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Identification of genes responsive to low-intensity pulsed ultrasound stimulations

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ABSTRACT

This study was designed to compare the temporal changes of gene expression profile in osteoblastic cell lines (SaOS-2) treated with low-intensity pulsed ultrasound stimulation (LIPUS) using complementary DNA (cDNA) microarrays. SaOS-2 cells were treated with LIPUS for 20 min. Thereafter, cells were harvested and RNA was extracted twice at 4 and 24 h, respectively. Using cDNA microarrays, 7488 genes with changes in expression in SaOS-2 cells were identified for comparison. Microarray analysis revealed a total of 165 genes in SaOS-2 cells were regulated at 4 and 24 h after LIPUS treatment. Except for 30 known LIPUS-regulated genes, our study demonstrated for the first time that over 100 genes were related to the underlying molecular mechanism of LIPUS and suggested that LIPUS might regulate a transient expression of numerous critical genes in osteoblastic cells. These results provide further understanding of the role of LIPUS in the regulation of osteoblastic gene expression potentially involved in the molecular mechanism of osteogenesis in fracture repair.

A number of clinical and experimental studies demonstrated that low-intensity pulsed ultrasound stimulations (LIPUS, 200-μs burst of 1.5 MHz and 30.0 ± 5.0 mW/cm^2) was able to stimulate osteogenesis and therefore was effective for acceleration of fracture healing and treatment of delayed fracture union and nonunion [1–3]. Despite its pronounced efficacy on connective tissue repair, the related underlying mechanisms of LIPUS action at both cellular and molecular level remain to a large extent unexplained. Without understanding the precise cellular mechanisms associated with osseous tissue transformation responses to LIPUS intervention, it would be futile to develop optimal therapeutic protocols for clinical applications.

In vitro models are standard for studying molecular and cellular mechanisms under the influence of either biological or biophysical interventions. A few studies showed that LIPUS induced transient expression of the immediate-early response gene c-fos and elevated gene expression for bone sialoprotein (BSP), insulin-like growth factor-1 (IGF-1), and osteocalcin (OC) using a mouse bone marrow stromal-derived cell line (ST2 cell), osteoblasts, and osteocytes [4,5]. Some other growth factors were also implicated to stimulate fracture repair processes, including transforming growth factor beta (TGF-β), bone morphogenetic protein (BMP) [6,7], and adenosine 5′-triphosphate (ATP) [8]. LIPUS was able to increase the production of these growth factors by stimulating bone cells and these studies reported that LIPUS had effects on regulating the expression of bone-specific genes although these genes only accounted for a very small proportion of the human genome.

Accordingly, we hypothesized that the use of LIPUS would accelerate and augment connective tissue healing processes through a variety of biological mechanisms, especially through changing a number of critical gene expressions that might be involved in acceleration of healing tissue remodeling. In this study, we used high-density complementary DNA (cDNA) microarrays to investigate the entire genome expression profile changes in osteoblastic cells SaOS-2. This cDNA microarray included most known genes, which might cover multiple biological functions regulated by LIPUS, such as those related to cytoskeleton and cell maturation. The significance of the present study was to provide essential information on the molecular mechanisms involved in osteogenesis mediated by LIPUS.

Materials and methods

Cell culture. Human osteoblastic osteosarcoma cell lines (SaOS-2) (HTB-85, American Type Culture Center, Manassas, VA, USA) were used and maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 0.8% penicillin–streptomycin–neomycin complex, and 2.5 mM L-glutamine

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(Life Technologies, Gaithersburg, MD, USA) in a 5% CO_2, 37 °C incubator with humidified atmosphere. Cells were harvested by trypsinization and resuspended in medium before intervention [8,9]. SaOS-2 cell number was 5×10^5 per well and cells were seeded into a six-well plate for 2 days prior to LIPUS treatment. This study was approved by the Research Ethics Committee and the Chemical and Safety Committee of the Chinese University of Hong Kong (Ref. CUHK4342/03M).

