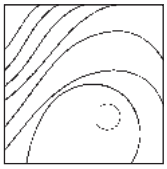
Evaluation of Gene Expression in MG63 Human Osteoblastlike Cells Exposed to Tantalum Powder by Microarray Technology



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Conventional orthopedic implants are composed from titanium. To improve some characteristics (ie, volumetric porosity, modulus of elasticity, frictional modulus), a new porous tantalum biomaterial has been developed and its biocompatibility reported. By using DNA microarrays containing 20,000 genes, several genes whose expression were significantly up- or down-regulated were identified in an osteoblastlike cell line (MG63) cultured with tantalum powder (TP). The differentially expressed genes cover a broad range of functional activities: signaling transduction; transcription; cell cycle regulation, proliferation, and apoptosis; and cytoskeleton formation. To the authors' knowledge, the data reported represent the first genetic portrait of TP. (Int J Periodontics Restorative Dent 2011;31:e17–e28.)

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The increase in orthopedic and dental prosthetic surgery has stimulated research on new materials for bone implants.[1–5] Generally, any implant has two major sets of properties: bulk material characteristics, which are responsible for mechanical and structural properties, and surface characteristics, which promote biocompatibility by interacting with the biologic environment.[3]

Conventional orthopedic implants are obtained from stainless steel, cobalt-chromium, and titanium alloys.[6] Titanium is the metal most commonly used for implants because of its excellent biocompatibility, ready availability, and sufficient strength for small specific gravity.[7] Although excellent clinical results have been shown with these materials, they have several inherent limitations (low volumetric porosity, relatively high modulus of elasticity, and low frictional characteristics). To solve the limitations of these solid metals and to improve the characteristics of prosthetic implants, a new porous tantalum biomaterial was developed (Zimmer Trabecular Metal Technology, Implex).[6]

Tantalum has been used as an implant material, and its biocompatibility has been reported.[7]

Porous tantalum is an open-cell tantalum structure of repeating dodecahedrons with an appearance similar to cancellous bone. Tantalum is a transition metal (atomic number, 73; atomic weight, 180.05) that remains relatively inert in vivo.[6] Tantalum-fashioned implants have shown good biocompatibility, high volumetric porosity, relatively high frictional characteristics, and excellent corrosion-erosion resistance.[6,8] The good mechanical properties and biocompatibility of tantalum could lead to less peri-implant stress shielding and could allow immediate loading, resulting in a significant clinical improvement in prosthetic surgery. One of the most important causes of aseptic loosening of a hip implant prosthesis is the debridement of the material the prosthesis is composed of.[1,2] Moreover, the particles generated from micromotion between the bone and cementless implant in the femoral bone bed at the bone-implant interface are believed to be a major cause of numerous osteoblast functional changes that eventually result in improper participation of bone bed formation and remodeling.[8,9] Tantalum has shown good biocompatibility when studied in bulk, although its eventual direct toxicity effect on cells in vitro has not been studied.

Tantalum can potentially be used to fabricate dental implants. However, residual powder derived from the turning of tantalum implants could alter osteoblast activity. Consequently, the authors decided to verify the molecular effect of tantalum powder (TP) on osteoblasts. Since microscopic particles of implant materials demonstrate nonbiocompatibility characteristics distinct from bulk quantities of the same material[3–7] and since there are no data on the biocompatibility of TP in previous in vitro studies,[6] the authors attempted to address this question by using microarray techniques.

DNA microarray is a molecular technology that enables the analysis of gene expression in parallel on a large number of genes, spanning a significant fraction of the human genome. Gene expression is performed by a process of RNA extraction, reverse transcription, and labeling of complementary DNA (cDNA). Reference and investigated (ie, cells cultured with tantalum) cDNA are labeled with different dyes and hybridized on slides containing cDNA fragments. Then, the slides are scanned with a laser system, and two false color images are generated for each cDNA hybridization from the investigated and reference cells. The overall result is the generation of a so-called "genetic portrait,"[9–13] corresponding to up- or down-regulated genes in the investigated cell system.

In the present study, the genetic effect of TP was defined on cells by using an osteoblastlike cell line (MG63) and microarray slides containing 20,000 different oligonucleotides.

