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BASIC RESEARCH

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

A Pilot Study

Vincenzo Sollazzo MD, Annalisa Palmieri PhD, Furio Pezzetti PhD, Leo Massari MD, Francesco Carinci MD

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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.

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V. Sollazzo (✉), L. Massari
Istituto di Clinica Ortopedica Università di Ferrara,
Corso Giovecca 203, 44100 Ferrara, Italy
e-mail: slv@unife.it

F. Pezzetti
Istituto di Istologia ed Embriologia Generale Università di Bologna, Bologna, Italy

A. Palmieri, F. Carinci
Istituto di Chirurgia Maxillo Facciale Università di Ferrara, Ferrara, Italy

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

defective production and use of this growth factor [10]. PEMF exposure induces cell proliferation in human osteoblasts and chondrocytes cultured in vitro [18, 20, 38, 44, 45]. PEMFs determine signal transduction by means of intracellular release of Ca^{2+} leading to an increase in cytosolic Ca^{2+} and an increase in activated cytoskeletal calmodulin [9]. 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% CO_2 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-cm^2, 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 1α2 (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

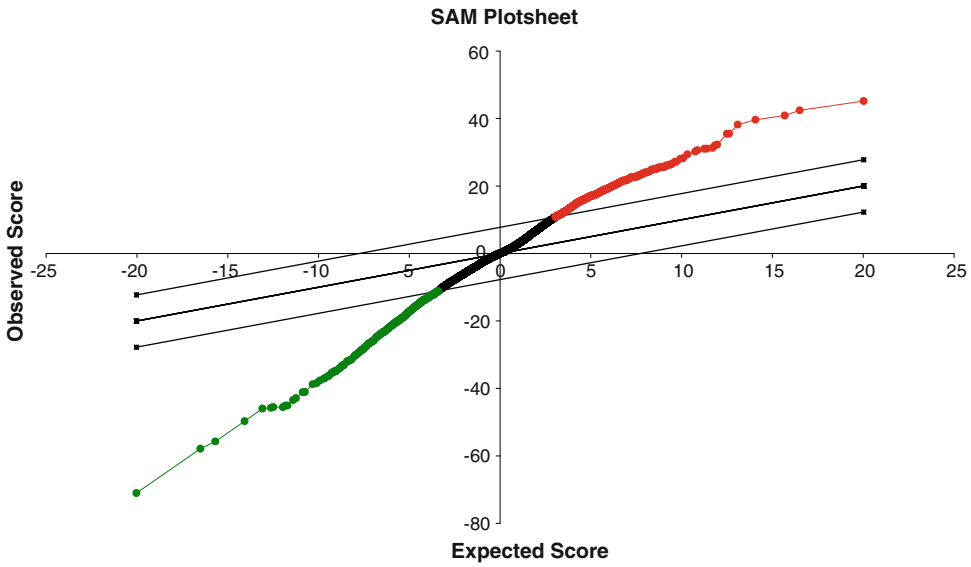

Fig. 1 A microarray (SAM) plot of MG63 exposed to PEMFs versus control is shown. Expected differentially expressed genes are reported on the x axis, whereas observed differentially expressed genes are reported on the y axis. Downregulated genes (green) are located in the lower left of the graph; upregulated genes (red) are in the upper right; genes with different expression but statistically insignificant are shown in black. Parallel lines drawn from the lower left to upper right squares are the cutoff limits. The solid line indicates the equal value of observed and expected differentially expressed genes.

