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1,25-Dihydroxy Vitamin D_3 Is an Autocrine Regulator of Extracellular Matrix Turnover and Growth Factor Release via ERp60-Activated Matrix Vesicle Matrix Metalloproteinases

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Key Words

$1\alpha,25(OH)_2D_3$ · Matrix vesicles · Extracellular matrix · TGF-β1, latent activation · Matrix metalloproteinases · MMP-3

Abstract

As growth plate chondrocytes mature and hypertrophy, they reorganize their proteoglycan-rich type II collagen extracellular matrix (ECM), involving $1,25(OH)_2D_3$-dependent regulation of matrix metalloproteinases (MMPs). Stromelysin-1 (MMP-3) and 72-kD gelatinase (MMP-2) are found in extracellular matrix vesicles (MVs) and release and activate ECM-bound latent TGF-β1 and TGF-β2, respectively. $1,25(OH)_2D_3$ regulates incorporation of MMP-2 and MMP-3 into MVs and release of these enzymes in the ECM. Plasma membranes (PMs) and MVs contain the $1\alpha,25(OH)_2D_3$ membrane receptor ERp60 (protein disulfide isomerase A3), phospholipase A_2 (PLA_2), PLA_2-activating protein, the nuclear vitamin D receptor and caveolin-1. $1,25(OH)_2D_3$ secreted by chondrocytes binds MV ERp60, activating PLA_2. Resulting lysophospholipids destabilize MV membranes, releasing active MMPs. We examined $1,25(OH)_2D_3$-dependent activation of latent TGF-β1 stored in cartilage ECM. Interestingly, TGF-β1 regulates $1,25(OH)_2D_3$ production. $1\alpha,25(OH)_2D_3$ activates PM protein kinase C (PKC)-α via ERp60-dependent PLA_2-signaling, lysophospholipid production and phospholipase C-γ. It also regulates distribution of phospholipids and PKC isoforms between MVs and PMs, enriching MVs in PKC-ζ. Direct activation of MV MMP-3 requires ERp60 based on blocking antibodies and PKC based on inhibitor studies. However, treatment of MVs with $1,25(OH)_2D_3$ decreases MV PKC-ζ activity, suggesting more complex feedback mechanisms, potentially involving MV lipid signaling. Our observations indicate that one role of MVs is to provide MMPs at sites distant from the cells. Chondrocytes secrete $1,25(OH)_2D_3$, which acts directly on MV-membranes via ERp60, releasing MMPs. MMP-specific ECM components are hydrolyzed, resulting in release and activation of growth factors that can act back on the cells.

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Abbreviations used in this paper	
$1\alpha,25(OH)_2D_3$	1,25-dihydroxy vitamin D_3
ECM	extracellular matrix
MMP	matrix metalloproteinase
MV	matrix vesicles
PDIA3	protein disulfide isomerase, family A, type 3
PKC	protein kinase C
PLA_2	phospholipase A_2
PM	plasma membrane
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinase

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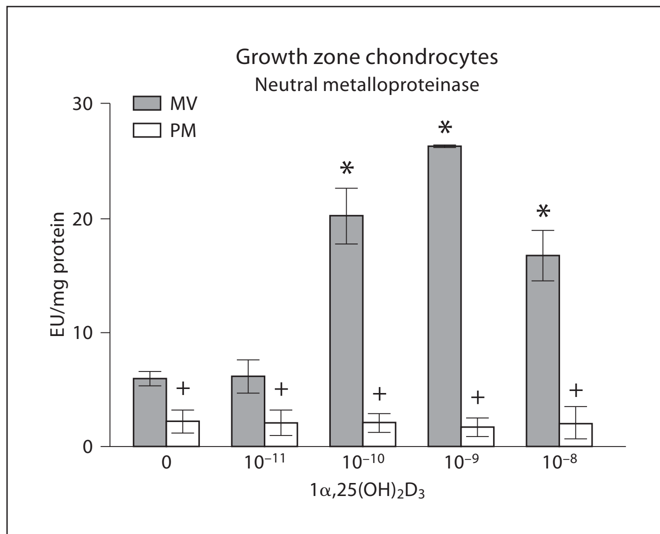