Method and materials

Cell culture

Osteoblastlike cells (MG63) were cultured in sterile Falcon wells (Becton Dickinson) containing Eagle minimum essential medium supplemented with 10% fetal calf serum (Sigma) and antibiotics (100 U/mL penicillin and 100 µg/mL treptomycin, Sigma). Cultures were maintained in a 5% carbon dioxide humidified atmosphere at 37°C. MG63 cells were collected and seeded at a density of 1×10^5 cells/mL into 9-cm^2 (3-mL) wells by using 0.1% trypsin and 0.02% ethylenediaminetetraacetic acid in Ca++- and Mg-free Eagle buffer for cell release. One set of wells was cultured with TP. After 24 hours, when the cultures were subconfluent, cells were processed for RNA extraction.

TP

The TP used in this study was obtained from a piece of porous tantalum (Zimmer Trabecular Metal Technology, Implex) used in orthopedic knee prosthetic surgery. Trabecular Metal consists of interconnecting pores resulting in a structural biomaterial that is 80% porous, allowing approximately 2 to 3 times greater bone ingrowth compared to conventional porous coatings and double the shear strength of the interface.[14] Trabecular Metal implants are fabricated using elemental tantalum metal and vapor deposition techniques that create

a metallic strut configuration similar to trabecular bone. The crystalline microtexture of a Trabecular Metal strut is conducive to direct bone apposition.[15] In this study, a portion of the tibial prosthetic component of a Trabecular Monoblock Tibial Component was grinded using a ball mill. TP was then sterilized using absolute ethanol and ultraviolet irradiation and added to the cell cultures at a concentration of 0.002 g/10 mL. The average diameter of the tantalum particles was in the range of 5 to 10 μm.

DNA microarray screening and analysis

The protocol was the same as that of previous experiments.[9–13] RNA was extracted from the cells by using RNAzol (Molecular Research Center). Ten micrograms of RNA were used for each sample. cDNA was synthesized by using Superscript II (Life Technologies, Invitrogen) and amino-allyl dUTP (Sigma). Monoreactive Cy3 and Cy5 esters (Amersham Pharmacia) were used for indirect cDNA labeling. RNA extracted from control cells was labeled with Cy3 and used as a control against the Cy5-labeled treated (TP) cDNA. Human 20 K DNA microarrays were used (MWG Biotech). For 20 K slides, 100 μL of the sample and control cDNA in DIG Easy hybridization solution (Roche) was 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 and 0.1% sodium dodecyl sulfate at 42°C and three times for 5 minutes with 0.1× saline sodium citrate at room temperature. Slides were dried by centrifugation for 2 minutes at 2,000 rpm. The experiment was repeated twice and the dyes switched. A GenePix 4000a DNA microarray scanner (Axon) was used to scan the slides, and data were extracted with GenePix Pro (Axon). Genes with expression levels of less than 1,000 after removing the local background were not included in the analysis since ratios are not reliable at that detection level.

After scanning the two slides containing the 20,000 human genes in duplicate, the local background was calculated for each target location. A normalization factor was estimated from median ratios. Normalization was performed by adding the $\log_2$ of the normalization factor to the $\log_2$ of the median ratio. The $\log_2$ ratios for all targets on the array were then calibrated using the normalization factor, and $\log_2$ ratios outside the 99.7% confidence interval (median ± 3 times the SD = 0.52) were determined to be significantly changed in the treated cells. Thus, genes are significantly modulated in expression when the absolute value of their $\log_2$ expression level is higher than 1.56 or there is a threefold difference in expression between treated and reference cells.

GenePix Pro software was used to report genes above the threshold and with less than a 10% difference in three different statistical evaluations of the intensity ratio, thus effectively enabling an automated quality control check of the hybridized spots. Furthermore, all positively passed spots were inspected visually. Statistical analysis of the microarray program (SAM) was then performed, and a score was obtained (T statistic value).[9–13]

The genes differentially expressed in cells treated with TP are reported in Tables 1 and 2, whereas Fig 1 is the reported SAM plot.

Results

Hybridization of cDNA (derived form MG63 cultured with TP) to cDNA microarrays allowed simultaneous systemic analysis of expression profiles for thousands of genes and provided primary information on transcriptional changes related to TP. The authors identified several genes whose expression was definitely up or down regulated.

