a significant effect of subculture on the time to confluence. Bonferroni-Dunn post hoc testing showed no significant difference among the three subcultures, Tx1-Tx3.

Immunohistochemistry revealed an intense staining for α-smooth muscle actin prominently displayed in stress fibers of the outgrowing cells in monolayer (Fig. 7(a)). Approximately 82% (range 63–100%) of the cells were positive for α-smooth muscle actin. Alkaline phosphatase staining of cell cultures showed a high percentage of positive cells confirming the osteoblast phenotype (Fig. 7(b)). Treatment with von Kossa stain revealed the typical appearance of widespread black-labeled punctate, needle-like, and plate-like features seen individually and in aggregates (Fig. 7(c)).

All protein extracts of outgrowing cells of different passages showed the presence of α-smooth muscle actin using Western blot analysis (Fig. 8). Interestingly, it appeared that the α-smooth muscle actin content of the cells was higher when the explants were cultured with β-glycerophosphate. However, it was not possible to definitively draw this conclusion or to establish the effect of time in culture or number of passages on α-smooth muscle actin content.

Cells grown from the explants were able to contract a type I collagen–glycosaminoglycan matrix. Matrix diameters decreased to 54 ± 1% (mean ± SEM) of their original diameter after 7 days. Accounting for the change in diameter of the non-seeded matrix, due to the
slight plasticizing effect of the aqueous medium, the cell-mediated contraction was calculated to be 40 ± 1%. Normalization to cell number (amount of DNA) revealed a cell-mediated contraction of 20 ± 2%/μg DNA.

Discussion

Our results demonstrate the expression of a contractile actin isoform, α-smooth muscle actin, in human bone. All 20 of the bone specimens analyzed contained cells on the surface of trabeculae, i.e., osteoblasts, that contained α-smooth muscle actin. A notable finding was that the percentage of active osteoblasts containing α-smooth muscle actin was sevenfold higher than the percentage of resting cells expressing α-smooth muscle actin, a highly statistically significant finding. In some areas of selected samples virtually all of the active osteoblasts contained α-smooth muscle actin. In contrast, in other regions of the samples from the same individual less than 10% of the inactive bone lining cells were α-smooth muscle actin-positive.
The difference in the percentage of flattened and plump cells that contained SMA was so great that even if the aspect ratio used to distinguish the cells was changed a difference in the percentage would have been detectable. In this regard, the current findings raise questions about the contribution of this particular actin isoform to maintaining the more rounded shape of the active osteoblast that heretofore has been principally...
Fig. 8. Western blot analysis of α-smooth muscle actin in cells growing out from explants of human bone in culture after passages 2 and 5, in medium with and without β-glycerophosphate. SMC = smooth muscle cell control.

associated with the increased cytoplasmic content of endoplasmic reticulum required for protein synthesis. Such questions relating the cytoskeletal make-up of the cell and its biosynthetic activity will require additional study for resolution.

A limitation of the simple morphological classification of cells on the bone surface as active or resting is that it was not possible to distinguish active cells just prior to or after matrix synthesis or in the middle of the process of matrix production. Histomorphometry of undecalcified material, which might have been useful in quantifying the amount of osteoid underlying the cells to better distinguish their stage of activity, would have required plastic embedding techniques that would not have readily accommodated the immunohistochemical method of detecting the presence of α-smooth muscle actin. This approach should be considered for future work to investigate the role of expression of this actin isoform in the activation of remodeling and in the process by which the active osteoblasts manipulate the newly synthesized matrix to create a specific architecture.

The trabecular bone used in this study was from the metaphyseal regions of patients undergoing total hip and knee arthroplasty for osteoarthritis. These specimens were employed because of their availability and because the samples could be obtained as blocks of cancellous bone that could be carefully dissected in the laboratory for allocation for histology or as explants for culture. Histologically there were no signs of abnormalities other than areas of low bone density as might be expected due to the reduced joint loading associated with the painful condition. Generally these specimens displayed low bone turnover as reflected in the low percentage of the surface covered by active osteoblasts and by the small number of osteoclasts. Although the specimens were obtained from patients undergoing arthroplasty, no major histological anomalies could be found. However, some pathology cannot be ruled out. This limits generalization of the findings to normal bone. However, that comparable observations were made in a prior study of normal canine bone samples [36] suggests that the findings of the current investigation likely apply to normal human bone.