Fig. 1. Effect of $1\alpha,25(OH)_2D_3$ on neutral metalloproteinase activity in growth plate chondrocyte cultures. Confluent cultures of rat costochondral growth zone cartilage cells were treated with 10^{-11} to 10^{-8} M $1\alpha,25(OH)_2D_3$ for 24 h. MVs and PMs were isolated and assayed for neutral MMP activity using aggrecan containing polyacrylamide gel beads. Data are means ± SEM for 6 independent cultures and are from 1 of 2 separate experiments, both with comparable results. * $p < 0.05$, treatment versus control; $^{+}$ $p < 0.05$, MV versus PM at each concentration of $1\alpha,25(OH)_2D_3$.

Introduction

Growth plate chondrocytes produce a proteoglycan-rich type II collagen extracellular matrix (ECM). As the cells in the growth plate mature and hypertrophy, there is a massive reorganization of the ECM to accommodate the changes in cell shape and to prepare the matrix for calcification and eventual vascular ingrowth. Concurrently, there is a shift in matrix-processing enzymes from neutral to acidic metalloproteinases [Dean et al., 2001]. In addition, enzyme activities that modulate the size of proteoglycan aggregate, including chondroitinases and ADAMTS, are increased [Cawston and Wilson, 2006].

In vivo studies have shown that activity of matrix processing enzymes in the growth plate is regulated by $1\alpha,25(OH)_2D_3$ [Dean et al., 2001; Lin et al., 2002]. This phenomenon has been confirmed in studies using growth plate chondrocyte cultures [Schmitz et al., 1996b; Maeda et al., 2000]. There are a number of mechanisms by which this occurs. Many of the enzymes are produced as zymogens, and $1\alpha,25(OH)_2D_3$ increases zymogen activation [Schmitz et al., 1996b]. $1\alpha,25(OH)_2D_3$ has been shown to regulate the levels of mineral ions that are required for metalloproteinase activity [Brown et al., 1993]. In addition, $1\alpha,25(OH)_2D_3$ modulates transcription of their mRNA [Schmitz et al., 1996a, b]. Because regulation of matrix metalloproteinase (MMP) activity is critical, chondrocytes produce inhibitors like tissue inhibitor of metalloproteinases (TIMP-1 and TIMP-2) [Dean et al., 1992] and $1\alpha,25(OH)_2D_3$ modulates levels of these inhibitors as well [Dean et al., 2001].

We have shown that growth plate chondrocytes also take advantage of extracellular organelles called matrix vesicles (MVs) to package MMPs and store them in the ECM separate from the matrix proteins [Schmitz et al., 1994; Dean et al., 1996a, b; D'Angelo et al., 2001]. MVs produced by cells in the prehypertrophic and hypertrophic cell zones contain higher levels of acidic metalloproteinases and this is further increased in animals treated with $1\alpha,25(OH)_2D_3$ [Dean et al., 1996a, b]. In addition, $1\alpha,25(OH)_2D_3$ increases activity of neutral MMPs in MVs produced by these cells (fig. 1). Cell culture studies have shown that incorporation of stromelysin-1 (MMP-3) and 72-kD gelatinase (MMP-2) into MVs is regulated by $1\alpha,25(OH)_2D_3$ [Schmitz et al., 1996b]. MMP-2 and MMP-3 are stored in MVs produced by growth zone chondrocytes together with TIMP-1 and TIMP-2, and are released from the MVs in an active form. $1\alpha,25(OH)_2D_3$ reduces MV TIMP levels [Dean et al., 2001], supporting the hypothesis that the MMPs are activated prior to their release in the matrix.