LIPUS treatment. Sonic Accelerated Fracture Healing System 2A (Smith & Nephew Inc., Memphis, TN, USA) was the LIPUS system used for this study that generated 1.5 MHz ultrasound in a pulsed-wave mode (200-μs pulse burst-width with repetition frequency of 1 kHz at the spatial-averaged temporal-averaged intensity of 30 mW/cm^2) [1–3]. The culture plate was placed on six-well ultrasound transducers with a thin smear of coupling gel (Acoustix, Conmed Corp., Utica, NY, USA) before LIPUS was delivered through the bottom of culture plates for 20 min at 37° C. Cells were harvested at 4 and 24 h after LIPUS treatment. Control samples were prepared and processed in the same manner but without LIPUS treatment. Twelve wells were used for each treatment and control group at each harvest. The experiment from stages of seeding SaOS-2 cells up to hybridization of cDNA with the array chip was performed three times.

RNA sample preparation. The cultured cells were lysed and homogenized with Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA present in the cell samples was extracted using Qiagen RNeasy Mini kit, (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's recommendations.

cDNA microarray analysis. cDNA microarray analysis was performed using Chipscreen Human Microarray Gene Chip (Shenzhen Chipscreen Biosciences, Ltd., Shenzhen, China), which detected 7488 cDNA fragments of human genes. These genes were listed in Chipscreen's website (http://www.chipscreen.com). cDNAs converted from total RNA were labeled with cyanine dye (Cy3 dye or Cy5 dye) by reverse transcription. Briefly, 10 μl volume of the reaction mixture was incubated for 5 min at 70 °C with 20 μg total RNA, 1 μl 18-mer oligonucleotide (1 μg/μl), and 2 μl Cy3 or Cy5 Spike RNA (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The following reagents were then added: 5× 1st strand buffer (Invitrogen Corp., Carlsbad, CA, USA) 4 μl, 0.1 M dithiothreitol 2 μl, dNTP mix (deoxy-ribonucleoside triphosphate) 1 μl, ribonuclease inhibitor (40 U/μl) 1 μl, Cy3 or Cy5 dCTP (1 mM deoxycytidine triphosphate, Amersham pharmacia) 1 μl, and Superscript II reverse transcriptase (200 U/μl, Invitrogen Corp.) 1 μl. The solution was stirred and briefly spun down before incubating it at 42 °C for 2 h. The probe was purified using a Qiagen Qiaquick PCR purification kit (Qiagen, Inc.). Hybridization and washing of the microarray were carried out according to the manufacturer's instructions. In brief, the probe solutions containing both Cy3- and Cy5-labeled cDNA probes were added to pretreated slides, and the slides were covered with cover slides. The slides were kept overnight in the hybridization chamber at 42 °C with gentle shaking, and then washed three times with 2× SSC (standard saline citrate, 150 mM NaCl and 15 mM sodium citrate) containing 0.1% SDS (sodium dodecyl sulphate). The slides were scanned with a Generation III array scanner (Amersham Pharmacia Biotech). The scanned images were converted to digital data with ArrayVision Software 6.0 (Image Research Inc., St. Catharines, Ontario, Canada) and ImageQuant software (GE Healthcare Life Sciences, Piscataway, NJ, USA) was used for analysis. The raw data was normalized with nonparametric Lowess regression before and MIDAS analysis (registered by ShenZhen Chipscreen Biosciences, Ltd., China). Valid data with a ratio greater than 2 or less than 0.5 between the Cy3 and Cy5 signals were considered significant change in gene expression.

Semiquantitative reverse transcription-polymerase chain reaction (RT- PCR). RT-PCR was performed to confirm the differential expression results obtained by the microarray experiments. Total RNA was extracted and purified using the experimental protocols described above. One microgram of total RNA was reverse-transcribed into cDNA, and followed by PCR amplification using human gene-specific primers (Table 1), which were designed by PrimerExpress Software (Applied Biosystems, Foster City, CA, USA). The parameters for RT-PCR cycling were set as follows: reverse transcription reaction at 70 °C for 5 min and at 42 °C for 2 h; and PCR at 95 °C for 2 min; followed by a regime of cycles (Table 1): at 95 °C for 15 s, at 55 °C for 15 s, and at 72 °C for 30 s. The PCR products were separated on a 1.5% agarose gel electrophoresis that contained ethidium bromide and visualized with UV-induced fluorescence. The gel picture was analyzed using Image-J software (NIH, Bethesda, MD, USA). All signals were quantified and the final value was obtained by calculating the target gene/GAPDH ratio values. The fold of induction was calculated as the increase in value over that in the corresponding control sample. Each RT-PCR experiment was performed three times.

