Effects of Pulsed Electromagnetic Fields on Human Osteoblastlike Cells (MG-63)
A Pilot Study

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Abstract

Background Although pulsed electromagnetic fields (PEMFs) are used to treat delayed unions and nonunions, their mechanisms of action are not completely clear. However, PEMFs are known to affect the expression of certain genes.

Questions/purposes We asked (1) whether PEMFs affect gene expression in human osteoblastlike cells (MG63) in vitro, and (2) whether and to what extent stimulation by PEMFs induce cell proliferation and differentiation in MG-63 cultures.

Methods We cultured two groups of MG63 cells. One group was treated with PEMFs for 18 hours whereas the second was maintained in the same culture condition without PEMFs (control). Gene expression was evaluated throughout cDNA microarray analysis containing 19,000 genes spanning a substantial fraction of the human genome.

Results PEMFs induced the upregulation of important genes related to bone formation (HOXA10, AKT1), genes at the transductional level (CALM1, P2RX7), genes for cytoskeletal components (FN1, VCL), and collagenous (COL1A2) and noncollagenous (SPARC) matrix components. However, PEMF induced downregulation of genes related to the degradation of extracellular matrix (MMP-11, DUSP4).

Conclusions and Clinical Relevance PEMFs appear to induce cell proliferation and differentiation. Furthermore, PEMFs promote extracellular matrix production and mineralization while decreasing matrix degradation and absorption. Our data suggest specific mechanisms of the observed clinical effect of PEMFs, and thus specific approaches for use in regenerative medicine.

Introduction

PEMFs have been used for many years [44]. They reportedly are effective for treating nonunions [1, 7, 10], delayed unions [1, 42, 44], osteotomies [32], avascular necrosis of the femoral head [5, 34], bone grafts [11], and spinal fusion [36]. Although the therapeutic properties of PEMFs are well known, the sequence of events by which electromagnetic stimulation can bring about its desirable effects on bone healing is not completely understood. PEMFs modify some important physiologic parameters of cells, such as proliferation, transduction, transcription, synthesis, and secretion of growth factors [24]. PEMFs induce cell proliferation in mitogen-stimulated lymphocytes [10] and improve IL-2 receptor expression and IL-2 use in lymphocytes from aged donors, which are characterized by
intracellular release of Ca^{2+} [45]. PEMFs determine signal transduction by means of osteoblasts and chondrocytes cultured in vitro [18, 20, 38, 44, 45]. PEMFs induce a dose-dependent increase in bone [2] and cartilage differentiation [2–4, 33], and upregulation of mRNA expression of extracellular matrix molecules, proteoglycan, and Type II collagen [3]. The acceleration of chondrogenic differentiation is associated with increased expression of TGF-β1 mRNA and protein [4], suggesting the stimulation of TGF-β1 may be a mechanism through which PEMFs affect complex tissue behavior such as cell differentiation and through which the effects of PEMFs may be amplified [4]. PEMFs also are postulated to act at a membrane level influencing signal transduction of several hormones or growth factors such as parathyroid hormone, IGF 2, and adenosine A2a, producing the amplification of their transmembrane receptors [1, 19, 21, 23, 31, 46]. Studies of single genes using RT-PCR suggest activation of osteocalcin, osteopontin, and TGF-β transcription during osteogenesis [22] and inhibition of cyclooxygenase 2 in synovial fibroblasts stimulated with TNFα or lipopolysaccharide [21]. A wide analysis of gene expression in cells exposed to PEMFs has not been performed: most studies focus on a few aspects of cell activities or they have been performed using different types of signals in different experimental conditions.

We therefore asked (1) whether PEMFs affected a wide array of genes in human osteoblastlike cells (MG63), and (2) whether and to what extent PEMFs induce proliferation and differentiation of osteoblasts.

Materials and Methods

We treated osteoblastlike cell cultures (MG-63) with PEMFs for 18 hours, and maintained similar nontreated controls. Gene expression of both groups therefore was evaluated with cDNA microarray analysis, containing 19,000 genes spanning a substantial fraction of the human genome. All experiments were performed in triplicate in the same culture conditions for control and treated cells.

Osteoblastlike cells (MG63) were grown in sterile Falcon wells (Becton & Dickinson, Franklin Lakes, NJ) containing Eagle’s minimum essential medium supplemented with 10% fetal calf serum (Sigma-Aldrich, St Louis, MO) and antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL; Sigma-Aldrich). Cultures were maintained in a 5% CO₂ humidified atmosphere at 37°C. For the assay, cells were collected and seeded at a density of 1 × 10^5 cells/mL in two multiwells (one for the control and one for the treated). Each multiwell was comprised of six wells, 9-cm², in which 3-mL of complete medium was added.

After 24 hours, cells were exposed to PEMFs for 18 hours using a PEMF generator system (Igea, Carpi, Italy). The PEMF used in this study is used clinically to treat nonunions or delayed unions and avascular necrosis of the femoral head [32–34]. The solenoids were powered using a Biostim pulse generator (Igea), a PEMF generator. The electromagnetic bioreactor applied to the cells has the following characteristics: intensity of the magnetic field, 2 ± 0.2 mT; amplitude of the induced electric tension, 5 ± 1 mV; signal frequency, 75 ± 2 Hz; and pulse duration, 1.3 ms. The stimulated multiwell was placed parallel between the two solenoids of the PEMF generator. The solenoids were placed at a distance of 10 cm and the multiwell was located on an acrylic support exactly at the center of the two solenoids. Control cultures were placed in the same incubator; nevertheless, the presence of the electromagnetic field was checked and its value was less than 0.05 mT. This value was ineffective in previous studies [38–46]. After 18 hours, when cultures were subconfluent, cells were processed for RNA extraction.

For DNA microarray screening and analysis, we used the same protocol as described previously [12–16]. Briefly, RNA was extracted from cells by using RNAzol. Ten micrograms of total RNA was used for each sample. cDNA was synthesized by using Superscript II (Life Technologies, Invitrogen, Milano, Italy) and amino-allyl dUTP (Sigma-Aldrich). Monoreactive Cy3 and Cy5 esters (Amersham Pharmacia, Little Chalfont, UK) were used for indirect cDNA labeling. RNA extracted from untreated cells was labeled with Cy3 and used as control against the Cy5-labeled treated (PG) cDNA in the first experiment and then switched. For 20 K human DNA microarrays slides (MWG Biotech AG, Ebersberg, Germany), 100 μL of the sample and control cDNAs in DIG Easy hybridization solution (Roche, Basel, Switzerland) were used in a sandwich hybridization of the two slides, constituting the 20 K set at 37°C overnight. Washing was performed three times for 10 minutes with 1 × saline sodium citrate (SSC) and 0.1% sodium dodecyl sulfate at 42°C and three times for 5 minutes with 0.1 × SSC at room temperature. Slides were dried by centrifugation for 2 minutes at 2000 rpm. Hybridized arrays were scanned with a GenePix 4000 scanner (Axon Instruments) at variable photomultiplier tube (PMT) voltage to obtain maximal signal intensities with less than 1% probe saturation.

The Foreground Median intensity for Cy3 and Cy5, Background Median intensity for Cy3 and Cy5, spot size data were imported into BRB-ArrayTools software [43] using the Import wizard function. Global normalization was used to median center the log-ratios on each array r to
adjust for differences in labeling intensities of the Cy3 and Cy5 dyes.

The normalized Log ratios also were imported to Significance Analysis of Microarray (SAM) [48] software to identify differentially expressed genes. SAM assigns a score to each gene on the basis of a change in gene expression relative to the standard deviation of repeated measurements. For genes with scores greater than an adjustable threshold, SAM uses permutations of the repeated measurements to estimate the percentage of genes identified by chance—the false discovery rate (FDR). Analysis parameters (Delta) were set to result in zero FDR.

**Results**

PEMF affected gene expression in MG-63 osteoblastlike cells (Fig. 1). The genes differentially expressed in cells treated with PEMFs were either upregulated (268 genes) (Table 1) or downregulated (277 genes) (Table 2). PEMF induced osteoblast proliferation and differentiation and regulated genes involved in bone formation in the direction of an enhancement of osteogenesis (Tables 3, 4).

In particular, PEMFs induced upregulation of several genes at the transcriptional level like STAT3, homeobox A10 (HOXA10), and V-akt murine thymoma viral oncogene homolog 1 (AKT1). Some genes acting at the transductional level also are upregulated including calmodulin (CALM1), activator protein 1 (AP-1), Nuclear factor kappaB (NF-KB), cAMP response element binding (CREB), and P2RX7 (Table 3). Several interesting overexpressed genes are components of cytoskeleton and involved in cell adhesion (Table 3). Examples are fibronectin (FN1) and vinculin (VCL). PEMF also increased the expression of genes encoding for collagenous and noncollagenous extracellular matrix proteins including collagen Type 1x2 (COL1A2), osteonectin (SPARC), and metallopeptidase inhibitor 1 (TIMP1) (Table 3).

Some genes downregulated by PEMFs are related to degradation of extracellular matrix (ECM) (Table 4), specifically, matrix metallopeptidase 11 (MMP11), or stromelysin 3 and dual specificity phosphatase 4 (DUSP4).

**Discussion**

The improvement of osteogenesis is important because of the wide clinical applications it may have. PEMFs reportedly restart osteogenesis in disorders in which it has stopped [34] and in disorders in which osteogenesis needs to be enhanced [32]. Although considerable basic and clinical research on PEMFs has been reported, their mechanism of action is not completely clear. Moreover, studies in the existing literature have so far focused only on a few aspects of cell activities [9, 10, 46], or they have been performed by using different types of signals in different experimental conditions [1, 9, 22, 23]. To address these limitations in the literature, we asked (1) whether PEMFs affected a wide array of genes in human osteoblastlike cells (MG63), and (2) whether and to what extent PEMFs induce proliferation and differentiation of osteoblasts.