Autocrine Regulation of MV MMPs by $1\alpha,25(OH)_2D_3$

Traditionally, $1\alpha,25(OH)_2D_3$ acts via the vitamin D receptor to regulate gene transcription, so genetic modulation of MMP content during MV formation is not an unexpected finding. However, we have also shown that $1\alpha,25(OH)_2D_3$ can act directly on MVs, resulting in release of active MMPs [Dean et al., 1996b]. This indicates that $1\alpha,25(OH)_2D_3$ may act in an autocrine manner.

Like the plasma membrane (PM), MVs contain the $1\alpha,25(OH)_2D_3$ membrane receptor ERp60 [also called protein disulfide isomerase, family A, type 3 (PDIA3), ER60, ERp57, GRP57 and 1,25-MARRS], as well as phospholipase A_2 (PLA_2), PLA_2-activating protein and caveolin-1 (fig. 2), but they do not possess DNA or RNA and therefore do not produce new protein. In chondrocytes, $1\alpha,25(OH)_2D_3$ acts via ERp60 by activating protein ki-

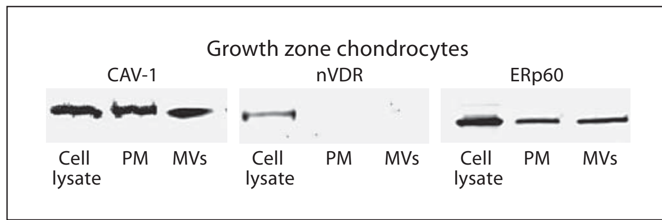

Fig. 2. Presence of caveolin-1 (CAV-1), ERp60 and nuclear vitamin D receptor (nVDR) in rat costochondral growth zone chondrocyte lysates and isolated PMs and MVs. MVs were isolated from trypsin digests of confluent cultures of growth zone chondrocytes. The cell pellet was lysed and PMs were isolated from the lysates by differential centrifugation. Each fraction (PMs, MVs and cell lysates) was separated on SDS-PAGE and then Western blots were probed with antibodies to caveolin-1, vitamin D receptor and ERp60.

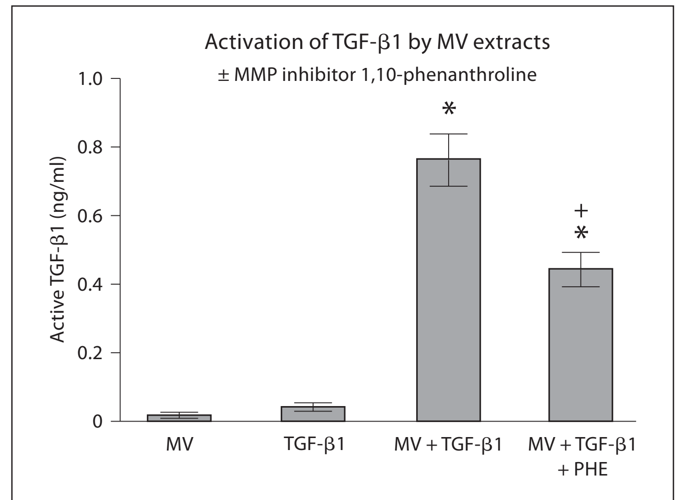

Fig. 3. Activation of latent TGF-β1 by MV extracts. MVs were isolated from confluent cultures of rat costochondral growth zone cartilage cells and assayed for TGF-β1 content by immunoassay (MV). MV extracts were then incubated in DMEM containing 10% fetal bovine serum without exogenous latent TGF-β1 and basal level of TGF-β1 in the reaction mixture was determined (TGF-β1). MV extracts were then incubated with latent TGF-β1 and active TGF-β1 was measured (MV+TGF-β1). Finally, MV extracts were treated with the MMP inhibitor 1,10-phenanthroline (PHE) and then incubated with latent TGF-β1 (MV+TGF-β1+PHE). Data are means ± SEM for 6 separate MV preparations. * $p < 0.05$, MV+TGF-β1 versus MV; + $p < 0.05$, MV+TGF-β1 versus MV+TGF-β1+PHE.

