Stretch-Induced Modulation of Matrix Metalloproteinases in Mineralizing Osteoblasts via Extracellular Signal-Regulated Kinase-1/2

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ABSTRACT: Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) produced by osteoblasts play an essential role in bone remodeling. Hence, these proteins could provide an interesting means by which mechanical loading leads to adaptation of bone. Here, we examined the effect of stretch on MMP-1, -2, -3, -8, -9, -13, and -14, as well as TIMP-1 and -2 gene expression in differentiating, mineralizing, and nonmineralizing human SV-40 immortalized preosteoblast cells. In the mineralizing osteoblast culture, but not in the nonmineralizing cultures, cyclic stretch for only 15 min resulted in an increase of MMP-1 (fourfold) and -3 (depending on differentiation stage up to 25-fold) transcript abundance. No clear effect was observed for other MMPs, TIMP-1 or -2. The increase of MMP-1 and -3 was confirmed on the protein level. Stretching experiments performed in the presence of a specific inhibitor of extracellular signal-regulated kinase (ERK) showed a strong suppression of the stretch-induced increase in MMP-1 and -3. In conclusion, we show that MMP-1 and MMP-3 are mechanosensitive genes in mineralizing the human osteoblast, and that the mechano-induction of these genes is mediated via the ERK pathway. Our findings implicate that these MMPs are important factors in the mechanoregulation of bone turnover. With the ability to generate MMPs at highly stretched sites, osteoblasts can potantially direct osteoclasts to specific bone surface areas prepared for resorption. © 2006 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 24:1480–1488, 2006

Keywords: matrix metalloproteinases; mineralizing osteoblasts; extracellular signal-regulated kinase-1/2

INTRODUCTION

Bone is continuously remodeled to meet the physical demands of its environment. In this process, temporal and spatial controlled breakdown of the extracellular matrix is essential for proper resorption and remodeling. Matrix metalloproteinases (MMPs) play an important role in the breakdown of collagenous extracellular matrices, including skeletal tissue.[1]

MMPs are a family of structurally and functionally related enzymes responsible for the proteolytic degradation of extracellular matrix components such as collagen, elastin, glycoproteins, proteoglycans, and glucosaminoglycans.[2] Currently, approximately 22 human MMPs have been identified, and classified into at least four groups, depending on their substrate specificity: collagenases, gelatinases, stromelysins, and matrilysins.

In bone, osteoblasts are considered to be the main source of MMP production,[3] indicating that these cells not only play a role in bone formation, but also can directly stimulate bone resorption.[4–6] Moreover, there's increasing evidence that MMPs are part of a coupling mechanism between osteoblast and osteoclasts, and finally osteoblasts again in initiating matrix degradation as well as finalizing the resorption before new bone formation.[7–10] MMPs are synthesized in a latent form, and become activated extracellularly by proteolytic cleavage requiring plasmin.[11] Their activity is tightly regulated by specific tissue inhibitors of metalloproteinases (TIMPs), which can also be produced

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by osteoblasts. MMPs and TIMPs are known to be involved in various physiological processes both in normal bone remodeling and under pathological conditions such as osteoporosis and rheumatoid arthritis.[12–14]

Direct regulation of MMPs or TIMPs by mechanical loading would provide an interesting means by which mechanical loading can regulate the spatial control of bone metabolism. Yet only very limited data are available on the interaction between mechanical loading and MMPs or TIMPs in osteoblasts. Tanaka et al.[15] found MMP-9 mRNA was elevated 1.3-fold after 7 days of vibration with sinusoidal strain on mouse osteoblasts in collagen gels. Recently, Yang et al.[16] reported induction of MMP-13 mRNA (2.8-fold) and MMP-13 activity (twofold) after uniaxial mechanical strain in mouse osteoblastic cell line MC3T3-E1.

The aim of the current study was to investigate the effect of mechanical loading on various MMPs and TIMPs in a well-defined human differentiating osteoblast cell line.[17–19] In addition, we examined whether effects observed were dependent on osteoblast mineralization and differentiation. Finally, following our recent observation that mechanical loading induces phosphorylation of extracellular signal-regulated kinase (ERK),[20] we examined the involvement of ERK phophorylation in MMP regulation by mechanical loading.