Table 1. Upregulated genes

GenBank	Name	Symbol	Cytoband	Score (d)*
W19447	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51	DDX51	12q24.33	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-p36.2	31.99
W44826	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
BQ108591	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	9p13.3	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	NPAL3	1p36.12-p35.1	23.55
W00391	Solute carrier family 11 member 2	SLC11A2	12q13	23.36
H12528	Annexin A5	ANXA5	4q26-q28l4q28-q32	23.13
N54759	Prenylcysteine oxidase 1	PCYOX1	2p13.3	23.08
T89646	ST3 beta-galactoside alpha-2,3-sialyltransferase 2	ST3GAL2	16q22.1	22.82
AA029517	KCNQ1 overlapping transcript 1	KCNQ1OT1	11p15	22.75
W47664	NAD(P)H dehydrogenase, quinone 1	NQO1	16q22.1	22.73
W02597	PMS1 postmeiotic segregation increased 1	PMS1	2q31-q33l2q31.1	22.61
R11416	Seryl-tRNA synthetase	SARS	1p13.3-p13.1	22.55
H67332	GTP binding protein 1	GTPBP1	22q13.1	22.43
H86020	NADH dehydrogenase	NDUFB5	3q26.33	22.21
AA031564	Chromosome 1 open reading frame 212	C1orf212	1p34.3	21.90
W67485	Zinc finger protein 136	ZNF136	19p13.2-p13.12	21.68
W32906	Zinc finger protein 702	ZNF702	19q13.41	21.49
BI492783	Zinc finger protein 207	ZNF207	17q11.2	21.43
BE278092	Ribosomal protein L10	RPL10	Xq28	21.42
N90960	Par-6 partitioning defective 6 homolog beta	PARD6B	20q13.13	21.32
BG565169	Ferritin, light polypeptide	FTL	19q13.3-q13.4	21.29
H75902	Complement component (3b/4b) receptor 1	CR1	1q32	21.15
W31736	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51 kDa	NDUFV1	11q13	20.96
AA417686	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 (Drosophila)	KLHL8	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

Table 1. continued

GenBank	Name	Symbol	Cytoband	Score (d)*
AI734239	Coiled-coil domain containing 120	CCDC120	Xp11.23	20.28
N72922	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	Hydroxysteroid (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-coil-helix-coiled-coil-helix domain containing 7	CHCHD7	8q12.1	19.07
BI092679	H19, imprinted maternally expressed untranslated mRNA	H19	11p15.5	18.99
N45602	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 (Drosophila)	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	5q21	18.07
AA047157	CD82 molecule	CD82	11p11.2	18.01
AA044701	ADAMTS-like 5	ADAMTSL5	19p13.3	17.82
BM477950	Ribosomal protein L8	RPL8	8q24.3	17.79
AI587328	Radical S-adenosyl methionine domain containing 2	RSAD2	2p25.2	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	PSMB4	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
AA004532	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

Table 1. continued

GenBank	Name	Symbol	Cytoband	Score (d)*
N40643	Chromosome 10 open reading frame 18	C10orf18	10p15.1	16.74
R82575	KIAA1704	KIAA1704	13q13-q14	16.51
BI196362	Tubulin, alpha 1a	TUBA1A	12q12-q14.3	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	GZF1	20p12.3-p11.21	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
T80698	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 I	SELI	2p23.3	15.62
T75376	Notch homolog 2 (Drosophila)	NOTCH2	1p13-p11	15.49
N46675	Unkempt homolog (Drosophila)	UNK	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	Neugrin, 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	TGOLN2	2p11.2	15.02
BI494911	Nck-associated protein 5	NAP5	2q21.2	14.89
N94192	Glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	GNS	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