We acknowledge several limitations. First, the experiment was performed using a human osteosarcoma cell line (MG63), whereas the use of a primary human osteoblast cell culture might better replicate what happens in humans in vivo. We chose the MG63 cell line because these cells
Table 1. Upregulated genes

| GenBank Name | Name | Symbol | Cytoband  | Score (d)* |
|--------------|------|--------|-----------|------------|
| W19447      | DEAD (Asp-Glu-Ala-Asp) box polypeptide 51 | DDX51  | 12q24.3   | 45.15      |
| BM908669    | Glyceraldehyde-3-phosphate dehydrogenase | GAPDH  | 12p13     | 42.40      |
| W33064      | Tubulin, alpha 4a | TUBA4A | 2q35      | 40.89      |
| BI258438    | Cofilin 1 (nonmuscle) | CFL1    | 11q13     | 39.61      |
| H80610      | Hypothetical protein LOC729176 | LOC729176 | 6q24.3    | 35.42      |
| R23641      | Vacuolar protein sorting 13 homolog A | VPS13A | 9q21      | 32.28      |
| BM006748    | Enolase 1 (alpha) | ENO1    | 1p36.3-3p36.2 | 31.99    |
| W44824      | Major histocompatibility complex, class I, E | HLA-E | 6p21.3    | 31.04      |
| BG547115    | Ferritin, heavy polypeptide 1 | FTH1   | 11q13     | 30.55      |
| BG288116    | Integrin, alpha 2 | ITGA2  | 5q23-q31  | 27.14      |
| BQ014343    | Family with sequence similarity 62 | FAM62B | 7q36.3    | 26.05      |
| R89805      | ELOVL family member 7 | ELOVL7  | 5q12.1    | 25.81      |
| BH108591    | Ribosomal protein S5 | RPS5   | 19q13.4   | 25.71      |
| H61302      | Hexose-6-phosphate dehydrogenase | H6PD    | 1p36      | 25.10      |
| AA151568    | Testis enhanced gene transcript (BAX inhibitor 1) | TEGT   | 12q12-q13 | 24.95      |
| H30300      | Small nuclear ribonucleoprotein polypeptide N | SNRPN  | 15q11.2   | 24.75      |
| N72456      | Similar to RIKEN cDNA A730055C05 gene | LOC388335 | 17p13.1   | 24.38      |
| H25618      | Chromatin modifying protein 5 | CHMP5  | 9q13.3    | 24.12      |
| AA059376    | Similar to phosphodiesterase 4D interacting protein isoform 2 | LOC653513 | 1q21.1    | 24.01      |
| W30787      | DnaJ (Hsp40) homolog, subfamily C, member 15 | DNAJC15 | 13q14.1   | 24.00      |
| BM801770    | Solute carrier family 35, member E3 | SLC35E3 | 12q15     | 23.64      |
| AA099240    | NIPA-like domain containing 3 | NPA1    | 10q11.2   | 23.55      |
| W00391      | Solute carrier family 11 member 2 | SLC11A2 | 12q13    | 23.36      |
| H12528      | Annexin A5 | ANXA5  | 4q26-q284q28-q29 | 23.13     |
| N54759      | Prenylcysteine oxidase 1 | PCYOX1 | 2p13.3    | 23.08      |
| T98646      | ST3 beta-galactoside alpha-2,3-sialyltransferase 2 | ST3GA2  | 1q32      | 22.82      |
| AA029517    | KCNQ1 overlapping transcript 1 | KCNQ1OT1 | 1p15      | 22.75      |
| W47664      | NADP(H) dehydrogenase, quinone 1 | NQ01    | 1q12.2    | 22.73      |
| W02597      | PMS1 postmeiotic segregation increased 1 | PMS1   | 2q31-q332q31 | 22.61     |
| R11416      | Seryl-tRNA synthetase | SARS   | 1p13.3-3p13.1 | 22.55     |
| H67332      | GTP binding protein 1 | GTPBP1  | 2q13.1    | 22.43      |
| H86020      | NADH dehydrogenase | NDUF5B | 3q26.3    | 22.63      |
| AA031564    | Chromosome 1 open reading frame 212 | C1orf212 | 1p34.3    | 21.90      |
| W67485      | Zinc finger protein 136 | ZNF136   | 9p13.2-p13.12 | 21.68     |
| W32906      | Zinc finger protein 702 | ZNF702  | 1q13.4    | 21.49      |
| BI492783    | Zinc finger protein 207 | ZNF207  | 1q11.2    | 21.43      |
| BE278092    | Ribosomal protein L10 | RPL10   | Xq28      | 21.42      |
| N90960      | Par-6 partitioning defective 6 homolog beta | PAR6B | 20q13.13  | 21.32      |
| BG545169    | Ferritin, light polypeptide | FTLL    | 19q13.3-4q13.4 | 21.29     |
| H75902      | Complement component (3b/4b) receptor 1 | CR1     | 1q32      | 21.15      |
| W31736      | NADH dehydrogenase (ubiquinone) flavoprotein 1, 5kDa | NDUVF1 | 11q13    | 20.96      |
| AA147868    | Casein kinase 1, gamma 3 | CSNK1G3 | 5q23      | 20.94      |
| R18627      | Amyloid beta precursor protein binding protein 2 | APPBP2 | 17q21-q23 | 20.81      |
| W38809      | Kelch-like 8 (Drosophilia) | KHLH8  | 4q22.1    | 20.76      |
| BM456402    | Hypothetical gene LOC96610 | LOC96610 | 22q11.22  | 20.70      |
| AA044942    | Eukaryotic translation initiation factor 4 gamma, 1 | EIF4G1 | 3q27-qter | 20.44      |
| BM041235    | Actin, alpha 2, smooth muscle, aorta | ACTA2   | 10q23.3   | 20.42      |
| AI690073    | Glutamate-cysteine ligase, catalytic subunit | GCLC   | 6p12      | 20.30      |
| GenBank      | Name                              | Symbol   | Cytoband   | Score (d)* |
|-------------|-----------------------------------|----------|------------|------------|
| AI734239    | Coiled-coil domain containing 120 | CCDC120  | Xp11.23    | 20.28      |
| N729222     | PDZ and LIM domain 5              | PDLIM5   | 4q22       | 20.23      |
| N50768      | Chromosome X open reading frame 57| CXorf57  | Xq22.3     | 20.05      |
| N76504      | Hypothetical protein LOC257407    | LOC257407| 2q37.1     | 19.69      |
| N45145      | Zinc finger, CCHC domain containing 4 | ZCCHC4   | 4p15.2     | 19.60      |
| BM922198    | Tubulin, beta 2C                 | TUBB2C   | 9q34       | 19.57      |
| H65175      | Solute carrier family 31 (copper transporters), member 1 | SLC31A1 | 9q31-q32   | 19.50      |
| H83172      | Cytochrome b5 domain containing 2 | CYB5D2   | 17p13.2    | 19.28      |
| N73208      | Zinc finger protein 207          | ZNF207   | 17q11.2    | 19.26      |
| H95413      | Hydroxy steroid (17-beta) dehydrogenase 7 | HSD17B7 | 1q23       | 19.21      |
| N72546      | Cathepsin S                      | CTSS     | 1q21       | 19.08      |
| BM705000    | Cold shock domain protein A       | CSDA     | 12p13.1    | 19.08      |
| W86495      | Coiled-helix-coiled-helix domain containing 7 | CHCHD7  | 8q12.1     | 19.07      |
| B1092679    | H19, imprinted maternally expressed untranslated mRNA | H19   | 11p15.5    | 18.99      |
| N54602      | Serine/threonine kinase 4         | STK4     | 20q11.2-q13.2 | 18.93     |
| H78769      | Interleukin-1 receptor-associated kinase 4 | IRAK4   | 12q12      | 18.88      |
| W35195      | Lethal giant larvae homolog 1 (Drosophilia) | LLGL1   | 17p11.2    | 18.88      |
| AA062617    | Myotubularin related protein 9    | MTMR9    | 8p23-p22   | 18.85      |
| BE315195    | Ribosomal protein L8              | RPL8     | 8q24.3     | 18.84      |
| BQ067508    | Glyceraldehyde-3-phosphate dehydrogenase | GAPDH   | 12p13      | 18.76      |
| R35530      | RAD23 homolog B (S cerevisiae)   | RAD23B   | 9q31.2     | 18.75      |
| H24644      | AlkB, alkylation repair homolog 5 (E coli) | ALKBH5  | 17p11.2    | 18.57      |
| BM010025    | Signal transducer and activator of transcription 3 | STAT3   | 17q21.31   | 18.53      |
| H08490      | Chloride channel 2               | CLCN2    | 3q27-q28   | 18.39      |
| H80175      | Radixin                          | RDX      | 11q23      | 18.27      |
| H46045      | Tripartite motif-containing 46    | TRIM46   | 1q22       | 18.18      |
| N25456      | Mutated in colorectal cancers    | MCC      | 5q11       | 18.07      |
| AA047157    | CD82 molecule                     | CD82     | 11p11.2    | 18.01      |
| AA044701    | ADAMTS-like 5                    | ADAMTS5  | 19p13.3    | 17.82      |
| BM477950    | Ribosomal protein L8             | RPL8     | 8q24.3     | 17.79      |
| AI587328    | Radical S-adenosyl methionine domain containing 2 | RSAD2   | 5p25.2     | 17.68      |
| W03282      | Dihydrofolate reductase          | DHFR     | 5q11.2-q13.2 | 17.62     |
| BQ072807    | Ribosomal protein L13a           | RPL13A   | 19q13.3    | 17.52      |
| H01638      | Coiled-coil domain containing 82  | CCDC82   | 11q21      | 17.48      |