MATERIALS AND METHODS

Cell Culture

At the start of experiments (day 0), 2×10^5 SV40-immortalized human fetal preosteoblast cells (SV-HFO)[18–20] per well were seeded on flexible, collagen type I coated six-well plates (BioFlex, Flexercell, McKeesport, PA) to ensure proper adherence of the cells. Cells were cultured in αMEM medium without phenol red (Gibco, Paisly, UK), supplemented with 20 mM HEPES (Sigma Chemical Co., St. Louis, MO), 2% charcoal-treated fetal calf serum, 1.8 mM $CaCl_2$, 100 μg/mL streptomycin and 100 IU/mL penicillin (Gibco). To trigger differentiation of SV-HFO cells into matrix forming and mineralizing osteoblasts, culture medium was supplemented with osteogenic factors: 1 μM dexamethasone (Sigma) and 10 mM β-glycerophosphate (Sigma). In the absence of dexamethasone and β-glycerophosphate, the cells do not produce a mineralized matrix. These two conditions will be referred to as "mineralizing" and "nonmineralizing."

DNA, Alkaline Phosphatase Activity, and Calcium Content

For characterization of the nonmineralizing and the mineralizing culture, DNA, alkaline phophatase (ALP) activity, and calcium content was determined. SV-HFO cell lysates in 0.1% PBS-Triton X-100 were treated with heparin and RNase A (50 mg/mL in PBS) for 30 min at 37°C. DNA content was measured according to the ethidium bromide method by Karsten and Wollenberger.[21] Alkaline phosphatase activity was determined by the colorimetric method of Lowry et al.[22] Calcium deposition into the matrix was determined after overnight extraction with HCl, using the Sigma calcium assay.

Histochemical Staining

For histochemical staining of calcium, cells were fixated in ice-cold 70% EtOH, stained with 0.1% Alizarin Red S solution (pH 4.0, saturated in MilliQ) for 30 min, and washed using 70% EtOH.

Stretching

Stretching experiments were performed using a loading unit (Flexercell, McKeesport, PA) inside a 37°C, 5% CO_2 incubator. In this setup vacuum is applied to the area under six-well plates, causing the flexible silicon bottom to stretch overloading posts underneath. Vacuum was generated through a Venturi valve using airflow regulated by a computer (using Labview software). The unit produces homogeneous biaxial stretch[23,24] with minimal vertical displacement of the silicon bottom and was adapted, using a vacuum of 650 mbar and 32-mm diameter loading posts, to produce physiological relevant strain levels of approximately 4000 μstrain.[25] Each six-well plate was placed in the loading unit 90 min prior to mechanical loading (preincubation period). Cyclic strains of 0.5 Hz were applied for 15 min on days 7, 14, and 21 of culture. The stretch protocol was based on previous ERK activation studies.[20] Nonstrained control groups were also placed in the loading unit for 90 min. Experiments were performed in the presence or absence of 10 μM U0126 (Promega, Madison, WI), a selective inhibitor of ERK through inhibition of the kinase activity of MAP Kinase Kinase.[26] The inhibitor, or its vehicle DMSO, was added to the medium 90 min prior to stretching, at the start of the preincubation period.

Quantification of Gene Expression

Cells were collected 2, 6, or 24 h after the 15-min stretching in 500 μL RNA-Bee™ (TEL-TEST, Friendswood, TX) for mRNA extraction. RNA was precipitated with 2-propanol and subsequently purified using 50 mM EDTA/2.5 M lithium chloride. Extracted total RNA was screened spectrophotometrically and 260 nm/280 nm ratios between 1.7 and 2.0 were considered reasonable pure. Total RNA was then quantified accurately using Ribogreen™ reagent (Molecular Probes BV, Leiden, The Netherlands) according to manufacturer's instructions. One microgra, of total RNA was reverse transcribed into cDNA using RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany) according to supplier's procedure.

All Taqman assays were performed in triplicates in 96-well optical plates using qPCR^{TM} Core Kit (Eurogentec, Maastricht, The Netherlands). Primer and probe nucleotide sequences for gene amplification are listed in Table 1. Real-time PCR conditions and normalization was adopted from Mandl et al.[27] Primer and probe nucleotide sequences for gene amplification are listed in table 1. The MMP-8 assay was obtained from Applied Biosystems (Hs 00233972_m1).