nase C (PKC)-α-dependent signaling through a mechanism that is mediated by PLA_2 and requires the presence of PLA_2-activating protein [Boyan et al., 2006]. Inhibition of PKC activity with chelerythrine blocks the effects of $1\alpha,25(OH)_2D_3$ on MMP-3 activity in isolated MVs [Maeda et al., 2000], suggesting that a similar mechanism to that which occurs in chondrocytes may be involved, but it is unlikely that this is the case. During MV production, $1\alpha,25(OH)_2D_3$ regulates the differential distribution of PKC isoforms between the PM and the MV membrane, resulting in higher levels of PKC-α in the PM and higher levels of PKC-ζ in the MVs [Sylvia et al., 1996]. However, when isolated PMs or MVs are treated directly with $1\alpha,25(OH)_2D_3$, PKC-α activity is increased in the PM but PKC-ζ activity is decreased in the MVs. This indicates that the direct action of the vitamin D metabolite is differentially distributed, allowing the responses to be specific to each membrane compartment.

In contrast to its effects on PKC isoforms, $1\alpha,25(OH)_2D_3$ stimulates PLA_2 activity in both membrane compartments [Schwartz et al., 2005], suggesting an alternate hypothesis. In this hypothesis, activation of PLA_2 in the PM is rapidly downregulated, but in the MVs this does not occur and the resulting production of lysophospholipids causes a loss of membrane integrity and eventual release of MMPs into the ECM. That this is receptor mediated is evidenced by the fact that antibodies to ERp60 prevent the release of MV MMPs [Maeda et al., 2000]. Moreover, growth plate chondrocytes produce and secrete $1\alpha,25(OH)_2D_3$ in a regulated manner at levels as high as 10^{-8} M, supporting the hypothesis that it acts in an autocrine manner.

Regulation of TGF-β Activation

Chondrocytes store TGF-β1 in the growth plate ECM as large latent TGF-β1 complexes, which consist of latent TGF-β1-binding protein, latency-associated peptide and latent TGF-β1 [Pedrozo et al., 1998]. Initial studies showed that $1\alpha,25(OH)_2D_3$ metabolites regulate expression of latent TGF-β1-binding protein and latent TGF-β1 and incorporation of latent TGF-β1 in the ECM of chondrocytes [Pedrozo et al., 1999b]. TGF-β1 regulates production of $1,25(OH)_2D_3$ by growth plate chondrocytes [Pedrozo et al., 1999a], suggesting the possibility of a feedback loop modulating TGF-β1 levels in the tissue.

The finding that $1\alpha,25(OH)_2D_3$ increased MMP activity suggested that $1\alpha,25(OH)_2D_3$ might also regulate availability of TGF-β1 by controlling activation of the latent growth factor. Initial experiments on inhibition of TGF-β1 activation by the MMP inhibitor 1,10-phenanthroline showed that MV extracts could activate latent TGF-β1 and that MMPs were responsible for this (fig. 3).