Table 1
PCR primers and conditions.

Primer	Sequences (5′–3′)	PCR cycles	GenBank No.
ARF1	GATGGCAAATCGGACCCT (sense) ACATGAGGTGGTGGTGGC (antisense)	25	W45572
GPS1	GACTGGCTGTTGGCTGGA (sense) AAGATGCTGGACGAGATGAAG (antisense)	26	AA521025
FLOT1	CTGGCAGGCGAGTTAGAAT (sense) AAGCAGCAGATTGAGGAGC (antisense)	26	AA488175
TGFB2	GGAGGGCAATAACATTAGCAG (sense) AGCAGAGTTCAGAGTCTTTCGTT (antisense)	25	AA233738
TUBB	AGCCGGGCATGAAGAAGT (sense) TCGGTCCTGGATGTGGTG (antisense)	23	AA427899
MAPK1	GCCAGAGCCTGTTCTACTTC (sense) CTGGACCTCAAGCCTTCC (antisense)	23	W45690
MAP2K2	GGAACTTGACGAGCAGCAGA (sense) TCACGAGGATGTTGGAGGG (antisense)	25	AA425826
BAX	GGATGCGTCCACCAAGAA (sense) CACCACTGTGACCTGCTCC (antisense)	30	AW072826
GAPDH	ATGCCATCACTGCCACCC (sense) GCCTGCTTCACCACCTTCTT (antisense)	18	BC083511

ARF1, ADP-ribosylation factor 1; GPS1, G protein pathway suppressor 1; FLOT1, flotillin 1; TGFB2, transforming growth factor-β2; TUBB, tubulin β-polypeptide; MAPK1, mitogen-activated protein kinase 1; MAP2K2, mitogen-activated protein kinase kinase 2; BAX, BCL2-associated X protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Data analysis. In order to qualify as a LIPUS-stimulated gene at a given time point, the gene should meet the following criteria: in each triplicate experiment the ratio between the LIPUS-treated sample signal and its untreated sample signal should be greater or less than 2-fold. The average ratio was then derived from the three different microarrays.

Results and discussion

After sorting through the analysis of the 7488 genes, it was apparent that a total of 165 genes showed at least a 2-fold change in expression after LIPUS treatment. To further validate the results, we used semiquantitative RT-PCR to examine the effect of LIPUS on representative genes. Seven genes from the 4-h LIPUS-regulated ones and eight genes from 24-h LIPUS-regulated ones were selected as targets for RT-PCR. Seven genes were regulated at both time points, except that tubulin-β (TUBB) was only regulated at 24 h. Microarray analysis revealed that only one gene did not achieve consistent change and at mRNA level RT-PCR showed GPS1 (1.2-fold) at 4 h (Table 2). Around 90% of the genes, i.e.,14 out of 15 genes from the 4- and 24-h treatments, were upregulated more than 2-fold by LIPUS.

Table 2
Alteration of target gene expression in SaOS-2 cells after LIPUS treatment detected by microarray and RT-PCR analysis.

Target gene	Ratio (microarray analysis)		Ratio (RT-PCR analysis)	
	4 h	24 h	4 h	24 h
MAPK1	3.0	3.1	2.4	2.2
MAP2K2	2.7	2.7	2.0	2.1
BAX	2.5	2.7	2.1	2.2
ARF1	3.9	4.3	6.2	3.9
GPS1	4.7	4.4	1.2[a]	2.6
TGFB2	1.3	2.8	1.3	3.3
TUBB	3.7	4.1	4.9	3.8
FLOT1	4.3	5.6	2.7	5.8

Ratio: target gene expression in LIPUS-treated cells/non-treated cells.
[a] Only GPS1 gene did not achieve the consistent change in mRNA level by RT-PCR detection at 4 h upon LIPUS treatment compared with by microarray analysis.