Western Blotting

For detection of MMP protein, measurements were performed at day 14 with osteoblast differentiating cultures. Cells were incubated on serum free medium 24 h prior to the stretching experiments. Next, cells were stretched for 15 min as described above and cell lysate (in 200 μL buffer containing 25 mM HEPES, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 0.5 M NaCl, 5 mM EDTA, 50 mM NaF, 1 mM PMSF, 1 mM Na_3VO_4, 10 μg/mL leupeptin, and 2 mM β-glycerophosphate) and serum free medium were collected 0.5, 2, or 40 h poststretch. After centrifugation of the cell lysate at $11{,}000 \times g$ for 15 min, supernatant was collected, and protein levels were determined using BCA protein assay kit (Pierce, Rockford, IL). Cell lysate (10 μg protein/lane) and medium were separated by SDS-PAGE and transferred to Hybond+ nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ). After overnight blocking with TBS + 0.1% Tween 20 containing 5% bovine serum albumin, membranes were incubated with monoclonal MMP-1 or -3 antibodies (1:500, Oncogene, San Diego, CA) for 3 h at room temperature and detected using horseradish peroxidase coupled secondary antibodies and the ECL detection system (Amersham Pharmacia Biotech, Piscataway, NJ). After exposure to Kodak HR film, immunoreactive bands were quantified using Quantity One (Bio-Rad, Hercules, CA) software.

For detection of activated ERK1/2, cells were immediately lysed in buffer after stretching. Protein levels were determined, and Western blotting technique was performed as described above, using monoclonal antibodies against ERK1/2-P (1:2000, Cell Signaling, Beverly, MA). Finally, all blots were stripped and blocked again for incubation with monoclonal antibodies against ERK1/2 (1:2000, Cell Signaling). ERK1/2-P levels were normalized for total ERK1/2 levels to correct for loading differences.

Statistics

Each condition was performed in triplicate ($n = 3$). Data are presented as means from at least three wells for each condition ± standard deviation. Differences between

Table 1. List with Genes, and the Primer and Probe Nucleotide Sequences

Gene	Acc.No.	Primer	nucleotide sequences
MMP1	NM_002421	HsMMP1_F	CTCAATTTCACTTCTGTTTTCTG
		HsMMP1_R	CATCTCTGTCGGCAAATTCGT
		HsMMP1_FAM	CACAACTGCCAAATGGGCTTGAAGC
MMP2	NM_004530	HsMMP2_F	TCAAGTTCCCCGGCGAT
		HsMMP2_R	TGTTCAGGTATTGCACTGCCA
		HsMMP2_FAM	TCGCCCCCAAAACGGACAAAGA
MMP3	NM_002422	HsMMP3_F	TTTTGGCCATCTCTTCCTTCA
		HsMMP3_R	TGTGGATGCCTCTTGGGTATC
		HsMMP3_FAM	AACTTCATATGCGGCATCCACGCC
MMP9	NM_004994	HsMMP9_F	TGAGAACCAATCTCACCGACAG
		HsMMP9_R	TGCCACCCGAGTGTAACCAT
		HsMMP9_FAM	CAGCTGGCAGAGGAATACCTGTACCGC
MMP13	NM_002427	HsMMP13_F	AAGGAGCATGGCGACTTCT
		HsMMP13_R	TGGCCCAGGAGGAAAAGC
		HsMMP13_FAM	CCCTCTGGCCTGCTGGCTCA
MMP14	NM_004995	HsMMP14_F	TGCCTGCGTCCATCAACACT
		HsMMP14_R	CATCAAACACCCAATGCTTGTC
		HsMMP14_FAM	AAGACGAATTTGCCATCCTTCCTCTCGT
TIMP1	NM_003254	HsTIMP1_F	TGCCGCATCGCCGAGAT
		HsTIMP1_R	ATGGTGGGTTCTCTGGTG
		HsTIMP1_FAM	CCAGCGCCCAGAGAGAC
TIMP2	NM_003255	HsTIMP1_F	CACCAGGCCAAGTTCTTC
		HsTIMP1_R	CGGTACCACGCACAGGA
		HsTIMP1_FAM	CCTGCATCAAGAGAAGTGAC
GAPDH	BC025925	HsGAPDH_F	ATGGGGAAGGTGAAGGTCG
		HsGAPDH_R	TAAAAGCAGCCCTGGTGACC
		HsGAPDH_FAM	CGCCCAATACGACCAAATCCGTTGAC

groups were analyzed using the Mann-Whitney *U*-test and were considered significant when $p < 0.05$.

RESULTS

Cell Growth, Differentiation, and Mineralization

During SV-HFO culture, osteoblast differentiation was monitored measuring DNA levels, ALP activity, and mineralization. Both in the presence or absence of osteogenic factors DNA levels gradually increased, albeit that DNA levels in the mineralizing cultures were higher than in nonmineralizing cultures (Fig. 1A). ALP activity in mineralizing osteoblast cell cultures peaked at day 14 of culture, and mineralization was strongly induced after 21 days. When cells were not induced to mineralize these changes did not occur (Fig. 1A). Calcium deposition was also stained in situ using alizarine red S staining (Fig. 1B), which confirmed the calcium assay measurements in both culture conditions. As can be seen from Figure 1, different stages in osteoblast differentiation can be discerned of which days 7 (no mineralization yet), 14 (onset of mineralization), and 21 (full mineralization) have been chosen as representative for the further analyses.