| BG529617    | Ribosomal protein, large, P1     | RPLP1    | 15q22      | 17.45      |
| H63198      | RAB interacting factor           | RABIF    | 1q32-q41   | 17.34      |
| BG397205    | Proteasome (prosome, macropain) subunit, beta type, 4 | PSMB4B | 1q21       | 17.33      |
| W31052      | Nephronophthisis 3 (adolescent)  | NPHP3    | 3q22.1     | 17.26      |
| BM925268    | Chromosome 12 open reading frame 32 | C12orf32 | 12p13.33   | 17.25      |
| H83233      | Malate dehydrogenase 1, NAD (soluble) | MDH1   | 2p13.3     | 17.23      |
| W19108      | UBX domain containing 4          | UBXD4    | 2p23.3     | 17.22      |
| AAA04532    | Fusion (involved in t(12;16) in malignant liposarcoma) | FUS   | 16p11.2    | 17.21      |
| R50299      | SHANK-associated RH domain interactor | SHARPIN | 8q24.3     | 17.01      |
| R47837      | Zinc finger, RAN-binding domain containing 2 | ZRANB2  | 1p31       | 16.99      |
| H85307      | V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog | KRAS   | 12p12.1    | 16.89      |
| N49567      | Agmatine ureohydrolase (agmatinase) | AGMAT  | 1p36.21    | 16.88      |
| N57076      | KIAA1909 protein                 | KIAA1909 | 5p15.33    | 16.84      |
| BI116974    | Ribosomal protein L18            | RPL18    | 19q13      | 16.83      |
| GenBank | Name | Symbol | Cytoband | Score (d)* |
|---------|------|--------|----------|------------|
| N40643  | Chromosome 10 open reading frame 18 | C10orf18 | 10p15.1  | 16.74      |
| R82575  | KIAA1704 | KIAA1704 | 13q13-1q14 | 16.51      |
| B1196362 | Tubulin, alpha 1a | TUBA1A | 12q12-1q13.3 | 16.36      |
| AA132192 | Pleckstrin homology domain containing, family H member 2 | PLEKHH2 | 2p21 | 16.34      |
| N31221  | Hypothetical protein DKFZp667M2411 | DKFZp667M2411 | 17q11.2 | 16.31      |
| H45243  | GDNF-inducible zinc finger protein 1 | GZFI1 | 20p12.3-11.21 | 16.29      |
| T86807  | Serine/threonine kinase 19 | STK19 | 6p21.3 | 16.28      |
| AA057270 | Choline kinase alpha | CHKA | 11q13.2 | 16.20      |
| R66209  | Synaptosomal-associated protein, 29 kDa | SNAP29 | 22q11.21 | 16.13      |
| R92306  | DnaJ (Hsp40) homolog, subfamily C, member 19 | DNAJC19 | 3q26.33 | 16.13      |
| T80969  | Glycine-N-acyltransferase-like 1 | GLYATL1 | 11q12.1 | 16.12      |
| N42722  | Guanine nucleotide binding protein (G protein), gamma 12 | GNG12 | 1p31.3 | 16.03      |
| BM911128 | Secreted protein, acidic, cysteine-rich (osteonectin) | SPARC | 5q31.3-q32 | 15.99      |
| N34619  | Coagulation factor II (thrombin) receptor-like 2 | F2RL2 | 5q13 | 15.97      |
| AA039528 | C-Maf-inducing protein | CMIP | 16q23 | 15.76      |
| N53715  | Neural precursor cell expressed | NEDD8 | 14q12 | 15.75      |
| BQ055308 | Ribosomal protein L4 | RPL4 | 15q22 | 15.73      |
| N44567  | Torsin A interacting protein 1 | TOR1AIP1 | 1q24.2 | 15.63      |
| AA046698 | Selenoprotein 1 | SEL1 | 2p23.3 | 15.62      |
| T75376  | Notch homolog 2 (Drosophila) | NOTCH2 | 1p13-p11 | 15.49      |
| N46675  | Unkempt homolog (Drosophila) | UNK1 | 17q25.1 | 15.44      |
| R93756  | Calmodulin 1 (phosphorylase kinase, delta) | CALM1 | 14q24-q31 | 15.42      |
| R67177  | Adenylate cyclase 1 (brain) | ADCY1 | 7p13-p12 | 15.39      |
| W24597  | Deoxyribonuclease II, lysosomal | DNASE2 | 19p13.2 | 15.28      |
| H43825  | HLA-B associated transcript 2 | BAT2 | 6p21.3 | 15.26      |
| N20577  | Leucine rich repeat containing 57 | LRRC57 | 15q15.1 | 15.24      |
| BI598074 | Neurin, neurite outgrowth associated | NGRN | 15q26.1 | 15.23      |
| W79562  | Arginyltransferase 1 | ATE1 | 10q26.13 | 15.20      |
| R68004  | Poly(rC) binding protein 2 | PCBP2 | 12q13.12-q13.13 | 15.19      |
| AA040826 | Major histocompatibility complex, class I, C | HLA-C | 6p21.3 | 15.12      |
| H52744  | Abhydrolase domain containing 12 | ABHD12 | 20p11.21 | 15.11      |
| N78350  | RAN binding protein 1 | RANBP1 | 22q11.21 | 15.10      |
| BQ026918 | Collagen, type I, alpha 2 | COL1A2 | 7q22.1 | 15.05      |
| BG109286 | COX18 cytochrome c oxidase assembly homolog | COX18 | 4q13.3 | 15.02      |
| W47525  | Trans-golgi network protein 2 | TGDN2 | 2p11.2 | 15.02      |
| BI494911 | Nick-associated protein 5 | NAP5 | 2q21.2 | 14.89      |
| N94192  | Glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IID) | GNIS | 12q14 | 14.84      |
| N28281  | Zinc finger protein 552 | ZNF552 | 19q13.43 | 14.61      |
| R84726  | Adenosine A1 receptor | ADORA1 | 1q32.1 | 14.59      |
| W40304  | Apoptosis inhibitor 5 | API5 | 11p11.2 | 14.54      |
| W63760  | Coilin | COIL | 17q22-q23 | 14.52      |
| T97408  | BCL2-associated athanogene | BAG1 | 9p12 | 14.51      |
| BM923884 | Glutathione S-transferase pi | GSTP1 | 11q13 | 14.49      |
| N46186  | Glutaredoxin 5 homolog (S cerevisiae) | GLRX5 | 14q32.13 | 14.40      |
| N57438  | Vitamin K epoxide reductase complex, subunit 1-like 1 | VKORC1L1 | 7q11.21 | 14.38      |
| W19461  | Abl interactor 2 | ABI2 | 2q33 | 14.33      |
| R74572  | Serine incorporator 1 | SERINC1 | 6q22.31 | 14.24      |
| N28330  | Melanoma cell adhesion molecule | MCAM | 11q23.3 | 14.23      |
| GenBank | Name                                      | Symbol | Cytoband   | Score (d)* |
|---------|------------------------------------------|--------|------------|------------|
| N20611  | GTP-binding protein 10 (putative)        | GTPBP10| 7q21.13    | 14.23      |
| H74119  | Sec61 beta subunit                       | SEC61B | 9q22.32-3q13.3 | 14.13      |
| N52748  | Zinc finger protein 536                   | ZNF536 | 1p13       | 14.13      |
| R48809  | Hypothetical gene supported by AK123662   | LOC388692 | 1p21.1    | 14.13      |
| AA005393| NADH dehydrogenase (ubiquinone) flavoprotein 2, 24 kDa | NDUFV2 | 18p11.31-p11.2 | 14.12      |
| R89913  | CD58 molecule                             | CD58   | 1p13       | 14.08      |
| W03395  | Elongation of very long chain fatty acids-like 1 | ELOVL1 | 1p43.2     | 13.97      |
| BM541374| Peptidylprolyl isomerase H (cyclophilin H) | PPIH   | 1p34.1     | 13.96      |
| AA046918| Splicing factor 3b, subunit 2, 145 kDa    | SF3B2  | 1q13.1     | 13.95      |
| R60604  | TAF5-like RNA polymerase II               | TAF5L  | 1p42.13    | 13.89      |
| H39844  | Small nuclear RNA activating complex, polypeptide 3 | SNACP3 | 9p22.3     | 13.84      |
| N39630  | Purinergic receptor P2X, ligand-gated ion channel, 7 | P2RX7 | 1q24       | 13.82      |
| H57205  | Vinculin                                 | VCL    | 10q22.1-q23 | 13.77      |
| N93274  | Hook homolog 3 (Drosophila)              | HOOK3  | 8p11.21    | 13.74      |
| H14054  | Beta-1,3-glucuronyltransferase 3 (glucuronosyltransferase I) | B3GAT3 | 11q12.3    | 13.59      |
| BG676419| Potassium channel tetramerisation domain containing 13 | KCTD13 | 16p11.2    | 13.59      |
| N31020  | Similar to Signal peptide complex subunit 2 | LOC653566 | 1p35.3     | 13.57      |
| BG110260| FK506 binding protein 14, 22 kDa         | FKBPI4 | 7p15.1     | 13.56      |
| H53224  | Transferrin receptor (p90, CD71)         | TFRC   | 3q29       | 13.54      |
| R31353  | Glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IID) | GNS | 1q14       | 13.51      |
| AA128133| Nexilin (F actin binding protein)        | NEXN   | 1p31.1     | 13.49      |
| BQ070812| Proteasome (prosome, macropain) 26S subunit, ATPase, 3 | PSMC3 | 11p12-p13  | 13.48      |
| H94761  | Disrupted in schizophrenia 1              | DISC1  | 1q42.1     | 13.48      |
| BQ050099| Ras homolog gene family, member A        | RHOA   | 3p21.3     | 13.39      |
| BG169474| UTP14, U3 small nucleolar ribonucleoprotein | UTP1A4 | Xq25       | 13.35      |
| R69639  | Carbohydrate (chondroitin 4) sulfotransferase 11 | CHST11 | 1q          | 13.35      |