Our key findings were that among the 165 genes, about 30 were LIPUS-regulated genes that were reported previously related to the underlying molecular mechanism of LIPUS. Over 100 genes were newly identified and belonged to more than 10 protein families based on their functions and involvement in the related signal transduction pathways (Table 3). A novel finding we believed was the up-regulated apoptotic gene, a key gene involved in acceleration of tissue remodeling (Table 3). Below were several important features about LIPUS signaling emerged from our evaluations and discussed accordingly.

Integrins and cytoskeleton genes

The microarray results of this study proved 2 integrins and 10 cytoskeleton genes or related genes (Table 3) responsive to LIPUS.

There was growing evidence that integrins were promising candidates for sensing extracellular matrix-derived mechanical stimuli and converting them into biochemical signals [10]. Integrins acted as mechanotransducers in response to shear stress [11], mechanical stretch [12], cycled magnetic fields [13], and transmitted acoustic pulsed energy [14] into intracellular biochemical signals inducing cell proliferation. Our findings further demonstrated that the integrin receptors on osteoblasts served as

Table 3
Key-regulated genes in SaOS-2 cells measured 4 and 24 h after LIPUS treatment.

No	GenBank No.	Gene function	Gene tag	Ratio change	
				4 h	24 h
Integrins and cytoskeleton genes					
1	AA424695	Integrin, α-3 (antigen CD49C, α3 subunit of VLA-3 receptor)	ITGA3	2.2	
2	AA445992	Integrin, β-like 1 (with EGF-like repeat domains)	ITGBL1	2.0	3.3
3	AI929773	Actinin, α2	ACTN2	3.1	3.7
4	AA427899	Tubulin, β-polypeptide	TUBB	3.7	4.1
5	AI973179	Smoothelin	SMTN	2.0	2.1
6	H08564	Transgelin 2	TAGLN2		2.6
7	AA598517	Keratin 8	KRT8	2.0	
8	AA464250	Keratin 19	KRT19	3.8	3.9
9	AI457164	Kinesin family member 1C	KIF1C	2.1	
10	AA436460	Kinesin family member C3	KIFC3		3.6
11	AA130870	Microtubule-associated protein 4	MAP4	2.0	
12	AA448400	Plectin 1, intermediate filament binding protein, 500 kDa	PLEC1	2.4	2.3
Transforming growth factor-beta (TGF-β) family					
13	R36467	Transforming growth factor-β1	TGFB1		2.1
14	AA233738	Transforming growth factor-β2	TGFB2		2.8
15	AA664389	Transforming growth factor-β-stimulated protein TSC-22	TSC22	−2.3	
Insulin-like growth factor (IGF) family					
16	T62547	Insulin-like growth factor 2 receptor	IGF2R		2.9
17	AA478724	Insulin-like growth factor binding protein 6	IGFBP6		2.2
Mitogen-activated protein kinase (MAPK) pathway					
18	W45690	Mitogen-activated protein kinase 1	MAPK1	3.0	3.1
19	AA425826	Mitogen-activated protein kinase kinase 2	MAP2K2	2.7	2.7
20	AA455056	Mitogen-activated protein kinase-activated protein kinase 2	MAPKAPK2		2.4
21	AA281945	MAP-kinase-activating death domain	MADD		2.2
Adenosine 5′-triphosphate (ATP)-related genes					
22	AA282230	Proteasome (prosome, macropain) 26S subunit, ATPase, 3	PSMC3		2.0
23	AA504809	arsA (bacterial) arsenite transporter, ATP-binding, homolog 1	ASNA1		2.8
24	W45572	ADP-ribosylation factor 1	ARF1	3.9	4.3
Guanine nucleotide binding protein (G proteins)					
25	AA521025	G protein pathway suppressor 1	GPS1	4.7	4.4
26	H49592	Guanine nucleotide-binding protein (G protein), α-activating activity polypeptide, olfactory type	GNAL		3.1
27	AA485734	Ran-GTPase-activating protein 1	RANGAP1	2.1	
28	AA151214	Ras-GTPase-activating protein SH3 domain-binding protein 2	KIAA0660		2.4
29	H07878	G protein-coupled receptor 19	GPR19	−2.2	
30	AA489523	Eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	EEF1D	2.6	2.5
31	AA481277	Rho guanine nucleotide exchange factor (GEF) 1	ARHGEF1	2.1	2.2
Lysyl oxidase (LO) gene					
32	AA452916	Lysyl oxidase	LOX		2.7
Apoptosis-associated genes					
33	AW072826	BCL2-associated X protein	BAX	2.5	2.7
34	AA460685	Baculoviral IAP repeat-containing 5 (survivin)	BIRC5	2.0	
35	T55353	TNF receptor-associated factor 2	TRAF2	2.2	
36	AI347622	Tumor necrosis factor (ligand) superfamily, member 7	TNFSF7		2.3