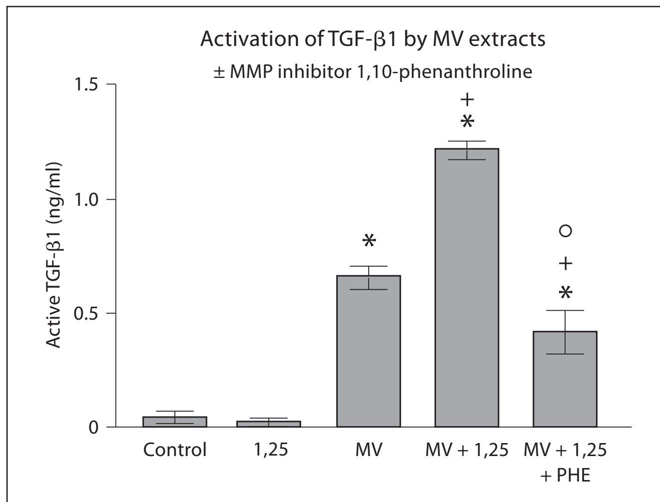

Fig. 4. Effect of $1\alpha,25(OH)_2D_3$ on activation of latent TGF-β1 by MVs. MVs were isolated from confluent cultures of rat costochondral growth zone cartilage cells and assayed for TGF-β1 content by immunoassay (control). MV extracts were then incubated in DMEM containing 10% fetal bovine serum and 10^{-8} M $1\alpha,25(OH)_2D_3$ without exogenous latent TGF-β1 and basal level of TGF-β1 in the reaction mixture was determined (1,25). MV extracts were then incubated with latent TGF-β1 and active TGF-β1 was measured (MV). MVs were treated with $1\alpha,25(OH)_2D_3$ and extracts of these MVs were incubated with latent TGF-β1 (MV+1,25). Finally, extracts from MVs treated with 10^{-8} M $1\alpha,25(OH)_2D_3$ were incubated with latent TGF-β1 in the presence of the MMP inhibitor 1,10 phenanthroline (MV+1,25+PHE). Data are means ± SEM for 6 separate MV preparations. * $p < 0.05$, MV versus control; $^{+}$ $p < 0.05$, MV+1,25 versus MV; ° $p < 0.05$, MV+1,25 versus MV+1,25+PHE.

A second set of experiments demonstrated that extracts of MVs that had been incubated directly with $1\alpha,25(OH)_2D_3$ were more effective than extracts of untreated MVs (fig. 4). Moreover, the effect of $1\alpha,25(OH)_2D_3$ was via activation of MMPs, based on the reduction in TGF-β1 activation by 1,10-phenanthroline.

$1\alpha,25(OH)_2D_3$-treated MVs activated latent TGF-β1 and to a lesser extent latent TGF-β2 [Boyan et al., 1994], suggesting that a similar mechanism was involved for both isoforms of this growth factor. Studies in other laboratories had shown that latent TGF-β could be activated by plasmin [Rosenthal et al., 2000] and we were able to show that plasmin could release active TGF-β1 from ECM produced by growth plate chondrocytes [Pedrozo et al., 1999c]. However, MVs do not contain plasmin, indicating that another enzyme was involved. When MV extracts were treated with antibodies to MMP-3, activation of TGF-β1 was blocked [Maeda et al., 2001], indicating that it was responsible for $1\alpha,25(OH)_2D_3$-dependent activation of latent TGF-β1. Others have shown that MMP-2 specifically activates TGF-β2 [Wang et al., 2005, 2006].

Taken together, these studies show that MVs contain enzymes that can result in the release of active TGF-β1 from the ECM produced by growth plate chondrocytes. Moreover, they indicate that $1\alpha,25(OH)_2D_3$ produced by the cells and secreted into the ECM is involved. Additional studies have shown that the effect of $1\alpha,25(OH)_2D_3$ is mediated by 2 pathways, both of which are ERp60 dependent, based on the ability of antibodies to ERp60 to block the effect. One mechanism involves the activation of PLA2, resulting in the formation of lysophospholipids. Lysophospholipids can act directly on latent TGF-β1, resulting in its activation [Gay et al., 2004]. They also function to destabilize the MV membrane, resulting in release of MMP-3 (an activator of TGF-β1). The other mechanism is mediated by PKC, since inhibition of PKC by chelerythrine also prevents $1\alpha,25(OH)_2D_3$-dependent activation of MV MMP-3 [Maeda et al., 2000].

Summary

The studies described above show that $1\alpha,25(OH)_2D_3$ regulates ECM turnover by activating both neutral and acidic matrix metalloproteinases. Part of this effect is via transcriptional regulation of MMP expression and part is via autocrine regulation of MMP release from extracellular MVs. MV MMP-3 specifically regulates activation of TGF-β1 stored in the ECM, providing a feedback loop regulating matrix turnover. Active TGF-β1 regulates $1\alpha,25(OH)_2D_3$ production and its secretion into the ECM. $1\alpha,25(OH)_2D_3$ acting as an autocrine factor acts directly on MVs via ERp60-dependent pathways, increasing local lysophospholipid content by stimulating PLA_2 activity and through control of PKC activity.