Growth of explants in culture stimulated an osteogenic process and likely degenerative changes, facilitating the investigation of the expression of α-smooth muscle actin in osteoblasts and related cells (viz., bone marrow stromal and vascular cell-derived) in different stages of differentiation and activity. The elevated state of osteogenesis was demonstrated by the increase in the percentage of cells that displayed the features of active osteoblasts. An advantage of the immunohistochemical evaluation of the explants in culture is that it afforded the opportunity of identifying cells expressing α-smooth muscle actin relative to their location in bone. This was especially useful considering the diversity of the cell populations. Reflecting the findings from analysis of the bone specimens (above), the percentage of active osteoblasts that were α-smooth muscle actin-positive was much higher than the percentage of inactive bone lining cells that contained α-smooth muscle actin. Of interest was the fact that the percentage of inactive cells that was α-smooth muscle actin-positive was fourfold higher than that found in vivo. This may be related to the general elevation of osteogenesis stimulated by the culture conditions. While the addition of β-glycerophosphate resulted in a dramatic increase in the percentage of active cells, there was no significant effect of this agent on the percentage of the active or inactive cells that contained α-smooth muscle actin in the explants. Preosteoblast-like cells away from the bone surface and fibroblast-like cells that formed a multiple cell layer enveloping the explant also stained for α-smooth muscle actin in relatively high percentages. Other work on articular cartilage explants [42] and on tendon cell-seeded collagen matrices [50] have demonstrated the presence of a similar enveloping layer of α-smooth muscle actin-containing fibroblast-like cells. The later study proposed that this cell layer was responsible for the contraction of the collagen matrices. Future work needs to consider if a similar layer of α-smooth muscle actin-expressing cells forms on fracture surfaces in vivo.

Also of interest was the α-smooth muscle actin expression of most of the cells growing out from the explants in monolayer. These cells may have been derived in part from the cell layer surrounding the explants and this may explain the shorter time to confluence after subculture of the explant. While these outgrowing cells stained positive for alkaline phosphatase and facilitated mineralization they likely included cells derived from the bone marrow stromal cells and vascular cells as well as preosteoblasts and osteoblasts. However, virtually all of the cells derived from the explants expressed α-smooth muscle actin as evidenced by immunohistochemistry. The expression of this actin isoform was confirmed by Western blot analysis. The actual amount of α-smooth muscle actin in each cell could not, however, be determined using these methods. These findings raise the question of whether cells migrating from bone into a lesion (e.g., at a microfracture-treated site) or fracture gap or into the pores of a porous coated implant in vivo will also be found to contain α-smooth muscle actin.
Of particular importance was the demonstration that human bone explant-derived cells displaying phenotypic traits of osteoblasts and expressing α-smooth muscle actin could contract a collagen–glycosaminoglycan analog of extracellular matrix. Prior preliminary findings demonstrated that α-smooth muscle actin-containing osteoblast-like cells of the MC3T3-E1 cell line were also capable of contracting a similar collagen–glycosaminoglycan scaffold [36]. The contractility of the cells growing out from the bone explants in this study (20% µg DNA after 7 days of culturing the cell-seeded matrix) can be placed in the context of the contractility of other cell types in type I collagen–glycosaminoglycan matrices of comparable composition, pore diameter, and size. Recent studies of the contractility of adult human articular chondrocytes [32] and lapine bone marrow stromal cells, expanded in monolayer culture, reported cell-mediated contractures of 15%µg DNA (after 21 days) and 50%µg DNA (after 14 days), respectively. Differences in the contractility of cells may be due to variations in the α-smooth muscle actin content of the cells, their adhesion to the matrix, and the changes in the modulus of the matrix effected by the cells.

The importance of α-smooth muscle actin-enabled contractile force generation in the process of bone formation will require additional study. One supposition is that it may facilitate the cell's organization of the newly synthesized extracellular matrix to impart a specific architecture. Moreover, results of a recent study suggested a correlation between α-smooth muscle actin expression in fibroblasts and their orientation to a solid surface [17]. The role of α-smooth muscle actin expression in osteoblasts warrants additional study.