mechanotransducers to transmit acoustic pulsed energy into intracellular biochemical signals through cytoskeleton systems. Integrin-associated signaling pathways included an increase in tyrosine phosphorylation of several signaling proteins, activation of serine/threonine kinases, and alterations in cellular phospholipid and calcium levels [15]. These events were associated with the formation of focal adhesions, which acted as a bridge to link integrin cytoplasmic domains to the cytoskeleton and activate integrin-associated signaling pathways, such as the MAPK pathway [16] and the Rho pathway [17].

Transforming growth factor-beta (TGF-β) family

In our study, LIPUS treatment resulted in elevated transient expression of TGF-β1, and TGF-β2 at 24 h, and decreased expression of TGF-β-stimulated clone-22 (TSC-22) at 4 h (Table 3).

Once released from the matrix and activated, TGF-β was reported to inhibit osteoclast activity, osteoblast proliferation, and stimulate the production of bone extracellular matrix (ECM) proteins [18]. TSC-22, a member of a transcription factor family, was shown to be induced in a variety of cell lines by many different factors, including TGF-β [19]. Not only TGF-β was demonstrated to be a pivotal factor in bone development and tissue repair, but that it also served as a primary candidate for a cell regulator in response to physical stimuli, such as the power frequency field [20], fluid-shear stress [21], and LIPUS [6]. The mRNA expression of TSC-22 in rat bone marrow-derived stromal cells was regulated by LIPUS [22]. Therefore, LIPUS might execute its role at least partially by modifying the signaling pathway of TGF-β family members.

Insulin-like growth factor (IGF) family

The researchers demonstrated that osteoblasts at various differentiation stages responded to ultrasound by transiently upregulating message levels of IGF-I after LIPUS stimulation [4,5]. We further found that the expression level of IGF-2 receptor and insulin-like growth factor binding protein 6 (IGFBP6) were higher in LIPUS-treated SaOS-2 cells compared with the non-treated control cells at 24 h (Table 3). Sant'Anna et al reported increased IGF-1 receptor mRNA expression in rat bone marrow stromal cells 3 days after LIPUS stimulation [7]. Therefore, the IGF pathway may be an important mechanism for cells to respond to LIPUS stimulation.

The growth potentiating effects of the IGF-1 and IGF-2 were reported to be modulated by a family of six insulin-like growth factor-binding proteins (IGFBPs) [23]. These findings and those of others implied that the most pronounced effect of LIPUS was on the expression of the receptor genes, rather than on the growth factor genes themselves.

Mitogen-activated protein kinase (MAPK) pathway

Our microarray experiments revealed that LIPUS induced the activation of four MAPK cascades (Table 3). Recently, it was suggested that MAPK signaling pathways might initiate downstream of the BMP receptor complex [24]. LIPUS activated ERK1/2 by a ROCK-dependent mechanism (ERK: extracellular signal-regulated kinase; ROCK: Rho-associated coiled-coil-containing protein kinase), which was required for LIPUS-induced cell proliferation [14]. To our knowledge, the mRNA expression of these four members involving MAPK pathway stimulated by LIPUS was our novel finding and reported herewith for the first time. In our study, we assumed that the beneficial effects of LIPUS on osteoblasts would partially be determined by the activation of MAPK pathway.