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References

Boyan, B.D., Z. Schwartz, S. Park-Snyder, D.D. Dean, F. Yang, D. Twardzik, L.F. Bonewald (1994) Latent transforming growth factor-β is produced by chondrocytes and activated by extracellular matrix vesicles upon exposure to 1,25-$(OH)_2D_3$. J Biol Chem *269:* 28374–28381.

Boyan, B.D., L. Wang, K.L. Wong, H. Jo, Z. Schwartz (2006) Plasma membrane requirements for 1α,25$(OH)_2D_3$ dependent PKC signaling in chondrocytes and osteoblasts. Steroids *71:* 286–290.

Brown, R.A., M. Kayser, B. McLaughlin, J.B. Weiss (1993) Collagenase and gelatinase production by calcifying growth plate chondrocytes. Exp Cell Res *208:* 1–9.

Cawston, T.E., A.J. Wilson (2006) Understanding the role of tissue degrading enzymes and their inhibitors in development and disease. Best Pract Res Clin Rheumatol *20:* 983–1002.

D'Angelo, M., P.C. Billings, M. Pacifici, P.S. Leboy, T. Kirsch (2001) Authentic matrix vesicles contain active metalloproteases (MMP). a role for matrix vesicle-associated MMP-13 in activation of transforming growth factor-beta. J Biol Chem *276:* 11347–11353.

Dean, D.D., B.D. Boyan, O.E. Muniz, D.S. Howell, Z. Schwartz (1996a) Vitamin D metabolites regulate matrix vesicle metalloproteinase content in a cell maturation-dependent manner. Calcif Tissue Int *59:* 109–116.

Dean, D.D., Z. Schwartz, O.E. Muniz, M.R. Carreno, S. Maeda, D.S. Howell, B.D. Boyan (2001) Effect of 1α,25-$(OH)_2D_3$ and 24R,25-$(OH)_2D_3$ on metalloproteinase activity and cell maturation in growth plate cartilage in vivo. Endocrine *14:* 311–323.

Dean, D.D., Z. Schwartz, O.E. Muniz, R. Gomez, L.D. Swain, D.S. Howell, B.D. Boyan (1992) Matrix vesicles are enriched in metalloproteinases that degrade proteoglycans. Calcif Tissue Int *50:* 342–349.

Dean, D.D., Z. Schwartz, J.P. Schmitz, O.E. Muniz, Y. Lu, F.J. Calderon, D.S. Howell, B.D. Boyan (1996b) Vitamin D regulation of metalloproteinase activity in matrix vesicles. Connect Tissue Res *35*: 331–336.

Gay, I., Z. Schwartz, V.L. Sylvia, B.D. Boyan (2004) Lysophospholipid regulates release and activation of latent TGF-β1 from chondrocyte extracellular matrix. Biochem Biophys Acta *1684:* 18–28.

Lin, R., N. Amizuka, T. Sasaki, M.M. Aarts, H. Ozawa, D. Goltzman, J.E. Henderson, J.H. White (2002) 1α,25-dihydroxyvitamin D3 promotes vascularization of the chondro-osseous junction by stimulating expression of vascular endothelial growth factor and matrix metalloproteinase 9. J Bone Miner Res *17:* 1604–1612.

Maeda, S., D.D. Dean, I. Gay, Z. Schwartz, B.D. Boyan (2001) Activation of latent transforming growth factor β1 by stromelysin 1 in extracts of growth plate chondrocyte-derived matrix vesicles. J Bone Miner Res *16:* 1281–1290.

Maeda, S., D.D. Dean, V.L. Sylvia, B.D. Boyan, Z. Schwartz (2000) Metalloproteinase activity in growth plate chondrocyte cultures is regulated by 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ and mediated through protein kinase C. Matrix Biol *20:* 87–97.