Table 1. continued

GenBank	Name	Symbol	Cytoband	Score (d)*
N20611	GTP-binding protein 10 (putative)	GTPBP10	7q21.13	14.23
H74119	Sec61 beta subunit	SEC61B	9q22.32-q31.3	14.13
N52748	Zinc finger protein 536	ZNF536	19q12	14.13
R48809	Hypothetical gene supported by AK123662	LOC388692	1q21.1	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	1p34.2	13.97
BM541374	Peptidylprolyl isomerase H (cyclophilin H)	PPIH	1p34.1	13.96
AA046918	Splicing factor 3b, subunit 2, 145 kDa	SF3B2	11q13.1	13.95
R60604	TAF5-like RNA polymerase II	TAF5L	1q42.13	13.89
H39844	Small nuclear RNA activating complex, polypeptide 3	SNAPC3	9p22.3	13.84
N39630	Purinergic receptor P2X, ligand-gated ion channel, 7	P2RX7	12q24	13.82
H57205	Vinculin	VCL	10q22.1-q23	13.77
N39274	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 peptidase complex subunit 2	LOC653566	1p35.3	13.57
BG110260	FK506 binding protein 14, 22 kDa	FKBP14	7p15.1	13.56
H53224	Transferrin receptor (p90, CD71)	TFRC	3q29	13.54
R31353	Glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	GNS	12q14	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	UTP14A	Xq25	13.35
R69639	Carbohydrate (chondroitin 4) sulfotransferase 11	CHST11	12q	13.35
T77351	Rotatin	RTTN	18q22.2	13.33
AA203284	Basic transcription factor 3	BTF3	5q13.2	13.33
AA056664	V-akt murine thymoma viral oncogene homolog 1	AKT1	14q32.32|14q32.32	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	15q22	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	14q11.2	12.84
W16514	Rho family GTPase 1	RND1	12q12-q13	12.81
AA021382	Secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	5q31.3-q32	12.76
H90355	Ubiquitin protein ligase E3 component n-recognin 1	UBR1	15q13	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

Table 1. continued

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 metallopeptidase inhibitor 1	TIMP1	Xp11.3-p11.23	12.34
R16054	HMG-box transcription factor 1	HBP1	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	C12orf32	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	Mercaptopyruvate sulfurtransferase	MPST	22q13.1	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 metallo-endopeptidase	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

Table 1. continued

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	14q24.3	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	Synaptojanin 1	SYNJ1	21q22.2	11.40
BE790941	Centromere protein O	CENPO	2p23.3	11.36
H66235	Ataxin 2	ATXN2	12q24.1	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
BQ050102	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 1	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
AA058638	ATPase, H + transporting, lysosomal 13 kDa, V1 subunit G1	ATP6V1G1	9q32	10.98
BG469305	Keratin 18	KRT18	12q13	10.96
T95392	Microfibrillar-associated protein 3-like	MFAP3L	4q32.3	10.91
AA037600	Regulator of chromosome condensation 1	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.

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

Table 2. Downregulated genes

GenBank	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	19p12	−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	11p13	−38.77
AA044149	Methylmalonyl CoA epimerase	MCEE	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 I, alpha	MAT1A	10q22	−34.96
AA136950	Plexin domain containing 2	PLXDC2	10p12.32-p12.31	−34.86
BG620850	Chorionic somatomammotropin hormone 2	CSH2	17q24.2	−34.04
H79911	Core-binding factor, runt domain	CBFA2T3	16q24	−33.25
N55596	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	18q22.2	−32.03
AA127799	FYVE and coiled-coil domain containing 1	FYCO1	3p21.31	−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	Xq24	−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
W47223	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	NCOR2	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
AI188464	Matrix metallopeptidase 11 (stromelysin 3)	MMP11	22q11.2|22q11.23	−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

Table 2. continued

GenBank	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	MBOAT2	2p25.1	−21.77
W19459	Dipeptidyl-peptidase 8	DPP8	15q22	−21.59
R61012	CDC42 binding protein kinase alpha (DMPK-like)	CDC42BPA	1q42.11	−21.38
H61387	Reticulon 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	6q23-q24	−21.03
N48524	TIA1 cytotoxic granule-associated RNA binding protein-like 1	TIAL1	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	EPH receptor B2	EPHB2	1p36.1-p35	−20.62
H10896	Dual specificity phosphatase 4	DUSP4	8p12-p11	−20.42
T87012	CD79a molecule, immunoglobulin-associated alpha	CD79A	19q13.2	−20.31
W16524	CDC42 binding protein kinase alpha (DMPK-like)	CDC42BPA	1q42.11	−20.30
R47766	Transient receptor potential cation channel, subfamily C	TRPC4AP	20q11.22	−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	10pter-q26.12	−19.77
R02669	Adaptor-related protein complex 3, beta 1 subunit	AP3B1	5q14.1	−19.77
H00518	Multiple inositol polyphosphate histidine phosphatase, 1	MINPP1	10q23	−19.73
R64061	Pregnancy specific beta-1-glycoprotein 5	PSG5	19q13.2	−19.62
T84786	TRNA splicing endonuclease 2 homolog (S cerevisiae)	TSEN2	3p25.1	−19.50
BI818657	Serine/threonine kinase 10	STK10	5q35.1	−19.49
H79636	KIAA1012	KIAA1012	18q12.1	−19.41
AA055329	Hypothetical locus LOC678655	LOC678655	12p13.31	−19.28
H80810	Formin-like 2	FMNL2	2q23.3	−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	PPP1R2	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