Adenosine 5′-triphosphate (ATP)-related genes

The present microarray study showed that after exposure to LIPUS, the expression of three genes related to ATP increased significantly (Table 3). Recent data suggested that ATP was an important extracellular signaling molecule in the bone microenvironment [25]. ATP was released constitutively from healthy osteoblasts [26] and this process was enhanced after mechanical stimulation from fluid-shear force [27]. It was suggested that nucleotides played an important role in bone mechanotransduction, where detection of mechanical deformation by skeletal cells resulted in bone remodeling [28]. These novel findings reported in our studies and of others suggested that osteoblasts might respond to LIPUS stimulation by increasing the release of ATP and activating ATP-related genes.

Guanine nucleotide binding protein (G proteins)

In the present microarray experiment, transient expression level of some genes involved G protein pathway that was regulated by LIPUS treatment (Table 3). As LIPUS stimulated the members of G protein, it would be logic to assume that promotion of osteogenic activity by LIPUS was linked to the activation of G proteins. G proteins were a family of GTP-binding proteins that mediated steady/transient fluid shear stress-stimulated nitric oxide production [29,30] and flow-induced prostaglandin E_2 production by osteoblasts [31]. Chen et al. reported that LIPUS treatment rapidly activated membrane-bound Gαi proteins and cytosolic ERK [32]. These reports implied that G proteins mediated the transduction of mechanical stimulation into intracellular signals that subsequently regulated cell proliferation and differentiation.

Lysyl oxidase (LO) gene

In our study, we observed upregulation of LO mRNA in the exposed cultures as compared with the controls 24 h after LIPUS treatment (Table 3). LO was reported to play a role in regulating the total number of cross-links. The mRNA expression and activity of LO, as well as the total amount of cross-links, were also found to be significantly higher in the osteoblastic cells exposed to 30 mW/cm^2 compared to the control cells [33]. Candidate upregulators of LO gene expression included TGF-β, connective tissue growth factor (CTGF), and IGF-I [34,35]. In our experiment, LIPUS intervention increased TGF-β1, TGF-β2, IGFBP6, and IGF2R mRNA expression when compared with the controls 24 h after exposure. However, the precise mechanism underlying the quantitative changes in cross-link formation after LIPUS stimulation remained for further investigations.

Genes of other categories

Apart from the upregulated genes that we highlighted for this microarray study, expression of some important genes in SaOS-2 cells were also regulated by LIPUS. These included genes associated with cell apoptosis, such as BCL2-associated X protein (BAX) at both 4 and 24 h (Table 3). The LIPUS effect on cellular apoptosis has not been specified in the past. Our findings may help to further understand the mechanism of LIPUS effects on activation of apoptotic genes and osteogenesis in acceleration of the healing tissue remodeling. Wiren et al. also suggested that an enhanced apoptosis in osteoblasts could be associated with the anabolic stimulation of new bone growth [36].

Since the purpose of the current microarray study was to determine whether the mechanical signals from LIPUS could stimulate osteoblasts by means of gene expression, the precise biological roles of these genes in vivo were not included into the scope of this

investigation. Further studies are needed to assess the downstream pathways and functions of these genes, i.e. LIPUS-stimulated proteins translated by these genes with proteome study.