| T77351  | Rotatin                                  | RTTN   | 1p42.2     | 13.33      |
| AA203284| Basic transcription factor 3              | BTF3   | 5q13.2     | 13.33      |
| AA056664| V-akt murine thymoma viral oncogene homolog 1 | AKT1 | 1q32.3214q32.32 | 13.31 |
| BE385427| Chromatin modifying protein 6             | CHMP6  | 17q25.3    | 13.26      |
| BI850411| Calnexin                                 | CANX   | 5q35       | 13.19      |
| BG687243| Similar to ribosomal protein S13         | LOC729236 | 1p32.3     | 13.16      |
| BE256276| Ribosomal protein L32                    | RPL32  | 3p25-p24   | 13.15      |
| W17368  | Hexose-6-phosphate dehydrogenase         | H6PD   | 1p36       | 13.04      |
| N56629  | Hypoxia upregulated 1                    | HYOU1  | 11q23.1-q23.3 | 13.02    |
| R48663  | Nuclear factor of activated T-cells, cytoplasmic | NFATC2IP | 16p11.2   | 12.98      |
| BQ052715| Pyruvate kinase, muscle                   | PKM2   | 1q22       | 12.97      |
| R02012  | Downstream neighbor of SON               | DONSON | 21q22.1    | 12.97      |
| AA203750| Dimethylglycine dehydrogenase            | DMGDH  | 5q14.1     | 12.96      |
| AA058399| Zinc finger protein 720                  | ZNF720 | 16p11.2    | 12.89      |
| H69509  | ATP-binding cassette, sub-family B (MDR/TAP) | ABCB10 | 1q42       | 12.86      |
| W20454  | Fibronectin 1                            | FN1    | 2q34       | 12.85      |
| N80357  | NDRG family member 2                     | NDRG2  | 1q11.2     | 12.84      |
| W16514  | Rho family GTPase 1                      | RND1   | 12q12-q13  | 12.81      |
| AA021382| Secreted protein, acidic, cysteine-rich (osteocin) | SPARC | 5q31.3-q32 | 12.76      |
| H90355  | Ubiquitin protein ligase E3 component n-recognin 1 | UBR1 | 1q13       | 12.67      |
| N44935  | B-cell receptor-associated protein 31     | BCAP31 | Xq28       | 12.66      |
| AA054571| Phosphatidylinositol glycan anchor biosynthesis, class V | PIGV | 1p36.11    | 12.65      |
| GenBank   | Name                              | Symbol | Cytoband  | Score (d)* |
|----------|-----------------------------------|--------|-----------|------------|
| W61045   | Polymerase (DNA-directed), delta 4 | POLD4  | 11q13     | 12.65      |
| R25725   | Cylindromatosis (turban tumor syndrome) | CYLD   | 16q12.1   | 12.63      |
| BM468576 | Chaperonin containing TCP1, subunit 6A (zeta 1) | CCT6A  | 7p11.2    | 12.61      |
| R55158   | V-ral simian leukemia viral oncogene homolog B | RALB   | 2cen-q13  | 12.58      |
| N77205   | RAN binding protein 2              | RANBP2 | 2q12.3    | 12.55      |
| AA121350 | DCN1, defective in cullin neddylation 1 | DCUN1D2 | 13q34   | 12.49      |
| H22871   | Peptidase D                       | PEPD   | 19q12-q13.2 | 12.41    |
| H71235   | Sialic acid binding Ig-like lectin 5 | SIGLEC5 | 19q13.3   | 12.41      |
| W25557   | Tripartite motif-containing 28     | TRIM28 | 19q13.4   | 12.37      |
| H78781   | Absent in melanoma 1              | AIM1   | 6q21      | 12.37      |
| N51173   | Spastin                           | SPAST  | 2p24-p21  | 12.36      |
| AA001324 | TIMP metalloproteinase inhibitor 1 | TIMP1  | Xp11.3-p11.23 | 12.34  |
| R16054   | HMG-box transcription factor 1     | HBPI   | 7q22-q31  | 12.34      |
| R88469   | Dipeptidyl-peptidase 6            | DPP6   | 7q36.2    | 12.34      |
| AA037249 | ATP synthase                       | ATP5C1 | 10p15.1   | 12.32      |
| T84763   | Cell division cycle associated 8  | CDCA8  | 1p34.3    | 12.30      |
| R69935   | Hypothetical protein FLJ10404      | FLJ10404 | 5q35.3    | 12.29      |
| H89836   | Phospholipase D1, phosphatidylcholine-specific | PLD1   | 3q26      | 12.28      |
| R48131   | SH3-domain binding protein 2       | SH3BP2 | 4p16.3    | 12.27      |
| AA007268 | Polyhomeotic homolog 2 (Drosophila) | PHC2  | 1p34.3    | 12.19      |
| H52288   | Metallothionein 1E                | MT1E   | 16q13     | 12.18      |
| AA044796 | Similar to BMS1-like, ribosome assembly protein | LOC729096 | 10q22.2   | 12.17      |
| T70535   | NUAK family, SNF1-like kinase, 1   | NUAK1  | 12q23.3   | 12.16      |
| BQ083501 | Ribosomal protein L12             | RPL12  | 9q34      | 12.16      |
| H72796   | Hexose-6-phosphate dehydrogenase   | H6PD   | 1p36      | 12.05      |
| N99693   | Chromosome 12 open reading frame 32 | C1orf32 | 12p13.33  | 12.05      |
| BQ063705 | Coiled-coil-helix-coiled-coil-helix domain containing 2 | CHCHD2 | 7p11.2    | 12.04      |
| AA040816 | Cleavage and polyadenylation specific factor 3, 73 kDa | CPSF3 | 2p25.1    | 12.03      |
| R50700   | Mercaptoptpyruvate sulfurtransferase | MPST | 2q13.1    | 11.95      |
| H38879   | Phosphoserine phosphatase          | PSPH   | 7p15.2-p15.1 | 11.93    |
| AA059211 | Male germ cell-associated kinase   | MAK    | 6p24      | 11.93      |
| W21187   | Thymidylate synthetase             | TYMS   | 18p11.32  | 11.92      |
| W49716   | GRAM domain containing 3           | GRAMD3 | 5q23.2    | 11.90      |
| W05242   | DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 | DDX5 | 17q21    | 11.90      |
| N28562   | Exportin, tRNA (nuclear export receptor for tRNAs) | XPOT | 12q14.2    | 11.86      |
| AA128587 | Zinc finger protein 629            | ZNF629 | 16p11.2   | 11.83      |
| N76529   | Membrane metalloendopeptidase      | MME    | 3q25.1-q25.2 | 11.72    |
| N44807   | NF-kappaB activating protein        | NKAP   | Xq24      | 11.67      |
| AA147560 | Hect domain and RLD 2 pseudogene  | LOC440248 | 15q13.1    | 11.67      |
| H62176   | E1A binding protein p300            | EP300  | 22q13.2   | 11.65      |
| AA030048 | Protein kinase, cAMP-dependent, regulatory, type I, beta | PRKAR1B | 7p22    | 11.63      |
| R82429   | Alpha-methylacyl-CoA racemase       | AMACR  | 5p13      | 11.63      |
| BM457262 | Non-metastatic cells 1, protein (NM23A) expressed in | NME1 | 17q21.3    | 11.61      |
| W19413   | Cytoskeleton-associated protein 4   | CKAP4  | 12q23.3   | 11.59      |
| AA054778 | Homeobox A10                       | HOXA10 | 7p15-p14  | 11.58      |
| N36197   | Proline-rich protein HaeIII subfamily 1 | PRH1 | 12p13.2   | 11.57      |
| W47528   | Overexpressed in colon carcinoma-1 | OCC-1 | 12q23.3   | 11.54      |
| BM559619 | MOB1, Mps One Binder kinase activator-like 1B (yeast) | MOBK1B | 2p13.1    | 11.53      |
show a phenotype similar to that of normal human osteoblasts, while also providing a reproducible experimental model suitable for the microarray analysis. Second, as it is still difficult to explain the roles of all genes, whose expression was modified, we focused on the role of genes with well-known functions related to osteogenesis. Third, although microarray technology is widely accepted as a valid approach to describe changes induced by a factor on cell environment, additional research using, for example RT-PCR, might be useful to provide supplementary support for the results obtained. Fourth, we studied responses at only one time. We chose 18 hours exposure time on the basis of a previous time experiment, in which a peak in DNA synthesis was seen after 18 hours of stimulation in MG63 cultures maintained in the presence of 10% FCS [45]. In contrast, Lohmann et al. reported PEMFs enhanced cell differentiation in MG63 cultures and reduced cell proliferation [30]. The differences existing between the two sets of data regarding cell proliferation could be related to the different experimental conditions used. Lohmann et al. exposed MG63 cultures when they reached confluence. When cultures are confluent they stop to proliferate. We exposed cells to PEMF when cultures were subconfluent, therefore, they responded with an enhancement of proliferation. We cannot extrapolate our findings to shorter or longer exposures to PEMFs.