Table 2. continued

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
AI927909	Homogentisate 1,2-dioxygenase (homogentisate oxidase)	HGD	3q13.33	−18.75
R05896	Sodium channel modifier 1	SCNM1	1q21.2	−18.59
N80988	GTP binding protein 2	GTPBP2	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
H38322	SET binding factor 1	SBF1	22q13.33	−17.69
R73417	Peptidase inhibitor 16	PI16	6p21.2	−17.60
T67154	IMP2 inner mitochondrial membrane peptidase-like	IMMP2L	7q31	−17.45
R96767	Phospholipase C, beta 4	PLCB4	20p12	−17.42
N35681	Diablo 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
N73236	Storkhead box 1	STOX1	10q21.3	−16.94
H39156	Myotubularin 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	1p34.3	−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	10q11.2	−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	IPO8	12p11.21	−15.68
R12736	Staufen, RNA binding protein, homolog 2 (Drosophila)	STAU2	8q13-q21.1	−15.61
N74741	BTG family, member 3	BTG3	21q21.1-q21.2	−15.61
AA069533	Chromosome 7 open reading frame 42	C7orf42	7q11.21	−15.55
N28267	Integrin, alpha X	ITGAX	16p11.2	−15.47
AA135718	Neuropilin 1	NRP1	10p12	−15.46

Table 2. continued

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	BDKRB1	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	EFCAB2	1q44	−14.96
W52509	ARV1 homolog (S cerevisiae)	ARV1	1q42.2	−14.95
H79753	Death-associated protein	DAP	5p15.2	−14.93
BI905854	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	16p13.1	−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	19p13.2	−14.15
AA481714	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 27	TRIM27	6p22	−13.93
AI056197	Amidohydrolase domain containing 2	AMDHD2	16p13.3	−13.92
BE871226	TAF6 RNA polymerase II	TAF6	7q22.1	−13.87
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	3p25	−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	EXTL2	1p21	−13.75
R86053	Nuclear factor of kappa light polypeptide gene enhancer	NFKB2	10q24	−13.71
AA203387	Trophinin associated protein (tastin)	TROAP	12q13.12	−13.70
H52351	Transmembrane protein 150	TMEM150	2p11.2	−13.70
W47015	Ts translation elongation factor, mitochondrial	TSFM	12q13-q14	−13.60
T89583	Spermatogenesis associated 21	SPATA21	1p36.13	−13.59
BM993318	Ubiquitin specific peptidase 24	USP24	1p32.3	−13.58
W88434	Carboxypeptidase B2 (plasma)	CPB2	13q14.11	−13.55
N48417	GA binding protein transcription factor, alpha subunit 60 kDa	GABPA	21q21-q22.1|21q21.3	−13.53
H81801	Phosphatidylinositol 3,4,5-trisphosphate-dependent RAC exchanger 1	PREX1	20q13.13	−13.52