Effect of Mechanical Loading on MMPs, TIMP-1, and -2

In both the nonmineralizing and the mineralizing culture, all tested genes were expressed. MMP-1, -2, -14, and TIMP-2 were expressed most abundantly (C_t values around 27, 23, 22, and 22, respectively), whereas MMP-8 was least expressed with C_t values around 36.

From these genes, only MMP-1 and MMP-3 responded to cyclic stretch. A significant increase of MMP-1 and MMP-3 expression was observed in the mineralizing osteoblast cultures. Analysis of stretch-induced expression of MMP-1 and MMP-3 in relation to the various osteoblast differentiation stages (i.e., days 7, 14, and 21) demonstrated differences between these two MMPs. Stretch induced MMP-1 expression fourfold at all days during differentiation, while expression of MMP-3 was clearly dependent on the mineralization stage (Fig. 2). Stretch induced the expression of MMP-3 25-fold at the early stage of differentiation when no mineralization has taken place yet (day 7). At the onset of mineralization (day 14), this induction was reduced to fivefold, while on day 21 MMP-3 expression was not affected.

When the cells were not induced to mineralize, stretch weakly induced MMP-1, and did not change MMP-3 expression (Fig. 3). None of the other MMPs tested were sensitive to stretch either in mineralizing osteoblasts and nonmineralizing cells. TIMP-1 and -2 were also not affected by stretch in either culture model.

To support our findings on RNA level, Western blots for MMP-1 and -3 were performed on cell lysate and serum-free medium samples collected after stretching. Experiments were performed on day 14 to obtain sufficient cell material for protein analyses. Stretching for 15 min resulted in an increase of latent MMP-1 and -3 both in cell lysate as well as in the medium 40 h poststretch (Fig. 6, lanes 1 and 2 for MMP-1, and 5 and 6 for MMP-3). No active forms of MMP-1 or MMP-3 were detected in either the cell lysate or medium samples.

In addition, a matrix metalloproteinases antibody array experiment showed abundant basal protein levels of TIMP-1 and 2 and paralleled RNA data that stretch does not regulate TIMP1 or -2 (data not shown).

Effect of the MEK1/2 Specific Inhibitor U0126

Previous studies showed an increase of phosphorylated ERK levels in mineralizing osteoblast cultures after mechanical stimulation.[20] Treatment with the MEK inhibitor U0126 90 min prior to stretching effectively suppressed stretch-induced increase in ERK1/2 phosphorylation as determined by phosphospecific ERK1/2 immunoblot while total ERK1/2 remained unchanged with U0126 treatments.[20]

Stretching experiments performed in the presence of the MEK1/2 inhibitor U0126 showed a strong suppression of the stretch-induced increase in MMP-1 and -3 mRNA expression at all tested days, except for MMP-3 on day 21 (Fig. 4). The control data (-U0126) are the same as the mineralizing data shown in Figure 3. Even the very potent induction of MMP-3 by stretch already after 6 h on day 7 was strongly inhibited by blocking ERK1/2 phosphorylation (Fig. 5). The control data (-U0126) of MMP-1 and -3 are the same as those shown in Figure 2. GAPDH and basal expression of the other genes in the nonstretched control group was not altered by the presence of U0126.

The effects of the MEK1/2 inhibitor on MMP-1 and MMP-3 mRNA level were confirmed on protein level (Fig. 6, lane 3 and 4, and 7 and 8, respectively).

DISCUSSION

This study shows mechanoregulation of two specific MMPs in mineralizing human osteoblasts via the ERK signaling pathway. In the mineralizing