PEMFs appear to act on bone formation by inducing upregulation of several genes related to osteoblast proliferation and differentiation. Among those genes, HOXA10, a transcriptional factor that acts positively on RUNX2, is the main transcriptional regulator of osteoblast differentiation [25]. HOXA10 controls osteoblastogenesis via RUNX2-promoted osteoprogenitor cell differentiation in immature osteoblasts [25]. This protein also is believed to be involved in activation of alkaline phosphatase, osteocalcin, and sialoprotein genes [25]. We also observed

| GenBank Name | Symbol | Cytoband | Score (d)* |
|--------------|--------|----------|------------|
| N46377 Galactose-3-O-sulfotransferase 4 | GAL3ST4 | 7q22.1 | 11.53 |
| AA033651 UDP-N-acetyl-alpha-D-galactosamine | GALNT6 | 12q13 | 11.50 |
| R97614 Ribosomal protein L32 pseudogene 3 | RPL32P3 | 3q21.3 | 11.49 |
| H82707 Protein phosphatase 2 (formerly 2A), regulatory subunit B’ | PPP2R3A | 3q22.1 | 11.48 |
| H16005 Niemann-Pick disease, type C2 | NPC2 | 1q42.3 | 11.46 |
| R23610 Zinc and ring finger 2 | ZNRF2 | 7p15.1 | 11.45 |
| H25541 Ring finger protein 138 | RNF138 | 18q12.1 | 11.44 |
| AA114919 Y box binding protein 1 | YBX1 | 1p34 | 11.43 |
| H57747 Betaine-homocysteine methyltransferase | BHMT | 5q13.1-q15 | 11.42 |
| BM974828 Ribosomal protein L18 | RPL18 | 19q13 | 11.41 |
| H97422 NOL1/NOP2/Sun domain family, member 3 | NSUN3 | 3q11.2 | 11.40 |
| BG545342 Synaptotagmin 1 | SYN1 | 21q22.2 | 11.40 |
| BE790941 Centromere protein O | CENPO | 2p23.3 | 11.36 |
| H66235 Ataxin 2 | ATXN2 | 1q24.1 | 11.31 |
| H82010 Transcription termination factor, RNA polymerase II | TTF2 | 1p22 | 11.30 |
| N40640 WW domain binding protein 5 | WBP5 | Xq22.1-q22.2 | 11.27 |
| H21773 Hypothetical protein LOC145758 | LOC145758 | 15q26.3 | 11.25 |
| T97204 Interleukin 6 receptor | IL6R | 1q21 | 11.22 |
| BQ505012 Proteasome (prosome, macropain) subunit, beta type, 2 | PSMB2 | 1p34.2 | 11.22 |
| W02584 Lysosomal trafficking regulator | LYST | 1q42.1-q42.2 | 11.06 |
| H61357 Tumor protein p53 (Li-Fraumeni syndrome) | TP53 | 17p13.1 | 11.03 |
| N38855 Cyclin B1 interacting protein | CCNB1IP1 | 14q11.2 | 11.03 |
| BM928663 Chromodomain helicase DNA binding protein 4 | CHD4 | 12p13 | 11.01 |
| BM905720 LSM12 homolog (S cerevisiae) | LSM12 | 17q21.31 | 10.99 |
| H64813 Ribosomal protein S28 pseudogene | LOC646195 | 11q14.1 | 10.98 |
| AA056368 ATPase, H + transporting, lysosomal 13 kDa, V1 subunit G1 | ATP6V1G1 | 9q32 | 10.98 |
| BG46932 Keratin 18 | KRT18 | 12q13 | 10.96 |
| T95392 Microfibrillar-associated protein 3-like | MFAP3L | 4q32.3 | 10.91 |
| AA037600 Regulator of chromosome condensation | RCC1 | 1p36.1 | 10.90 |

* SAM assigns a score to each gene on the basis of a change in gene expression relative to the standard deviation of repeated measurements.
Table 2. Downregulated genes

| GenBank Name | Name                        | Symbol | Cytoband | Score (d)* |
|--------------|-----------------------------|--------|----------|------------|
| BG700671     | Potassium inwardly rectifying channel | KCNJ13 | 2q37     | −57.88     |
| H88081       | Otoraplin                   | OTOR   | 20p12.1-p11.23 | −55.73     |
| H81127       | Protein kinase, AMP-activated | PRKAB2 | 1q21.1   | −49.72     |
| H44375       | Myocyte enhancer factor 2B  | MEF2B  | 1q12     | −45.98     |
| N48215       | Solute carrier family 20    | SLC20A1| 2q11-q14 | −45.74     |
| AA099522     | MORC family CW-type zinc finger 4 | MORC4 | Xq22.3   | −45.56     |
| BE904276     | Protein tyrosine phosphatase, non-receptor type 3 | PTPN3 | 9q31     | −45.11     |
| H03729       | Epidermal growth factor receptor | EGFR  | 7p12     | −41.14     |
| T84537       | Fanconi anemia, complementation group D2 | FANCD2 | 3p26     | −41.10     |
| BG776239     | Wilms tumor 1               | WT1    | 1p13     | −38.77     |
| AA044149     | Methylmalonyl CoA epimerase | MCHE   | 2p13.3   | −38.44     |
| BF437100     | Transmembrane protein 87B   | TMEM87B| 2q13     | −37.68     |
| AA010608     | Parvalbumin                 | PVALB  | 22q12-q13.1/22q13.1 | −36.84 |
| BG818724     | Solute carrier family 7     | SLC7A1 | 13q12-q14 | −36.44     |
| AA156812     | Collagen, Type XVIII, alpha 1 | COL18A1| 21q22.3  | −35.48     |
| N76723       | Hypothetical protein LOC150166 | LOC150166| 22q11.21 | −35.03     |
| N50000       | Methionine adenosyltransferase 1, alpha | MAT1A | 10q22    | −34.96     |
| AA136950     | Plexin domain containing 2  | PLXDC2 | 10p12.32-p12.31 | −34.86 |
| BG620850     | Chorionic somatomammatropin hormone 2 | CS2H2 | 1q24.2   | −34.04     |
| H79911       | Core-binding factor, runt domain | CBFA2T3| 1q24     | −33.25     |
| N55960       | NOL1/NOP2/Sun domain family, member 7 | NSUN7 | 4p14     | −33.05     |
| W44535       | Neurochondrin               | NCDN   | 1p34.3   | −33.00     |
| N42329       | Suppressor of cytokine signaling 6 | SOCS6 | 1q22.2   | −32.03     |
| AA127799     | FYVE and coiled-coil domain containing 1 | FYCO1 | 3q21.31  | −31.87     |
| BG622452     | ADAM metallopeptidase domain 12 (meltrin alpha) | ADAM12 | 10q26.3  | −31.86     |
| BQ073808     | Proteasome (prosome, macropain) | PSMC4 | 19q13.11-q13.13 | −31.65 |
| BM466167     | Septin 6                    | SEP6   | 4q24     | −31.55     |
| H52445       | Leucine rich repeat containing 31 | LRRC31 | 3q26.2   | −31.41     |
| W87840       | Helicase with zinc finger   | HELZ   | 17q24.2  | −29.81     |
| R53682       | SH2 domain containing 3C    | SH2D3C | 9q34.11  | −29.56     |
| H69334       | Pirin (iron-binding nuclear protein) | PIR | Xp22.2  | −29.15     |
| W05657       | E74-like factor 1 (ets domain transcription factor) | ELF1 | 13q13    | −28.60     |
| W472223      | Mitochondrial trans-2-enoyl-CoA reductase | MECR | 1p36.1-p35.1 | −28.59 |
| AA053903     | FRY-like                    | FRYL   | 4p12     | −28.54     |
| N44611       | Transmembrane protein 50B   | TMEM50B| 21q22.11 | −28.28     |
| R99229       | Hydroxymethylbilane synthase | HMBS  | 11q23.3  | −28.12     |
| BM857788     | Nuclear receptor co-repressor 2 | NCO2R2 | 12q24    | −27.72     |
| N52672       | Nuclear receptor subfamily 1, group D, member 2 | NR1D2 | 3p24.2   | −27.52     |
| H17037       | Similar to CG4502-PA        | FLJ25076 | 5p15.31 | −26.95     |
| BE779318     | Transcription elongation factor B (SIII) | TCEB3 | 1p36.1   | −26.77     |
| T90862       | Remodeling and spacing factor 1 | RSF1 | 11q14.1  | −26.74     |
| AA455435     | Chromosome 9 open reading frame 5 | C9orf5 | 9q31     | −26.67     |
| A1188464     | Matrix metallopeptidase 11 (stromelysin 3) | MMP11 | 22q11.222q11.23 | −26.36 |
| N54724       | Chromosome 14 open reading frame 24 | C14orf24 | 14q13.2 | −26.14     |
| W38932       | Heme oxygenase (decycling) 2 | HMox2  | 16p13.3  | −25.94     |
| N51855       | Poly (ADP-ribose) polymerase family, member 2 | PARP2 | 14q11.2-q12 | −25.87 |
| R99225       | Keratin associated protein 4-7 | KRTAP4-7 | 17q12-q21 | −25.75     |
| T78280       | Histone acetyltransferase 1 | HAT1   | 2q31.2-q33.1 | −24.75 |