Up-regulated genes

Among the up-regulated genes (Table 1) are those involved in signal transduction, such as TRPM3 (which modulates the activity of Ca++ selective channels), MAP3K2 (which activates the mitrogen-activated protein kinase signaling pathway), RASA3 and RASGRP3 (which control cellular proliferation and differentiation), and WISP3 (a member of the WNT1 inducible signaling pathway). TP acts also on genes related with the immune system, such as

Table 1 **Up-regulated genes in treated osteoblasts**

Symbol	Name	Cytoband	Score (d)
FLJ23790	Family with sequence similarity 91	8q24.13	2.18
TRPM3	Transient receptor potential cation channel	9q21.11	2.16
F11	Coagulation factor XI	4q35	2.11
MAP3K2	Mitogen-activated protein kinase kinase kinase 2	2q14.3	1.75
KLK10	Kallikrein 10	19q13.3-q13.4	1.72
RASA3	RAS p21 protein activator 3	13q34	1.71
RASGRP3	RAS guanyl releasing protein 3	2p25.1-p24.1	1.67
GTF3C3	General transcription factor IIIC	2q33.1	1.59
VARS2	Valyl-tRNA synthetase	6p21.3	1.35
MGC10334	Hypothetical protein MGC10334	1p36.33	1.24
NOLC1	Nucleolar and coiled-body phosphoprotein 1	10q24.32	1.19
KIAA1244	KIAA1244	6q23.3	1.18
DKFZP564K2062	Chromosome 16 open reading frame 51	16p13.13-p12.3	1.16
VIL1	Villin 1	2q35-q36	1.16
Raptor	Raptor	17q25.3	1.14
NRIP2	Nuclear receptor interacting protein 2	12p13.33	1.10
PCDHGC3	Protocadherin gamma subfamily A, 12	5q31	1.09
DPYSL4	Dihydropyrimidinase-like 4	10q26	1.08
TPD52L2	Tumor protein D52-like 2	20q13.2-q13.3	1.06
FLJ13195	Stromal antigen 3-like	7p11.2-q11.2	1.02
NEIL1	Nei endonuclease VIII-like 1 (E. coli)	15q23	1.02
DFNA5	Deafness, autosomal dominant 5	7p15	1.00
INDO	Indoleamine-pyrrole 2,3 dioxygenase	8p12-p11	1.00
DONSON	Downstream neighbor of SON	21q22.1	1.00
C6orf166	Chromosome 6 open reading frame 166	6q15	0.99
ZNF443	Zinc finger protein 443	19p13.2	0.98
VCL	Vinculin	10q22.1-q23	0.97
DST	Dystonin	6p12-p11	0.97
LOC91137	Hypothetical protein BC017169	5q22.1	0.97
DEDD	Death effector domain containing	1q23.3	0.96

Table 1 continued **Up-regulated genes in treated osteoblasts**

Symbol	Name	Cytoband	Score (d)
GNB1	Guanine nucleotide binding protein (G protein)	1p36.33	0.96
DKFZP564A022	Ring finger protein 170	8p11.21	0.95
RKHD2	Ring finger and KH domain containing 2	18q21.1	0.94
FLJ10156	Family with sequence similarity 64, member A	17p13.2	0.93
KIAA1272	Chromosome 20 open reading frame 74	20p11.22	0.93
SLAMF1	Signaling lymphocytic activation molecule	1q22-q23	0.92
KIRREL2	Kin of IRRE like 2 (Drosophila)	19q13.12	0.91
PRIM1	Primase, polypeptide 1, 49kDa	12q13	0.91
DSCAML1	Down syndrome cell adhesion molecule like 1	11q23	0.91
WISP3	WNT1 inducible signaling pathway protein 3	6q22-q23	0.91
VSIG2	V-set and immunoglobulin domain containing 2	11q24	0.91
FLJ21628	Zinc finger protein 2 homolog (mouse)	5q35.3	0.91
MOBK1B	Mps One Binder kinase activator-like 1B (yeast)	2p13.1	0.90
CECR1	Cat eye syndrome chromosome region, candidate 1	22q11.2	0.90
IRF3	Interferon regulatory factor 3	19q13.3-q13.4	0.90
BLCAP	Bladder cancer associated protein	20q11.2-q12	0.90
VNN3	Vanin 3	6q23-q24	0.89
SGNE1	Secretory granule, neuroendocrine protein 1	15q13-q14	0.89
NUP37	Nucleoporin 37kDa	12q23.3	0.89