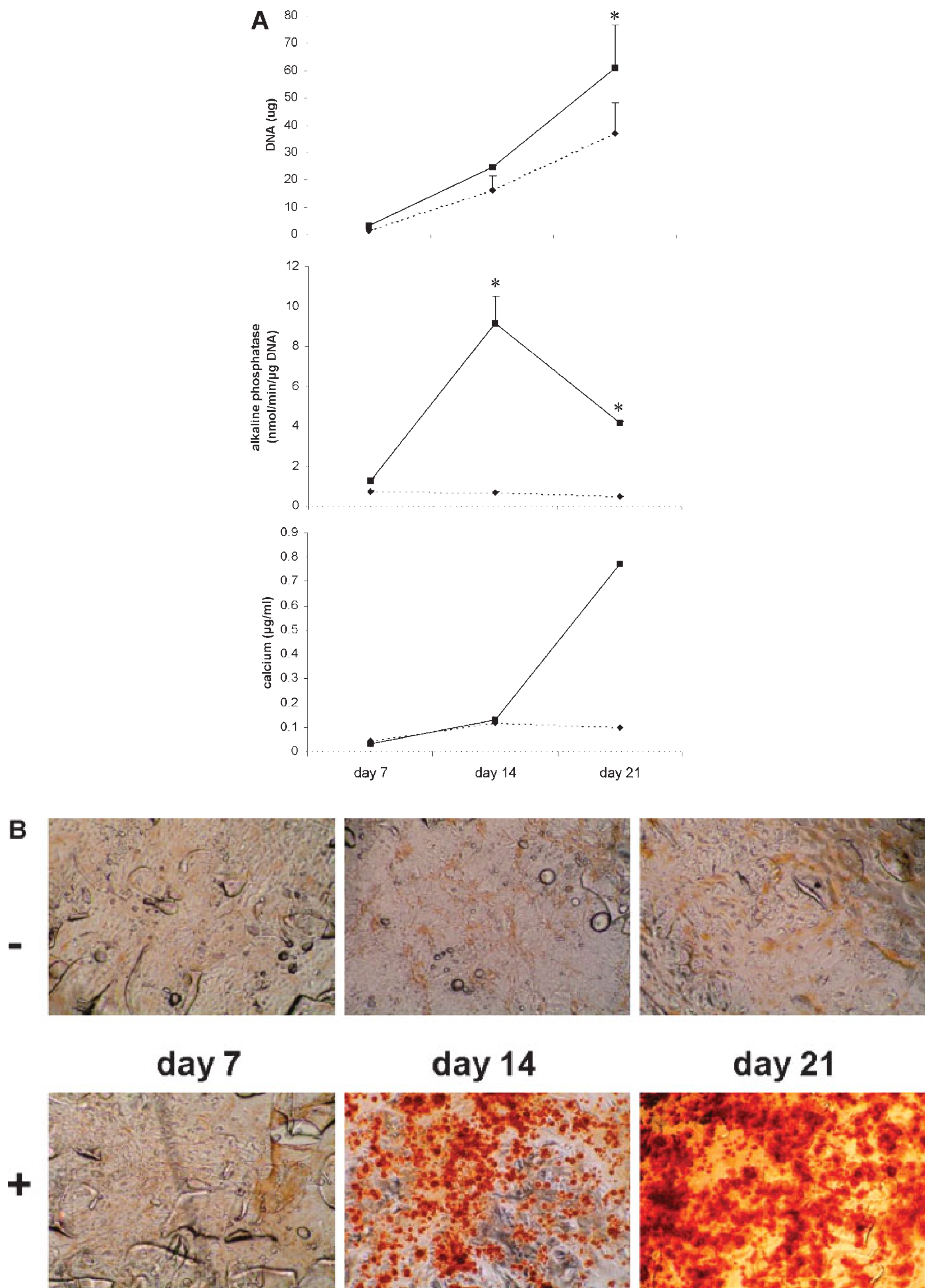

Figure 1. (A) DNA, alkaline phosphatase activity, and calcium accumulation during culture in the absence (dashed line) or presence (continuous line) of the osteogenic factors dexamethasone and β-glycerophosphate. (B) Mineralization was also demonstrated using Alizarin Red S staining and is depicted in red. Based on these characteristics, conditions will be referred to as nonmineralizing (−), or mineralizing (+), respectively. Significant differences due to osteogenic factors are marked by an asterisk.

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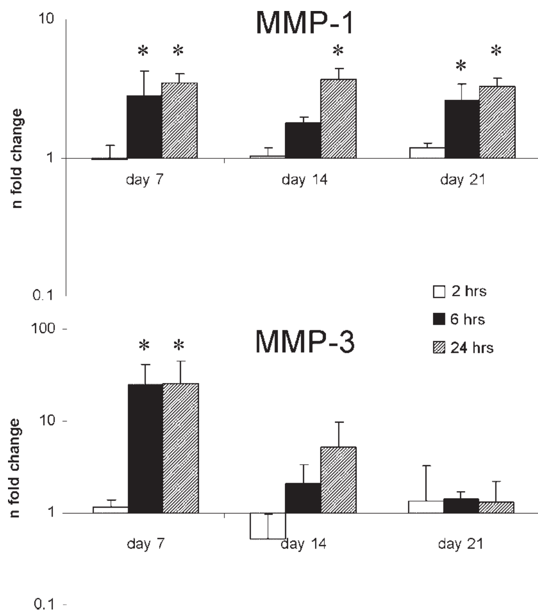

Figure 2. Effect of 15 min of cyclic stretch on change in MMP-1 and -3 mRNA expression levels in the mineralizing culture. On day 7, 14, and 21, cell material was collected 2 (open columns), 6 (filled columns), and 24 (striped columns) h after mechanical loading. The n-fold change is relative to the static control. Data shown were obtained from three experiments and normalized to GAPDH. *Statistical significant increase over static control.