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| GenBank Name | Name | Symbol | Cytoband | Score (d)* |
|--------------|------|--------|----------|------------|
| R16431       | Chromosome 4 open reading frame 29 | C4orf29 | 4q28.2    | -24.61     |
| H91396       | Bile acid coenzyme A: amino acid N-acyltransferase | BAAT | 9q22.3    | -24.49     |
| T66756       | Sprouty homolog 3 (Drosophila) | SPRY3 | Xq28 and Yq12 | -24.16 |
| BG565707     | Fibrinogen gamma chain | FGG | 4q28 | -24.00 |
| AA031920     | Cytochrome b-245, alpha polypeptide | CYBA | 16q24 | -23.63 |
| H77390       | Golgi autoantigen, golgin subfamily a, 1 | GOLGA1 | 9q33.3 | -23.34 |
| R98300       | KIAA0286 protein | KIAA0286 | 12q13.3 | -23.28 |
| H25352       | Serum response factor binding protein 1 | SRFBP1 | 5q23.1 | -22.98 |
| H67225       | Solute carrier family 7 | SLC7A2 | 8p22-p21.3 | -22.56 |
| AA151360     | Rho GTPase activating protein 12 | ARHGAP12 | 10q11.1 | -22.36 |
| N34285       | Solute carrier family 26 | SLC26A2 | 5q31-q34 | -22.34 |
| H59530       | CHK1 checkpoint homolog (S pombe) | CHEK1 | 11q24-q24 | -22.25 |
| H68793       | Yip1 interacting factor homolog B (S cerevisiae) | YIF1B | 19q13.2 | -22.09 |
| H75715       | Membrane bound O-acyltransferase domain containing 2 | MOBAT2 | 2p25.1 | -21.77 |
| W19459       | Dipetidyl-peptidase 8 | DPP8 | 15q22 | -21.59 |
| R61012       | CDC42 binding protein kinase alpha (DMPK-like) | CDC42BPA | 1q42.11 | -21.38 |
| H61387       | Reticulin 4 receptor | RTN4R | 22q11.21 | -21.33 |
| H28872       | Aspartyl-tRNA synthetase | DARS | 2q21.3 | -21.21 |
| W47361       | Folate receptor 3 (gamma) | FOLR3 | 11q13 | -21.16 |
| T92079       | Proteasome (prosome, macropain) activator subunit 2 | PSME2 | 14q11.2 | -21.15 |
| N55035       | Peroxisomal biogenesis factor 3 | PEX3 | 6q33-q24 | -21.03 |
| N48524       | TIA1 cytosolic granule-associated RNA binding protein-like 1 | TIA1L | 10q | -20.94 |
| BG770889     | RAB11 family interacting protein 2 (class I) | RAB11FIP2 | 10q26.11 | -20.75 |
| H97449       | Integrin, beta 5 | ITGB5 | 3q21.2 | -20.71 |
| T78739       | EPB receptor B2 | EPB2 | 1p36.1-p35 | -20.62 |
| H10896       | Dual specificity phosphatase 4 | DUSP4 | 8p12-p11 | -20.42 |
| T87012       | CD79A molecule, immunoglobulin-associated alpha | CD79A | 1q32.3 | -20.31 |
| W16524       | CDC42 binding protein kinase alpha (DMPK-like) | CDC42BPA | 1q42.11 | -20.30 |
| R47766       | Transient receptor potential cation channel, subfamily C | TRPC4AP | 2q11.22 | -20.23 |
| T64848       | Period homolog 3 (Drosophila) | PER3 | 1p36.23 | -20.16 |
| N54874       | Chromosome 20 open reading frame 39 | C20orf39 | 20p11.21 | -20.16 |
| T95182       | Chromosome 6 open reading frame 86 | C6orf86 | 6p25.2 | -20.14 |
| BM994830     | UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase | B4GALT1 | 9p13 | -19.97 |
| N44094       | Cyclin J | CCNJ | 10qter-q26.12 | -19.77 |
| R02669       | Adaptor-related protein complex 3, beta 1 subunit | AP3B1 | 5q14.1 | -19.77 |
| H00518       | Multiple inositol polyphosphate histidase-phosphatase, 1 | PHPS1 | 10q23 | -19.73 |
| R64061       | Pregnancy specific beta-1-glycoprotein 5 | PSG5 | 19q13.2 | -19.62 |
| T84786       | TRNA splicing endonuclease 2 homolog (S cerevisiae) | TSEN2 | 3p21.5 | -19.50 |
| B181657      | Serine/threonine kinase 10 | STK10 | 5q35.1 | -19.49 |
| H79636       | KIAA012 | KIAA012 | 18q12.1 | -19.41 |
| AA055329     | Hypothetical locus LOC678655 | LOC678655 | 12p13.31 | -19.28 |
| H80810       | Formin-like 2 | FMNL2 | 2q35.3 | -19.26 |
| R91604       | Solute carrier family 38, member 2 | SLC38A2 | 12q | -19.21 |
| T77428       | ELOVL family member 5, elongation of long chain fatty acids | ELOVL5 | 6p21.1-p12.1 | -19.17 |
| H85608       | Protein phosphatase 1, regulatory (inhibitor) subunit 2 | PP1R2 | 3q29 | -19.08 |
| T98709       | Major facilitator superfamily domain containing 11 | MFSD11 | 17q25 | -19.04 |
| AA033653     | Major histocompatibility complex, class II, DR beta 1 | HLA-DRB1 | 6p21.3 | -19.03 |
| W78787       | Complement component 5 | C5 | 9q33-q34 | -19.01 |
| GenBank Name | Symbol | Cytoband | Score (d)* |
|-------------|--------|----------|------------|
| AA001996 MutS homolog 6 (E coli) | MSH6 | 2p16 | -18.87 |
| N32361 PQ loop repeat containing 3 | PQLC3 | 2p25.1 | -18.85 |
| H45525 Ras homolog gene family, member G (rho G) | RHOG | 11p15.5-p15.4 | -18.79 |
| A1927909 Homogentisate 1,2-dioxygenase (homogentisate oxidase) | HGD | 3q13.33 | -18.75 |
| R05896 Sodium channel modifier 1 | SCN1M | 1q21.2 | -18.59 |
| N80988 GTP binding protein 2 | GTPB2 | 6p21-p12 | -18.53 |
| N40600 SUMO1/sentrin specific peptidase 7 | SENP7 | 3q12 | -18.47 |
| R23473 PAK1 interacting protein 1 | PAK1IP1 | 6p24.2 | -18.40 |
| BQ063621 Calsyntenin 1 | CLSTN1 | 1p36.22 | -18.36 |
| H58311 Coagulation factor V (proaccelerin, labile factor) | F5 | 1q23 | -18.29 |
| H08311 DTW domain containing 2 | DTWD2 | 5q23.1 | -18.26 |
| W02106 Solute carrier family 26 (sulfate transporter), member 2 | SLC26A2 | 5q31-q34 | -18.22 |
| H39162 1-acylglycerol-3-phosphate O-acyltransferase 1 | AGPAT1 | 6p21.3 | -17.90 |
| AI368607 Family with sequence similarity 13, member A1 | FAM13A1 | 4q22.1 | -17.89 |
| AA040364 Hypothetical protein LOC284513 | LOC284513 | 1p36.13 | -17.84 |
| H38222 SET binding factor 1 | SBF1 | 22q13.33 | -17.69 |
| R73417 Peptidase inhibitor 16 | PIH1 | 6p21.2 | -17.60 |
| T67154 IMP2 inner mitochondrial membrane peptidase-like | IMP2L | 7q31 | -17.45 |
| R96767 Phospholipase C, beta 4 | PLCB4 | 20p12 | -17.42 |
| N35681 Diabo homolog (Drosophila) | DIABLO | 12q24.31 | -17.29 |
| W25288 SNAP-associated protein | SNAPAP | 1q21.3 | -17.27 |
| AA203442 Chromosome 9 open reading frame 39 | C9orf39 | 9p22.2 | -17.21 |
| R73337 Zinc finger protein 777 | ZNF777 | 7q36.1 | -17.08 |
| N73263 Storkhead box 1 | STOX1 | 10q21.3 | -16.94 |
| H39156 Myotubulin related protein 6 | MTMR6 | 13q12 | -16.93 |
| T77015 GSG1-like | GSG1L | 16p11.2 | -16.66 |
| R23489 Zinc finger protein 354A | ZNF354A | 5q35.3 | -16.62 |
| H64555 S100 calcium binding protein A2 | S100A2 | 1q21 | -16.52 |
| W90519 Zinc finger protein 652 | ZNF652 | 17q21.32 | -16.50 |
| W19130 Plexin A2 | PLXNA2 | 1q32.2 | -16.39 |
| H78273 Sperm associated antigen 9 | SPAG9 | 17q21.33 | -16.33 |
| AA156879 Zinc finger protein 615 | ZNF615 | 19q13.33 | -16.30 |
| N53192 Hypothetical protein MGC22014 | hCG_40738 | 2p13.1 | -16.00 |
| BG682138 Secreted protein, acidic, cysteine-rich (osteonectin) | SPARC | 5q31.3-q32 | -15.87 |
| H41974 Integrin, alpha 3 | ITGA3 | 17q21.33 | -15.84 |
| H79050 Protein tyrosine phosphatase, receptor type, E | PTPRE | 10q26 | -15.83 |
| W48559 Zinc finger, MYM-type 1 | ZMYM1 | 1q34.3 | -15.78 |
| H87048 ADP-ribosylation factor GTPase activating protein 3 | ARFGAP3 | 22q13.2-q13.3 | -15.76 |
| T84174 Eukaryotic translation initiation factor 3 | EIF3S9 | 7p22.2 | -15.76 |
| N40120 Zinc finger protein 33B | ZNF33B | 1q11.2 | -15.74 |
| W35313 Sterile alpha motif and leucine zipper containing kinase AZK | ZAK | 2q24.2 | -15.70 |
| AA040656 Zinc finger protein 502 | ZNF502 | 3p21.31 | -15.70 |
| H18810 Importin 8 | IPOP | 1p21.21 | -15.68 |
| R12736 Staufen, RNA binding protein, homolog 2 (Drosophila) | STAU2 | 8q13-q21.1 | -15.61 |
| N74741 BTG family, member 3 | BTG3 | 21q11.2 | -15.61 |
| AA069533 Chromosome 7 open reading frame 42 | C7orf42 | 7q11.2 | -15.55 |
| N28267 Integrin, alpha X | ITGAX | 6p11.2 | -15.47 |
| AA135718 Neuropilin 1 | NRPI | 10p12 | -15.46 |
| GenBank     | Name                                                                 | Symbol    | Cytoband       | Score (d)* |
|------------|----------------------------------------------------------------------|-----------|----------------|------------|
| R50902     | Tubulin, gamma complex associated protein 6                         | TUBGCP6   | 22q13.31-q13.33| -15.46     |
| BQ020504   | Translocase of outer mitochondrial membrane 20 homolog              | TOMM20    | 1q42           | -15.43     |
| BM545369   | Hect domain and RLD 2 pseudogene 2                                 | HERC2P2   | 15q11.2        | -15.39     |
| W58640     | SECIS binding protein 2                                             | SECISBP2  | 9q22.2         | -15.21     |
| W49512     | Bradykinin receptor B1                                              | BDKB1     | 14q32.1-q32.2  | -15.19     |
| N46282     | PiggyBac transposable element derived 2                            | PGBD2     | 1q44           | -15.12     |
| W31642     | Early B-cell factor 3                                               | EBF3      | 10q26.3        | -15.05     |
| BG623586   | ADAM metallopeptidase with thrombospondin type 1 motif             | ADAMTS5   | 21q21.3        | -14.97     |
| W87709     | Kelch-like 23 (Drosophila)                                          | KLHL23    | 2q31.1         | -14.97     |
| N44005     | EF-hand calcium binding domain 2                                    | EFCA2     | 1q44           | -14.96     |
| W52509     | ARV1 homolog (S cerevisiae)                                         | ARV1      | 1q44           | -14.95     |
| H79753     | Death-associated protein                                            | DAP       | 5p15.2         | -14.93     |
| BI005854   | Glycerophosphodiester phosphodiesterase domain containing 3        | GDPD3     | 16p11.2        | -14.71     |
| H53660     | HLA-B associated transcript 3                                       | BAT3      | 6p21.3         | -14.71     |
| AA037312   | ATP synthase mitochondrial F1 complex assembly factor 1             | ATPAF1    | 1p33-p32.3     | -14.68     |
| N57425     | DAZ associated protein 2                                            | DAZAP2    | 12q12          | -14.67     |
| BM545099   | Lectin, galactoside-binding, soluble, 9 (galectin 9)                | LGALS9    | 17q11.1        | -14.66     |
| W16685     | N-glycanase 1                                                       | NGLY1     | 3p24.2         | -14.64     |