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References

[1] L. Qin, Fracture healing—the effect of low intensity pulsed ultrasound (LIPUS), Orthop. Trauma Direct. 3 (2005) 17–23.
[2] H. Lu, L. Qin, P. Fok, W. Cheung, K. Lee, X. Guo, W. Wong, K. Leung, Low-intensity pulsed ultrasound accelerates bone–tendon junction healing: a partial patellectomy model in rabbits, Am. J. Sports Med. 34 (2006) 1287–1296.
[3] Y. Azuma, M. Ito, Y. Harada, H. Takagi, T. Ohta, S. Jingushi, Low-intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on the various cellular reactions in the fracture callus, J. Bone Miner. Res. 16 (2001) 671–680.
[4] K. Naruse, Y. Mikuni-Takagaki, Y. Azuma, M. Ito, T. Oota, K. Kameyama, M. Itoman, Anabolic response of mouse bone-marrow-derived stromal cell clone ST2 cells to low-intensity pulsed ultrasound, Biochem. Biophys. Res. Commun. 268 (2000) 216–220.
[5] K. Naruse, A. Miyauchi, M. Itoman, Y. Mikuni-Takagaki, Distinct anabolic response of osteoblast to low-intensity pulsed ultrasound, J. Bone Miner. Res. 18 (2003) 360–369.
[6] S. Mukai, H. Ito, Y. Nakagawa, H. Akiyama, M. Miyamoto, T. Nakamura, Transforming growth factor-beta1 mediates the effects of low-intensity pulsed ultrasound in chondrocytes, Ultrasound Med. Biol. 31 (2005) 1713–1721.
[7] E.F. Sant'Anna, R.M. Leven, A.S. Virdi, D.R. Sumner, Effect of low intensity pulsed ultrasound and BMP-2 on rat bone marrow stromal cell gene expression, J. Orthop. Res. 23 (2005) 646–652.
[8] M.J. Hayton, J.P. Dillon, D. Glynn, J.M. Curran, J.A. Gallagher, K.A. Buckley, Involvement of adenosine 5′-triphosphate in ultrasound-induced fracture repair, Ultrasound Med. Biol. 31 (2005) 1131–1138.
[9] S.M. McCormick, V. Saini, Y. Yazicioglu, Z.N. Demou, T.J. Royston, Interdependence of pulsed ultrasound and shear stress effects on cell morphology and gene expression, Ann. Biomed. Eng. 34 (2006) 436–445.
[10] D.E. Ingber, Mechanosensation through integrins: cells act locally but think globally, Proc. Natl. Acad. Sci. USA 100 (2003) 1472–1474.
[11] F.A. Weyts, Y.S. Li, J. van Leeuwen, H. Weinans, S. Chien, ERK activation and alpha v beta 3 integrin signaling through Shc recruitment in response to mechanical stimulation in human osteoblasts, J. Cell. Biochem. 87 (2002) 85–92.
[12] S. Kippenberger, A. Bernd, S. Loitsch, M. Guschel, J. Muller, J. Bereiter-Hahn, R. Kaufmann, Signaling of mechanical stretch in human keratinocytes via MAP kinases, J. Invest. Dermatol. 114 (2000) 408–412.
[13] C. Schmidt, H. Pommerenke, F. Durr, B. Nebe, J. Rychly, Mechanical stressing of integrin receptors induces enhanced tyrosine phosphorylation of cytoskeletally anchored proteins, J. Biol. Chem. 273 (1998) 5081–5085.
[14] S. Zhou, A. Schmelz, T. Seufferlein, Y. Li, J. Zhao, M.G. Bachem, Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts, J. Biol. Chem. 279 (2004) 54463–54469.
[15] F.G. Giancotti, E. Ruoslahti, Integrin signaling, Science 285 (1999) 1028–1032.
[16] D.D. Schlaepfer, T. Hunter, Integrin signalling and tyrosine phosphorylation: just the FAKs?, Trends Cell Biol 8 (1998) 151–157.
[17] D. Riveline, E. Zamir, N.Q. Balaban, U.S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, A.D. Bershadsky, Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism, J. Cell Biol. 153 (2001) 1175–1186.
[18] L.F. Bonewald, Regulation and regulatory activities of transforming growth factor beta, Crit. Rev. Eukaryot. Gene Expr. 9 (1999) 33–44.
[19] S. Ohta, Y. Shimekake, K. Nagata, Molecular cloning and characterization of a transcription factor for the C-type natriuretic peptide gene promoter, Eur. J. Biochem. 242 (1996) 460–466.