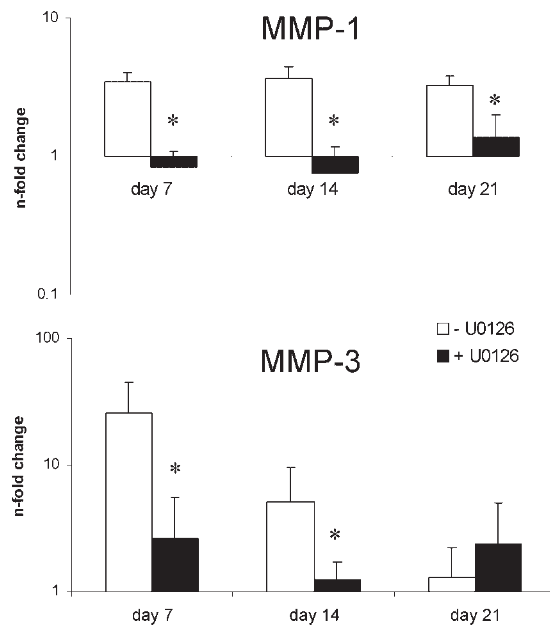

Figure 3. Effect of 15 min of cyclic stretch on change in MMP-1 and -3 mRNA expression levels in the nonmineralizing culture (open columns) or the mineralizing culture (filled columns). On day 7, 14, and 21, cell material was collected 24 h after mechanical loading. The n-fold change is relative to the static control. Data shown were obtained from three experents and normalized to GAPDH. *Significant increase over control.

osteoblast culture, MMP-1 and MMP-3 were both significantly upregulated in response to cyclic strain. Interestingly, no effects of stretch on either MMP were observed in the nonmineralizing culture. This implicates that differentiation into fully active osteoblasts, which mineralize, is important for mechanoregulation of MMPs. The difference in responsiveness between the mineralizing and nonmineralizing cultures was already present after 7 days of culture when mineralization was not yet initiated. Apparently, in this early phase the cells are already in different differentiation stage that without yet manifest mineralization affects the responsiveness of the cells to mechanical loading and regulation of MMP-1 and MMP-3. A possible explanation may be a difference in matrix properties between the two culture systems, as described previously by Weyts et al.[25] Within the mineralizing osteoblast culture, the differentiation stage appeared to be important for the mechanosensitivity and MMP regulation of the osteoblasts. To culture in a tightly controlled model of osteoblastic differentiation, we used SV40-immortalized osteoblasts. A possible disadvantage of these cells may be that they have a

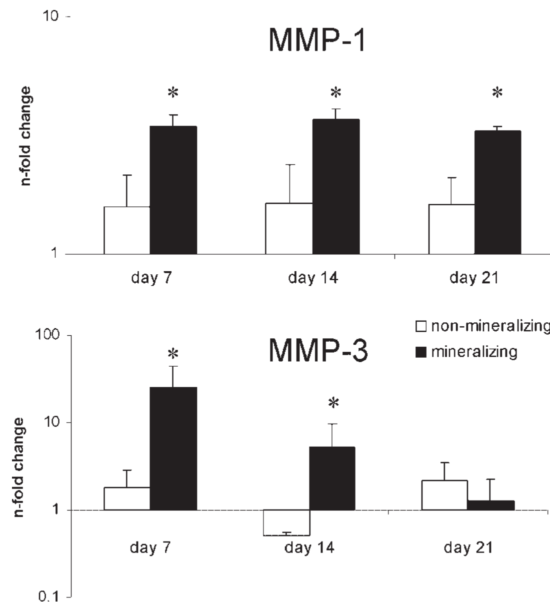

Figure 4. Change in MMP-1 and MMP-3 mRNA expression 24 h after stretch in the absence (open columns) or presence (filled columns) of the MEK1/2 inhibitor U0126 at days 7, 14, and 21 of mineralizing culture. U0126, or its vehicle DMSO, was added to the medium 90 min prior to 15 min stretch. Significant differences due to the presence of U0126 are marked with an asterisk ($n = 3$).