| N76853     | Golgi autoantigen, golgin subfamily b, macrogolgin                  | GOLGB1    | 3q13           | -14.59     |
| AA001311   | Hypothetical protein LOC129293                                     | LOC129293 | 2p11.2         | -14.54     |
| W40439     | Forkhead box J1                                                     | FOXJ1     | 17q22-q25      | -14.44     |
| H61030     | REX2, RNA exonuclease 2 homolog (S cerevisiae)                      | REXO2     | 11q23.1-q23.2  | -14.39     |
| H03728     | G1 to S phase transition 1                                          | GSPT1     | 1p31.1         | -14.37     |
| W48584     | Procollagen-proline, 2-oxoglutarate 4-dioxygenase                   | P4HA2     | 5q31           | -14.30     |
| BI861012   | Mannosidase, alpha, class 1B, member 1                              | MAN1B1    | 9q34           | -14.24     |
| AA203133   | DNA (cytosine-5-)methyltransferase 1                                | DNMT1     | 9p13.2         | -14.15     |
| AA481714   | Lectin, galactoside-binding, soluble, 9 (galectin 9)                | LGALS9    | 17q11.1        | -14.66     |
| H08319     | Zinc finger protein 783                                             | ZNF783    | 7q36.1         | -14.01     |
| N44142     | 3-hydroxy-3-methylglutaryl-coenzyme A reductase                     | HMGCR     | 5q13.3-q14     | -13.97     |
| R15789     | Tumor suppressing subtransferable candidate 1                       | TSSC1     | 2p25.3         | -13.97     |
| H79770     | Tripartite motif-containing 2                                       | TRIM27    | 6p22           | -13.93     |
| A1056197   | Amidohydrolase domain containing 2                                  | AMDHD2    | 16p13.3        | -13.92     |
| BE871226   | Chromosome 6 open reading frame 62                                  | C6orf62   | 6p22.2         | -14.02     |
| H08319     | Zinc finger protein 783                                             | ZNF783    | 7q36.1         | -14.01     |
| N44142     | 3-hydroxy-3-methylglutaryl-coenzyme A reductase                     | HMGCR     | 5q13.3-q14     | -13.97     |
| R15789     | Tumor suppressing subtransferable candidate 1                       | TSSC1     | 2p25.3         | -13.97     |
| H79770     | Tripartite motif-containing 2                                       | TRIM27    | 6p22           | -13.93     |
| A1056197   | Amidohydrolase domain containing 2                                  | AMDHD2    | 16p13.3        | -13.92     |
| BE871226   | Chromosome 6 open reading frame 62                                  | C6orf62   | 6p22.2         | -14.02     |
| BM468475   | Keratin 8 pseudogene 12                                             | KRT8P12   | 3q26.1         | -13.84     |
| H10533     | Plasminogen activator, tissue                                       | PLAT      | 8p12           | -13.82     |
| H93653     | Collagen-like tail subunit of asymmetric acetylcholinesterase      | COLQ      | 3q25           | -13.82     |
| BG333273   | CD47 molecule                                                       | CD47      | 3q13.1-q13.2   | -13.80     |
| H44717     | Cytochrome c oxidase subunit 8A (ubiquitous)                        | COX8A     | 11q12-q13      | -13.79     |
| W38526     | Exostoses (multiple)-like 2                                        | EXT2L     | 1p21           | -13.75     |
| R86053     | Nuclear factor of kappa light polypeptide gene enhancer             | NFKB2     | 10q24          | -13.71     |
| AA203387   | Trophinin associated protein (tastin)                                | TROAP     | 1q13.12        | -13.70     |
| H52351     | Transmembrane protein 150                                           | TMEM150   | 2p11.2         | -13.70     |
| W47015     | Ts translation elongation factor, mitochondrial                     | TSFM      | 1q13.1-q14     | -13.60     |
| T95583     | Spermatogenesis associated 21                                       | SPATA21   | 1p36.13        | -13.59     |
| BM993318   | Ubiquitin specific peptidase 24                                     | USP24     | 1p32.3         | -13.58     |
| W88434     | Carboxypeptidase B2 (plasma)                                        | CPB2      | 3q14.11        | -13.55     |
| N48417     | GA binding protein transcription factor, alpha subunit 60 kDa       | GABPA     | 21q12-q22.121q21.3 | -13.53 |
| H81801     | Phosphatidylinositol 3,4,5-trisphosphate-dependent RAC exchanger 1  | PREX1     | 20q13.13       | -13.52     |
| GenBank Name                           | Symbol       | Cytoband  | Score (d)* |
|----------------------------------------|--------------|-----------|------------|
| Al478910 FERM domain containing 6     | FRMD6        | 14q22.1   | −13.45     |
| AA450143 WD repeat domain 27          | WDR27        | 6q27      | −13.41     |
| H61757 ELK4, ETS-domain protein       | ELK4         | 1q32      | −13.31     |
| BM459914 Serine/threonine kinase 4    | STK4         | 20q11.2-q13.2 | −13.31 |
| W60673 CREB regulated transcription coactivator 3 | CRTC3       | 15q26.1   | −13.28     |
| R32668 Component of oligomeric golgi complex 3 | COG3        | 13q14.12  | −13.27     |
| AA045300 CDC42 small effector 2      | CDC42SE2     | 5q31.1    | −13.17     |
| W56454 Furin (paired basic amino acid cleaving enzyme) | FURIN       | 15q26.1   | −13.12     |
| W61099 Chromosome X open reading frame 36 | CXorf36      |           | −13.06     |
| AA037834 Methylmalonic aciduria (cobalamin deficiency) cbIB type | MMAB        | 12q24     | −13.06     |
| W90717 Solute carrier family 24 (sodium/potassium/calcium exchanger) | SLC24A4     | 14q32.12  | −13.05     |
| N44262 Pecanex homolog (Drosophila)  | PCNX         | 14q24.2   | −12.99     |
| T70417 REC8 homolog (yeast)           | REC8         | 14q11.2-q12 | −12.98 |
| BE899110 Family with sequence similarity 105, member B | FAM105B     | 5p15.2    | −12.94     |
| R06564 UDP-galactose-4-epimerase      | GALE         | 1p36-p35  | −12.92     |
| R50922 Neurilin 4, X-linked           | LNGN4X       | Xp22.32-p22.31 | −12.89 |
| H15612 COX10 homolog, cytochrome c oxidase assembly protein | COX10       | 17p12-p11.2 | −12.88    |
| R18433 Opioid binding protein/cell adhesion molecule-like | OPCML       | 11q25     | −12.85     |
| AA143060 Melanoma associated antigen (mutated) 1 | MUM1        | 19p13.3   | −12.81     |
| BF969700 Chromosome 12 open reading frame 35 | C12orf35    | 12p11.21  | −12.79     |
| N43949 Mitogen-activated protein kinase kinase kinase kinase 4 | MAP4K4      | 2q11.2-q12 | −12.77     |
| N71526 Inhibitor of kappa light polypeptide gene enhancer in B-cells | IKKB        | 5p15.2    | −12.73     |
| W78799 Nudix (nucleoside diphosphate linked moiety X)-type motif 13 | NUDT13      | 10q22.1   | −12.69     |
| W20458 Tripartite motif-containing 59 | TRIM59       | 3q26.1    | −12.69     |
| H89618 WNK lysine deficient protein kinase 1 | WNK1        | 12p13.3   | −12.67     |
| R65820 SLC24A4 regulator              | SLC24A4RG    | 20q13.33  | −12.61     |
| R87913 Potassium voltage-gated channel, delayed-rectifier, subfamily S | KCNS1       | 20q12     | −12.60     |
| N57603 Solute carrier organic anion transporter family, member 1C1 | SLCO1C1     | 12p12.2   | −12.55     |
| AA045905 Forkhead box P1              | FOXP1        | 3p14.1    | −12.55     |
| H29349 Abelson helper integration site 1 | AH1I        | 6q23.3    | −12.54     |
| H08988 Ubiquitin specific peptidase 7 (herpes virus-associated) | USP7        | 16p13.3   | −12.43     |
| R23677 Nucleolar protein 4            | NOL4         | 18q12     | −12.43     |
| H46899 Adenosine deaminase, RNA-specific, B2 (RED2 homolog rat) | ADARB2      | 10p15.3   | −12.41     |
| B667959 Reticulin 1                   | RTN1         | 14q23.1   | −12.39     |
| BG323782 Coiled-coil domain containing 14 | CCDC14      | 3q21.1    | −12.36     |
| R38905 Dihydropyrimidinase-like 5     | DLPYSL5      | 2p23.3    | −12.35     |
| N23456 Cytooglobin                    | CYGB         | 17q25.3   | −12.24     |
| R11685 COP9 constitutive photomorphogenic homolog subunit 5 | COP55       | 8q13.2    | −12.33     |
| R06410 O-6-methylguanaine-DNA methyltransferase | MGMT      | 10q26     | −12.31     |
| H63698 N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D | NAPE-PLD | 7q22.1    | −12.27     |
| H93191 Ubiquitin specific peptidase 3 | USP3         | 15q22.3   | −12.26     |
| AA069502 Hypothetical protein DKFZp434H1419 | DKFZp434H1419 | 2q35     | −12.24     |
| AA043530 MORN repeat containing 2    | MORN2        | 2p22.1    | −12.21     |
| N57399 LMBR1 domain containing 1      | LMBRD1       | 6q13      | −12.20     |
| H40732 PPARG binding protein          | PPARGP       | 17q12-q21.1 | −12.19 |
| AI822112 Similar to SR protein related family member (rsr-1) | LOC728676  | 1p42.13   | −12.18     |
| R12743 HECT domain containing 1      | HECTD1       | 14q12     | −12.14     |
| N48445 RAB33A, member RAS oncogene family | RAB33A      | Xq25      | −12.13     |
It has been suggested that osteoblast-specific disruption of STAT3 results in an osteopenic phenotype [27, 41]. STAT3, involved in bone turnover [27], regulates the transcription of various genes that modulate cell proliferation and differentiation in a cell-specific manner [27]. P2RX7 is a purinergic receptor, which is correlated with calcium channels and interacts with the calmodulin-dependent protein [37]. Activation of P2RX7 receptors by exogenous nucleotides stimulates expression of osteoblast markers and enhances mineralization in cultures of rat calvarial cells promoting osteogenesis [37]. V-akt murine thymoma viral oncogene homolog 1 (AKT1), is a phosphoinositide-dependent serine-threonine protein kinase, and one of the key players in the signaling of potent bone anabolic factors [29]. The disruption of AKT1 in mice led to low-turnover osteopenia through dysfunction [29]. AKT1 deficiency causes decreased bone mass and formation [29],