[20] R.K. Aaron, D.M. Ciombor, H. Keeping, S. Wang, A. Capuano, C. Polk, Power frequency fields promote cell differentiation coincident with an increase in transforming growth factor-beta(1) expression, Bioelectromagnetics 20 (1999) 453–458.
[21] P. Malaviya, R.M. Nerem, Fluid-induced shear stress stimulates chondrocyte proliferation partially mediated via TGF-beta1, Tissue Eng. 8 (2002) 581–590.
[22] K. Sena, R.M. Leven, K. Mazhar, D.R. Sumner, A.S. Virdi, Early gene response to low-intensity pulsed ultrasound in rat osteoblastic cells, Ultrasound Med. Biol. 31 (2005) 703–708.
[23] S. Mohan, Y. Nakao, Y. Honda, E. Landale, U. Leser, C. Dony, K. Lang, D.J. Baylink, Studies on the mechanisms by which insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) and IGFBP-5 modulate IGF actions in bone cells, J. Biol. Chem. 270 (1995) 20424–20431.
[24] A. Suzuki, J. Guicheux, G. Palmer, Y. Miura, Y. Oiso, J.P. Bonjour, J. Caverzasio, Evidence for a role of p38 MAP kinase in expression of alkaline phosphatase during osteoblastic cell differentiation, Bone 30 (2002) 91–98.
[25] A. Hoebertz, T.R. Arnett, G. Burnstock, Regulation of bone resorption and formation by purines and pyrimidines, Trends Pharmacol. Sci. 24 (2003) 290–297.
[26] K.A. Buckley, S.L. Golding, J.M. Rice, J.P. Dillon, J.A. Gallagher, Release and interconversion of P2 receptor agonists by human osteoblast-like cells, FASEB J. 17 (2003) 1401–1410.
[27] M. Romanello, B. Pani, M. Bicego, P. D'Andrea, Mechanically induced ATP release from human osteoblastic cells, Biochem. Biophys. Res. Commun. 289 (2001) 1275–1281.
[28] N.R. Jorgensen, S.T. Geist, R. Civitelli, T.H. Steinberg, ATP- and gap junction-dependent intercellular calcium signaling in osteoblastic cells, J. Cell Biol. 139 (1997) 497–506.
[29] T.N. McAllister, J.A. Frangos, Steady and transient fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways, J. Bone Miner. Res. 14 (1999) 930–936.
[30] F. Hara, K. Fukuda, S. Asada, M. Matsukawa, C. Hamanishi, Cyclic tensile stretch inhibition of nitric oxide release from osteoblast-like cells is both G protein and actin-dependent, J. Orthop. Res. 19 (2001) 126–131.
[31] K.M. Reich, T.N. McAllister, S. Gudi, J.A. Frangos, Activation of G proteins mediates flow-induced prostaglandin E2 production in osteoblasts, Endocrinology 138 (1997) 1014–1018.
[32] Y.J. Chen, C.J. Wang, K.D. Yang, P.R. Chang, H.C. Huang, Y.T. Huang, M.C. Farach-Carson, Pertussis toxin-sensitive Galphai protein and ERK-dependent pathways mediate ultrasound promotion of osteogenic transcription in human osteoblasts, FEBS Lett. 554 (2003) 154–158.
[33] M. Saito, S. Soshi, T. Tanaka, K. Fujii, Intensity-related differences in collagen post-translational modification in MC3T3-E1 osteoblasts after exposure to low- and high-intensity pulsed ultrasound, Bone 35 (2004) 644–655.
[34] H.H. Hong, M.I. Uzel, C. Duan, M.C. Sheff, P.C. Trackman, Regulation of lysyl oxidase, collagen, and connective tissue growth factor by TGF-beta1 and detection in human gingiva, Lab. Invest. 79 (1999) 1655–1667.
[35] K. Reiser, P. Summers, J.F. Medrano, R. Rucker, J. Last, R. McDonald, Effects of elevated circulating IGF-1 on the extracellular matrix in "high-growth" C57BL/6J mice, Am. J. Physiol. 271 (1996) R696–R703.
[36] K.M. Wiren, A.R. Toombs, A.A. Semirale, X. Zhang, Osteoblast and osteocyte apoptosis associated with androgen action in bone: requirement of increased Bax/Bcl-2 ratio, Bone 38 (2006) 637–651.