Pedrozo, H.A., B.D. Boyan, J. Mazock, D.D. Dean, R. Gomez, Z. Schwartz (1999a) TGF-β1 regulates 25-hydroxyvitamin D_3 1α- and 24-hydroxylase activity in cultured growth plate chondrocytes in a maturation-dependent manner. Calcif Tissue Int *64:* 50–56.

Pedrozo, H.A., Z. Schwartz, R. Gomez, A. Ornoy, W. Xin-Sheng, S.L. Dallas, L.F. Bonewald, D.D. Dean, B.D. Boyan (1998) Growth plate chondrocytes store latent TGF-β1 in their matrix through latent TGFβ binding protein-1. J Cell Physiol *177:* 343–354.

Pedrozo, H.A., Z. Schwartz, T. Mokeyev, A. Ornoy, W. Xin-Sheng, L.F. Bonewald, D.D. Dean, B.D. Boyan (1999b) Vitamin D_3 metabolites regulate LTBP1 and latent TGF-β1 expression and latent TGF-β1 incorporation in the extracellular matrix of chondrocytes. J Cell Biochem *72:* 151–165.

Pedrozo, H.A., Z. Schwartz, M. Robinson, R. Gomez, D.D. Dean, L.F. Bonewald, B.D. Boyan (1999c) Potential mechanisms for the plasmin mediated release and activation of latent TGF-β1 from the extracellular matrix of growth plate chondrocytes. Endocrinology *140:* 5806–5816.

Rosenthal, A.K., C.M. Gohr, L.A. Henry, M. Le (2000) Participation of transglutaminase in the activation of latent transforming growth factor β1 in aging articular cartilage. Arthritis Rheum *43:* 1729–1733.

Schmitz, J.P., D.D. Dean, F.J. Calderon, Z. Schwartz, B.D. Boyan (1994) Matrix vesicles contain active stromelysin-1 regulated by vitamin D_3 metabolites. J Bone Miner Res *9:* S379.

Schmitz, J.P., D.D. Dean, Z. Schwartz, D.L. Cochran, G.M. Grant, R.J. Klebe, H. Nakaya, B.D. Boyan (1996a) Chondrocyte cultures express matrix metalloproteinase mRNA and immunoreactive protein: stromelysin-1 and 72kDa gelatinase are localized in extracellular matrix vesicles. J Cell Biochem *61:* 375–391.

Schmitz, J.P., Z. Schwartz, V.L. Sylvia, D.D. Dean, F. Calderon, B.D. Boyan (1996b) Vitamin D_3 regulation of stromelysin-1 (MMP-3) in chondrocyte cultures is mediated by protein kinase C. J Cell Physiol *168:* 570–579.

Schwartz, Z., E.J. Graham, L. Wang, S. Lossdorfer, I. Gay, T.L. Johnson-Pais, D.L. Carnes, V.L. Sylvia, B.D. Boyan (2005) Phospholipase A2 activating protein (PLAA) is required for 1α,25$(OH)_2D_3$ signaling in growth plate chondrocytes. J Cell Physiol *203:* 54–70.

Sylvia, V.L., Z. Schwartz, E.B. Ellis, S.H. Helm, R. Gomez, D.D. Dean, B.D. Boyan (1996) Nongenomic regulation of protein kinase C isoforms by the vitamin D metabolites 1α,25-$(OH)_2D_3$ and 24R,25-$(OH)_2D_3$. J Cell Physiol *167:* 380–393.

Wang, L., S. Clutter, J. Benincosa, J. Fortney, L.F. Gibson (2005) Activation of transforming growth factor-beta1/p38/Smad3 signaling in stromal cells requires reactive oxygen species-mediated MMP-2 activity during bone marrow damage. Stem Cells *23:* 1122–1134.

Wang, M., D. Zhao, G. Spinetti, J. Zhang, L.Q. Jiang, G. Pintus, R. Monticone, E.G. Lakatta (2006) Matrix metalloproteinase 2 activation of transforming growth factor-β1 (TGF-β1) and TGF-β1-type II receptor signaling within the aged arterial wall. Arterioscler Thromb Vasc Biol *26:* 1503–1509.