INDO (which has an antiproliferative effect on many tumor cells) and IRF3 (which activates the transcription of interferon alpha and beta). Several up-regulated proteins are enzymes contained in cytoskeleton, cell adhesion, and extracellular matrix components, such as VCL (a cytoskeletal protein associated with cell-cell and cell-matrix junctions) and DST (a member of the plakin protein family of adhesion junction plaque proteins). Other up-regulated genes are involved in negative regulation of the metabolic process, such as VIL1 (a member of a family of calcium-regulated actin-binding proteins) and NRIP2 (nuclear receptor interacting protein 2). Other genes promote cell death, such as death effector domain–containing ptoyein (DEDD) and the zinc finger protein ZNF443.

Table 2 Down-regulated genes in treated osteoblasts

Symbol	Gene name	Cytoband	Score (d)
MRPL23	Mitochondrial ribosomal protein L23	11p15.5-p15.4	–1.96
JPH3	Junctophilin 3	16q24.3	–1.90
PNPO	Pyridoxine 5′-phosphate oxidase	17q21.32	–1.80
RPS5	Ribosomal protein S5	19q13.4	–1.79
LBX1	Ladybird homeobox homolog 1 (Drosophila)	10q24	–1.77
MGC10744	Hypothetical protein MGC10744	17p13.1	–1.66
BARD1	BRCA1 associated RING domain 1	2q34-q35	–1.61
EYA2	Eyes absent homolog 2 (Drosophila)	20q13.1	–1.61
APG3L	ATG3 autophagy related 3 homolog	3q13.2	–1.55
MLLT2	AF4/FMR2 family, member 1	4q21	–1.53
AP4S1	Adaptor-related protein complex 4	14q12	–1.52
SARDH	Sarcosine dehydrogenase	9q33-q34	–1.51
TEAD2	TEA domain family member 2	19q13.3	–1.51
TGFBR3	Transforming growth factor, beta receptor III	1p33-p32	–1.48
TAGLN	Transgelin	11q23.2	–1.48
SMARCAL1	SWI/SNF related, matrix associated	2q34-q35	–1.46
HSPA4L	Heat shock 70kDa protein 4-like	4q28	–1.40
LCAT	Lecithin-cholesterol acyltransferase	16q22.1	–1.39
GTPBP6	GTP binding protein 6 (putative)	Xp22.33	–1.37
TM4SF11	Plasma membrane proteolipid (plasmolipin)	16q13	–1.36
RBMX	RNA binding motif protein, X-linked	Xq26.3	–1.34
C14orf11	Chromosome 14 open reading frame 11	14q13.1	–1.32
LOC113251	La ribonucleoprotein domain family	12q13.12	–1.30
NR1D2	Nuclear receptor subfamily 1	3p24.2	–1.25
HARS	Histidyl-tRNA synthetase	5q31.3	–1.25
KIR3DL2	Killer cell immunoglobulin-like receptor	19q13.4	–1.24
DNAJA1	DnaJ (Hsp40) homolog, subfamily A	9p13-p12	–1.21
C2orf29	Chromosome 2 open reading frame 29	2q11.2	–1.19
SHD1	SAC3 domain containing 1	11q13.1	–1.18
TULP4	Tubby like protein 4	6q25-q26	–1.17