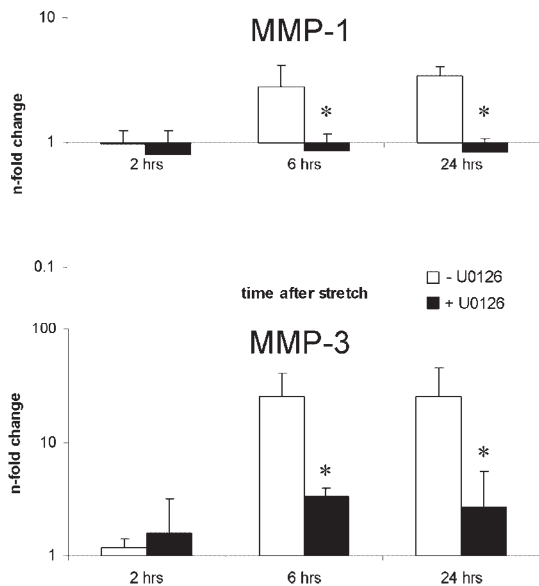

Figure 5. Change in MMP-1 and MMP-3 mRNA expression after stretch in the absence (open columns) or presence (filled columns) of the MEK1/2 inhibitor U0126 at day 7 of mineralizing culture. U0126, or its vehicle DMSO, was added to the medium 90 min prior to 15 min stretch. Cell material was collected 2, 6, and 24 h after mechanical loading. Significant differences due to the presence of U0126 are marked with an asterisk ($n = 3$).

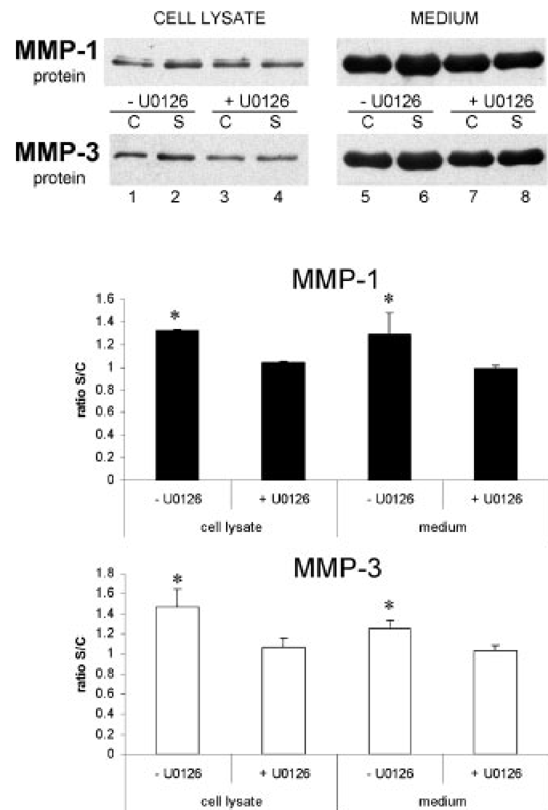

Figure 6. Western blot showing typical MMP-1 and -3 protein levels in total cell lysate and serum-free medium. Cell material and medium of mineralizing cultures was collected 40 h after 15 min of stretch for protein analysis, and was obtained from three separate experiments. Cells were stretched in the presence or absence of the MEK1/2 inhibitor U0126. The position of the bands correlated with the latent form of MMP-1 and -3 (52 kb). Immunosignals were quantified using analysis software, and the stretch over control ratio was calculated (B: MMP-1 and C: MMP-3). *Statistical significant increase over static control.

different sensitivity to strain compared to primary osteoblasts. However, we demonstrate here that mechanosensitivity of osteoblast cells is highly dependent on differentiation stage of these cells.

MMP-1 and MMP-3 are likely candidates to play an important role in bone remodeling in vivo. Approximately 90% of the organic matrix of bone is type I collagen. MMP-1 is a human collagenase that can degrade collagen type I, III, and to a lesser extent, type II. MMP-1 immunolocalizes in vivo to the osteoblast in high-rate bone-remodeling states in rat calvaria,[28] suggesting a role in the bone-remodeling process. MMP-3, often referred to as stromelysin-1, has structural homology with collagenases,[29] and is similar in molecular weight to MMP-1. Although not able to directly degrade the triple helical regions of interstitial collagens, MMP-3 can activate the inactive procollagenases MMP-1, -8, and -13.[30–32]

We have previously shown that stretch is able to induce ERK phosphorylation at all stages of differentiation and mineralization,[20,33] and induction was strongest at later stages of differentiation (days 14 and 21). Blocking this pathway did result in a strong suppression of both MMP-1 and -3 mRNA expression levels, demonstrating the significance of this signaling pathway for mechanotransduction in human osteoblasts. It can also be concluded that the diminishing effect of stretch on MMP-3 at day 14 and 21 is not due to inactivity of the ERK1/2 pathway. Indeed, MMP-1 is mechanically induced throughout the differentiation process, while it can be suppressed by the presence of an ERK inhibitor. However, the suppression of the stretch-induced increase of MMP-3 by U0126 was not complete. Apparently, there are different mechanisms why MMP-3 becomes "(in)sensitive" for stretch.