Table 2. continued

| GenBank | Name | Symbol | Cytoband | Score (d)* |
|---------|------|--------|----------|------------|
| H03305  | Bromodomain containing 1 | BRD1 | 22q13.33 | −12.13 |
| AA010089 | Hypothetical protein LOC157860 | LOC157860 | 8p11.23 | −12.09 |
| H93176  | 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 | PFKFB3 | 10p14-p15 | −12.07 |
| R12649  | Solute carrier family 13 (sodium-dependent dicarboxylate transporter) | SLC13A3 | 20q12-q13.1 | −12.05 |
| R14154  | Hypothetical protein LOC286063 | LOC286063 | 8q11.21 | −12.03 |
| R61444  | Thyroid adenoma associated | THADA | 2p21 | −12.02 |
| W17278  | Solute carrier family 25 (mitochondrial oxodicarboxylate carrier) | SLC25A21 | 14q11.2 | −12.00 |
| T77303  | Leucine-rich repeat LGI family, member 2 | LGI2 | 4p15.2 | −11.98 |
| AA043837 | Coiled-coil domain containing 45 | CCDC45 | 17q24.1 | −11.93 |
| R52735  | THAP domain containing 8 | THAP8 | 19q13.12 | −11.92 |
| W46207  | UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1 | B4GLT1 | 9p13 | −11.79 |
| B814695 | Immunoglobulin superfamily containing leucine-rich repeat 2 | ISLR2 | 15q24.1 | −11.78 |
| BQ000722 | Deoxyribonucleoside 1-like 1 | DNAE1L1 | Xq28 | −11.73 |
| N52657  | MYC binding protein 2 | MYCBP2 | 13q22 | −11.68 |
| AI340082 | Sulfotransferase family 1E, estrogen-prefering, member 1 | SULT1E1 | 4q13.1 | −11.67 |
| N54717  | Acyl-coenzyme A binding domain containing 5 | ACBD5 | 10p12.1 | −11.67 |
| R16400  | Elongation factor, RNA polymerase II, 2 | ELL2 | 5q15 | −11.63 |
| AA151264 | ALS2 C-terminal like | ALS2CL | 3p21.31 | −11.59 |
| T80372  | Calcium channel, voltage-dependent, alpha 2/delta subunit 2 | CACNA2D2 | 3p21.3 | −11.58 |
| AW872398 | Amphipathin | AMPH | 7p14-p13 | −11.53 |
| H08101  | Glutaminase 2 (liver, mitochondrial) | GLS2 | 1q21 | −11.51 |
| AA134026 | Ubiquitin-conjugating enzyme E2A (RAD6 homolog) | UBE2A | Xq24-q25 | −11.49 |
| R72472  | HLA-B associated transcript 1 | BAT1 | 6p21.3 | −11.47 |
| BM450631 | Heat shock protein 90 kDa alpha (cytosolic), class A member 2 | HSP90AA2 | 11p14.1 | −11.45 |
| AA534429 | Chromosome 1 open reading frame 38 | C1orf38 | 1p35.3 | −11.44 |
| BI753390 | Amyloid beta (A4) precursor protein-binding, family A | APBA2 | 15q11-q12 | −11.44 |
| BF112255 | Tousled-like kinase 2 | TLK2 | 17q23 | −11.42 |
| T78737  | KIAA2026 | KIAA2026 | 9p24.1 | −11.42 |
| R39428  | Protein tyrosine phosphatase, receptor type, G | PTPRG | 3p21-p14 | −11.40 |
| N42943  | PHD finger protein 17 | PHF17 | 4q26-q27 | −11.38 |
| H86918  | Pleckstrin homology domain containing, family B (evectins) | PLEKHB1 | 1q13.5-q14.1 | −11.38 |
| R23434  | Membrane-bound transcription factor peptidase, site 2 | MBTPS2 | Xp22.1-p22.2 | −11.37 |
| R17293  | GLE1 RNA export mediator-like (yeast) | GLE1L | 9q34.11 | −11.33 |
| BI522504 | Zinc finger protein 605 | ZNF605 | 12q24.33 | −11.29 |
| AA056155 | SEC24 related gene family, member C (S. cerevisiae) | SEC24C | 10q22.2 | −11.28 |
| AA055164 | Low density lipoprotein receptor-related protein 6 | LRP6 | 12p11-p13 | −11.21 |
| R27647  | KIAA1333 | KIAA1333 | 14q12 | −11.18 |

* SAM assigns a score to each gene on the basis of a change in gene expression relative to the standard deviation of repeated measurements.
RUNX2-dependent differentiation and function of osteoblasts [29], and impairs bone resorption via dysfunction of osteoblasts and osteoclasts [29]. AKT1 suppresses osteoblasts apoptosis through inhibition of Fox03a and Bim; Mediation of the osteoblastic bone formation by IGF-1 and insulin. IGF-1/AKT1 pathway might be a common pathway for bone anabolic action of parathyroid, thyroid, and growth hormone [29].

We also observed upregulation of genes involved in connective and bone tissue formation (COL1A2) and noncollagenous extracellular matrix (ECM) synthesis (SPARC, FN1, VCL). COL1A2 encodes for collagen Type 1α2. Collagen Type 1 is the most represented collagen in the human organism and is important for ECM stability [6].

Osteonectin (SPARC), the most abundant noncollagenous protein in bone tissue, modulates cell-matrix interaction and is involved in the tissue-remodeling process [47]. FN1 is important for ECM stability and involved in adhesion and migration cellular processes such as tissue healing [39]. VCL is a cytoskeletal protein associated with the intercellular junctions between the cells and the matrix [49].

The effect of TIMP1 upregulation and of MMP-11 and DUSP4 downregulation can be interpreted as a decrease in the degradation process. TIMP1 promotes apposition of ECM by inhibiting collagen and other components of ECM degradation operated by the metalloproteinase [26]. DUSP4 inactivates the superfamily of MAP kinase, which is involved with proliferation and differentiation. DUSP4 downregulation, then, stimulates proliferation [17]. MMPs potentially can degrade almost all components of the periprosthetic ECM and contribute to prosthetic loosening and osteolysis through pathologic ECM degradation and bone remodeling around prostheses [28, 35]. The stromelysins especially have broad substrate specificity, including proteoglycans, laminin, and fibronectin [35]. Stromelysin-1 determines the release and activation ECM-bound latent TGF-B1 and is involved with ECM turnover [8].

Upregulation of CALM1 promotes enhancement of calmodulin1, a protein involved in proliferative cell activation [40]. Calmodulin also is involved in the transduction mechanism of PEMFs [9].

Our data suggest many effects of PEMFs on human osteoblastlike cells in vitro. PEMFs seem to exert an...
anabolic effect on cells. In particular, they are consistent with abundant preclinical and clinical findings showing a positive effect of PEMFs on osteogenesis. Stimulation by PEMFs induces bone healing in patients, shortens the time of healing processes, and stimulates healing of nonunions. Exposure to PEMFs acts on cell behavior in different ways. More specifically, PEMFs stimulate cell proliferation and induce osteoblastogenesis and differentiation of osteoblasts. Moreover, PEMFs promote ECM apposition and induce osteoblastogenesis and differentiation of osteoblasts. These data suggest a more comprehensive explanation of the observed clinical effect of PEMFs on the induction of osteogenesis. Given their broad effects, PEMFs might be useful in other fields such as regenerative medicine.

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