There have been several investigations that have suggested contractile behavior in osteoblast-like cells. An early observation that explants of skin in culture were found to be attracted to each other [46] prompted later work investigating the behavior of bone explants in vitro [28]. James and Taylor described "fibroblasts" growing out from the bone explants. When these cells reached confluence, connecting a pair of explants in the same culture dish, one explant was drawn to the other. The computed stress generated by these cells was estimated to be 3.4 ± 0.23 × 10^4 dynes/cm^2 and the stress exerted per cell 1.65 × 10^-2 dynes/cm^2. The contraction of collagen gels by rat embryonic calvaria and osteosarcoma cells was later reported [5]. The calvarial bone cells that were employed were shown in a previous study to be responsive to parathyroid hormone and PGE_1 [43]. While there was no measurable contraction by the osteosarcoma cells, the calvarial cells greatly contracted the gel, albeit less than periodontal ligament cells to which they were Compared. The authors noted that the bone cells “efficiently aligned collagen fibers and established a reticular-like cell network chiefly through the elongation of cell processes along collagen fibers during the pre-contractile phase” [5]. More recent studies demonstrated the contraction of similar collagen gels by an osteoblast cell line, ROS 17/2.8 cells [8]. Moreover, it was found that gap junctional communication contributed to the lattice contraction by the osteoblasts.

The finding of α-smooth muscle actin in osteoblasts raises the question of whether this expression should be considered a phenotypic characteristic of this cell type, conferred by its precursor cell. The osteoblast is considered by many to be derived from the local mesenchymal stem cell (the bone marrow stromal stem cell) [3,11,22,24]. In vitro studies of mouse [40] and human [14,15] marrow stroma-derived cells have demonstrated that they expressed the gene for α-smooth muscle actin. Also of interest was the fact that stromal cells behaved like smooth muscle cells in other ways [15]: they ingest and store into their lysosomes large amounts of protein from the medium [16] and synthesize a similar profile of proteoglycans [60]. In vivo, stromal cells expressing the gene for α-smooth muscle actin have been found during fetal and early stages of development of bone marrow and in certain pathological conditions in the adult marrow, but only rarely in normal adult tissue [47]. Additionally, some stromal cells in certain pathologic conditions affecting bone marrow have been found to have ultrastructural features consistent with those found in smooth muscle cells [6]. A recent study [10] has revealed α-smooth muscle actin-enabled contraction of a collagen–glycosaminoglycan analog of extracellular matrix by lapine and canine marrow stromal cells. Freshly isolated lapine marrow stromal cells had approximately 30% of the amount of α-smooth muscle actin found in smooth muscle cells. The α-smooth muscle actin was incorporated into stress fibers that were prominently displayed by the cells.

Other α-smooth muscle actin-expressing cells have also been implicated as precursors of osteoblasts: pericytes and vascular smooth muscle cells. For many years the pericyte has been considered an osteoblast progenitor cell [9,18,53]. Brighton et al. [9] showed that α-smooth muscle actin-containing pericytes exhibit phenotypic expressions in vitro that are similar to those of bone cells, including the synthesis of alkaline phosphatase and osteocalcin and the formation of mineralizing colonies. This work was supported by later studies that showed that pericytes from the microvasculature formed a mineralized matrix in vitro even in the absence of β-glycerophosphate [49]. In more recent investigations [19], cultured bovine pericytes from retinal capillaries, inoculated into diffusion chambers implanted into athymic mice, induced the formation of cartilage and bone. This study provided the first direct evidence of the osteogenic potential of microvascular pericytes in vivo. There have been other observations made in animal studies that have suggested that pericytes are a source of osteoblasts [12,13].
Interest in associating smooth muscle cells with calcification and ossification processes stems from the importance of arterial calcification in vascular diseases. Findings of mRNA for bone-related proteins (e.g., BMP-2a) in calcified plaques [7] in arteries has fueled this interest. Studies have demonstrated that a subpopulation of cells from the bovine aortic media expressed high levels of alkaline phosphatase and type I collagen, and also expressed osteopontin and osteonectin and bone-specific osteocalcin [57]. Moreover, the matrix synthesized by these cells calcified [57]. While the α-smooth muscle actin expression of this subpopulation of vascular cells was not investigated, later work showed that α-smooth muscle actin-expressing vascular smooth muscle cells expressed matrix Gla protein and formed calcifying nodules in vitro [41]. These studies have led to the conclusion that vascular smooth muscle cells can differentiate to form osteoblast-like cells [41,52,58].

Collectively these investigations make a case for considering α-smooth muscle actin expression a phenotypic characteristic of osteoblasts that is up-regulated in active cells. This expression may be related to the retraction of osteoblastic cell processes that is a requirement for the initiation of the osteoclastic resorption process. Moreover, α-smooth muscle actin-enabled contractile forces may play a role in the cell-mediated organization of the extracellular matrix to impart the tissue-specific architecture.

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References