The finding that stretch did not result in a clear induction of phosphorylated ERK levels in the non-mineralizing culture further subscribes the difference in cell mechanosensitivity between the two culture systems.

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The differentiation-dependent effect of stretch on MMP gene expression could, in part, explain differences between findings from studies from Tanaka,[15] Yang,[16] and our study. However, there are also other possible causes, that is, the nature of mechanical stimulation or origin of the cells. Tanaka et al. used mouse MC3T3-E1 cells seeded in collagen gels and found that MMP-9 was slightly elevated after 7 days of intermittent mechanical stimulation, whereas no differences due to loading were observed in MMP-1A, -3, -13. Yang et al. focused on MMP-13, and report an increase of this gene already after 5 min of 8% mechanical strain in MC3T3 cells using PCR technique.

In this study we demonstrated that mechanical loading can lead to increased production of MMPs, first on RNA level (within 24 h), and subsequently on protein level (after 40 h). Another possible mechanism is the direct activation of latent MMPs in the extracellular environment, facilitating instant bone adaptation. Yang et al. indeed reported an increase of zymographic activity after 30 min. Although we used antibodies able to detect activated MMPs, we have not been able to show enzymatic activity in the conditioned medium within 30 min. A possible reason might be the ominous presence of TIMPs in the medium, as we found in an additional experiment using antibody arrays. As such, the MMP activity might be inhibited by the high TIMP levels.

There is still an unanswered question how osteoclasts break down the collagen matrix. Osteoclasts express MMP-9, -12, and -14, but these enzymes are not critical for collagen breakdown and resorption. Therefore, Hou et al.[3] hypothesized that the critical MMP for bone solubilization is produced by nonosteoclastic cells. Thus, the collagen turnover might be regulated by osteoblasts that are stimulated by loading.

MMPs may influence bone remodeling via various mechanisms. They are thought to play an important role initiating osteoclastic bone resorption, because osteoclasts are unable to attach to the bone surface if the osteoid layer covering the mineralized bone matrix has not been removed by proteinases.[8,10] Moreover, collagen fragments generated by MMP activity might attract and activate osteoclasts.[35] Another important role of MMPs in the reversal phase[34] of bone remodeling has been stipulated by Everts et al.,[9] who demonstrated removal of bone collagen left by osteoclasts in Howship's lacunae by bone lining cells producing MMPs. This process is followed by formation of new bone by osteoblasts. These studies indicate that MMPs are part of a coupling mechanism between osteoblasts and osteoclasts and finally osteoblasts again in initiating matrix degradation as well as finalizing the resorption before new bone formation. On the other hand, there are also studies performed by Rubin et al.,[36,37] who demonstrated downregulation of RANKL expression after stretch, indicating a decrease in osteoclast formation. This could ultimately lead to a decrease in bone remodeling. Although efforts have been made to repeat these results, RANKL was not sufficiently expressed in our culture model to verify these findings.

An important issue in bone turnover is the spatial localization. The increase of MMPs in response to stretch suggests that osteoblasts exposed to mechanical loading try to digest their matrix more than nonstretched cells. This can lead to spatially directed turnover of the extracellular matrix by directing the osteoclast to a specific location, ultimately resulting in an accurate adaptation of matrix architecture and composition that enables the osteoblasts to better resist mechanical deformation as a result of the forces that act upon them. This theory is supported by findings by Mudera et al.,[38] who showed that fibroblasts unable to align to applied loads remodel their matrix far more rapidly than orientated cells.

In conclusion, we show an interaction between stretch and MMPs in human osteoblasts. Short-term (15 min) cyclic stretch induces MMP-1 and MMP-3 expression with that for MMP-3 being dependent on osteoblast mineralization and differentiation stage. We have demonstrated a functional role for the ERK1/2 pathway in this mechanotransduction pathway. Being regulated by mechanical stimuli, MMP-1 and MMP-3 can provide an interesting means by which mechanical loading can regulate bone turnover and guide osteoclasts to a specific location.

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