Table 2 continued **Down-regulated genes in treated osteoblasts**

Symbol	Gene name	Cytoband	Score (d)
TNFRSF19L	Tumor necrosis factor receptor superfamily	11q13.4	–1.17
GOLGA4	Golgi autoantigen, golgin subfamily a, 4	3p22-p21.3	–1.16
KIAA1600	KIAA1600	10q25.3	–1.16
EDG2	Endothelial differentiation	9q31.3	–1.16
EAF2	ELL associated factor 2	3q13.33	–1.16
AAMP	Angio-associated, migratory cell protein	2q35	–1.16
CSNK1E	Casein kinase 1, epsilon	22q13.1	–1.14
3'HEXO	Three prime histone mRNA exonuclease 1	8p23.1	–1.14
ATP10D	ATPase, Class V, type 10D	4p12	–1.14
THBS4	Thrombospondin 4	5q13	–1.13
ANKS1	Ankyrin repeat and sterile alpha motif domain containing 1	6p21.31	–1.13
SEMA5A	Sema domain, seven thrombospondin repeats	5p15.2	–1.12
RAB11FIP1	RAB11 family interacting protein 1	8p11.22	–1.11
RNF150	Ring finger protein 150	4q31.21	–1.10
EPN2	Epsin 2	17p11.2	–1.10
C14orf93	Chromosome 14 open reading frame 93	14q11.2	–1.09
PCOLN3	Procollagen (type III) N-endopeptidase	16q24.3	–1.08
TAF1A	TATA box binding protein (TBP)-associated factor	1q42	–1.07
SPTBN4	Spectrin, beta, non-erythrocytic 4	19q13.13	–1.06
LSM10	LSM10, U7 small nuclear RNA associated	1p34.3	–1.05
LRPAP1	Low density lipoprot. receptor-relat. Protein	4p16.3	–1.04
ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2	17q11.2-q12	–1.04
TUBB3	Tubulin, beta 3	16q24.3	–1.03
HIRIP3	HIRA interacting protein 3	16p11.2	–1.03
SEMA5B	Sema domain	3q21.1	–1.02
C14orf156	Chromosome 14 open reading frame 156	14q24.3	–1.02
CDRT1	CMT1A duplicated region transcript 1	17p12	–1.02
C6orf72	Chromosome 6 open reading frame 72	6q25.1	–1.01
GBA2	Glucosidase. beta (bile acid) 2	9p13.3	–1.00
CALB2	Calbindin 2. 29kDa (calretinin)	16q22.2	–1.00

Table 2 continued **Down-regulated genes in treated osteoblasts**

Symbol	Gene name	Cytoband	Score (d)
RANGAP1	Ran GTPase activating protein 1	22q13	–1.00
DNAPTP6	DNA polymerase-transactivated protein 6	2q33.1	–0.99
WNT9B	Wingless-type MMTV integration site family	17q21	–0.98
FLJ10006	Hypothetical protein FLJ10006	2q14.3	–0.98
HADHA	Hydroxyacyl-Coenzyme A dehydrogenase	2p23	–0.97
WDR33	WD repeat domain 33	2q14.3	–0.97
MYO5A	Myosin VA (heavy polypeptide 12. myoxin)	15q21	–0.96
WNT10A	Wingless-type MMTV integration site family	2q35	–0.96
ATRN	Attractin	20p13	–0.95
KIRREL3	Kin of IRRE like 3 (Drosophila)	11q24	–0.95
EPB41	Erythrocyte membrane protein band 4.1	1p33-p32	–0.95
ARPC5	Actin related protein 2/3 complex	1q25.3	–0.94
MYH4	Myosin. heavy polypeptide 4	17p13.1	–0.94
MARCH-IX	Membrane-associated ring finger (C3HC4) 9	12q14.1	–0.94
SSTR1	Somatostatin receptor 1	14q13	–0.94
PCDHA6	Protocadherin alpha subfamily C. 1	5q31	–0.94
SAV1	Salvador homolog 1 (Drosophila)	14q13-q23	–0.93
NCOA3	Nuclear receptor coactivator 3	20q12	–0.92
CD5	CD5 antigen (p56-62)	11q13	–0.92
VPS28	Vacuolar protein sorting 28 (yeast)	8q24.3	–0.92
PRSS3	Protease. serine. 3 (mesotrypsin)	9p11.2	–0.91
PTRF	Polymerase I and transcript release factor	17q21.2	–0.91
ANTXR1	Anthrax toxin receptor 1	2p13.1	–0.90
LU	Lutheran blood group	19q13.2	–0.90
EIF3S6	Eukaryotic translation initiation factor 3	8q22-q23	–0.90
MCOLN2	Mucolipin 2	1p22	–0.90
UFD1L	Ubiquitin fusion degradation 1 like (yeast)	22q11.21	–0.90
SECISBP2	SECIS binding protein 2	9q22.2	–0.89
CELSR1	Cadherin. EGF LAG seven-pass G-type receptor 1	22q13.3	–0.88