Table 2. continued

GenBank	Name	Symbol	Cytoband	Score (d)*
AI478910	FERM domain containing 6	FRMD6	14q22.1	−13.45
AA450143	WD repeat domain 27	WDR27	6q27	−13.41
H61757	ELK4, ETS-domain protein (SRF accessory protein 1)	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) cblB 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	Neuroligin 4, X-linked	NLGN4X	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	IKBKB	8p11.2	−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	SLC2A4 regulator	SLC2A4RG	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	AHI1	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
BI667959	Reticulon 1	RTN1	14q23.1	−12.39
BG323782	Coiled-coil domain containing 14	CCDC14	3q21.1	−12.36
R38905	Dihydropyrimidinase-like 5	DPYSL5	2p23.3	−12.35
N23456	Cytoglobin	CYGB	17q25.3	−12.34
R11685	COP9 constitutive photomorphogenic homolog subunit 5	COPS5	8q13.2	−12.33
R06410	O-6-methylguanine-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
N57339	LMBR1 domain containing 1	LMBRD1	6q13	−12.20
H40732	PPAR binding protein	PPARBP	17q12-q21.1	−12.19
AI822112	Similar to SR protein related family member (rsr-1)	LOC728676	1q42.13	−12.18
R12743	HECT domain containing 1	HECTD1	14q12	−12.14
N48445	RAB33A, member RAS oncogene family	RAB33A	Xq25	−12.13

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-biphosphatase 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	B4GALT1	9p13	−11.79
BI914695	Immunoglobulin superfamily containing leucine-rich repeat 2	ISLR2	15q24.1	−11.78
BQ000722	Deoxyribonuclease I-like 1	DNASE1L1	Xq28	−11.73
N52657	MYC binding protein 2	MYCBP2	13q22	−11.68
AI340082	Sulfotransferase family 1E, estrogen-preferring, 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	Amphiphysin	AMPH	7p14-p13	−11.53
H08101	Glutaminase 2 (liver, mitochondrial)	GLS2	12q13	−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	11q13.5-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
AA056151	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.

STAT3, P2RX7, and AKT1 upregulation. 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], impairs

Table 3. Main upregulated genes related to bone formation

Gene	Function	Effect	Reference
STAT 3	Transcriptional factor, activation of the MAP kinase	Bone turnover	Itoh et al. [27]
HOXA 10	Activation of Runx2, alkaline phosphatase, osteocalcin, and bone sialoprotein	Osteogenic response	Hassan et al. [25]
AKT1	Suppression of osteoblasts apoptosis through inhibition of Fox03a and Bim; Mediation of the osteoblastic bone formation by IGF-1 and insulin.	Bone formation	Kawamura et al. [29]
CALM1	Signal transduction, stimuli to proliferation	Bone formation	Rhymer et al. [40]
P2RX7	Activation of P2RX7 receptors stimulates expression of osteoblast markers and enhances mineralization in cultures cells	Promote osteogenesis	Ohlendorff et al. [37]
FN1	Adhesion and migration cellular process	Extracellular matrix stability; tissue healing	Potts and Campbell [39]
COL1A2	Collagen 1α2, chain of the most abundant collagen in the human organism	Extracellular matrix stability	Antoniv et al. [6]
SPARC	The most abundant noncollagenous protein in the bone tissue.	Modulation of the cell-matrix interaction and production of the matrix	Yan and Sage [47]
VCL	Associated with the intercellular junctions between the cells and the matrix	Anchorages the actin to the cellular membrane	Ziegler et al. [49]
TIMP1	Inhibits collagen and other components of extracellular matrix degradation operated by the metalloproteinase	Decrease matrix degradation	Hatori et al. [26]

Table 4. Main downregulated genes related to bone formation

Gene	Function	Effect	Reference
MMP-11	Metallopeptidase with substrate specificity, including proteoglycans, lamini, and fibronectin	Degradation of extracellular matrix	Matziari et al. [35]
DUSP4	Inactivates the superfamily of MAP kinase	Inhibition of proliferation and differentiation	Caunt et al. [17]

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 FOXO3a and Bim [29], and may mediate the osteoblastic bone formation by IGF-1 [29]. The 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-ß1 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 mineralization, and decrease degradation and absorption processes of ECM. 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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