Table 2 continued **Down-regulated genes in treated osteoblasts**

Symbol	Gene name	Cytoband	Score (d)
TMEM2	Transmembrane protein 2	9q13-q21	–0.88
DKFZP434F0318	Hypothetical protein DKFZP434F0318	12p13.2	–0.87
ROBO4	Roundabout homolog 4. magic roundabout	11q24.2	–0.87
TH1L	TH1-like (Drosophila)	20q13	–0.87
SYNCOILIN	Syncoilin. intermediate filament 1	1p34.3-p33	–0.87
CSMD1	CUB and Sushi multiple domains 1	8p23.2	–0.86
AQP6	Aquaporin 6. kidney specific	12q13	–0.86
DFFA	DNA fragmentation factor. 45kDa. alpha polypeptide	1p36.3-p36.2	–0.86
NKD2	Naked cuticle homolog 2 (Drosophila)	5p15.3	–0.86
FLJ12505	Hypothetical protein FLJ12505	1q32.3	–0.86
SEPHS2	Selenophosphate synthetase 2	16p11.2	–0.85
TP53TG3	TP53TG3 protein	16p13	–0.84
CSEN	Calsenilin. presenilin binding protein	2q21.1	–0.84
VEGFC	Vascular endothelial growth factor C	4q34.1-q34.3	–0.84
LPGAT1	Lysophosphatidylglycerol acyltransferase 1	1p36.13-q42.3	–0.84
SFRP5	Secreted frizzled-related protein 5	10q24.1	–0.84
ROR2	Receptor tyrosine kinase-like orphan receptor 2	9q22	–0.83
LRDD	Leucine-rich repeats and death domain containing	11p15.5	–0.83
MLL	Myeloid/lymphoid or mixed-lineage leukemia	11q23	–0.83
PCDHGC3	Protocadherin gamma subfamily A. 12	5q31	–0.82
MAP4K1	Mitogen-activated protein kinase 1	19q13.1-q13.4	–0.82
CDCA7	Cell division cycle associated 7	2q31	–0.82
RRAS2	Related RAS viral (r-ras) oncogene homolog 2	11p15.2	–0.82
TRUB2	TruB pseudouridine (psi) synthase homolog 2 (E. coli)	9q34.11	–0.82
ZNF2	Zinc finger protein 2	2q11.2	–0.82
SH3KBP1	SH3-domain kinase binding protein 1	Xp22.1-p21.3	–0.81

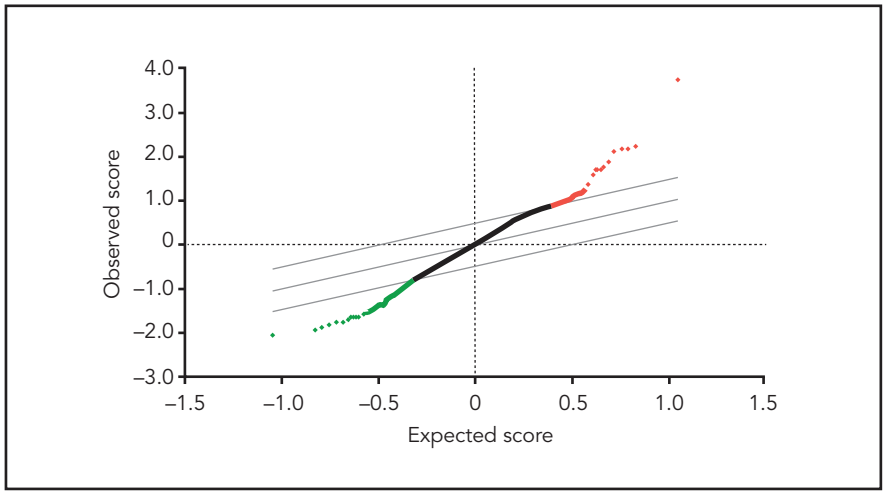

Fig 1 *SAM plot of MG63 cultured for 24 hours with TP. Expected differentially expressed genes are reported on the x-axis, whereas observed differentially expressed genes are on the y-axis. Green = down-regulated genes; red = up-regulated genes; black = genes with different expression but are not statistically significant; parallel lines = cut-off limits.*

Down-regulated genes

Among the down-regulated genes (Table 2) are those involved in signaling transduction, such as EDG2 (which mediates diverse biologic functions, including proliferation, chemotaxis, and tumor cell invasion), ERBB2 (a member of the epidermal growth factor receptor family), SSTR1 (a component of the superfamily of somatostatin receptors), VEGFC (a member of the platelet-derived growth factor/vascular endothelial growth factor family active in angiogenesis and endothelial cell growth), and ROR2 (a receptor protein tyrosine kinase involved in the early formation of the chondrocytes). TP down-regulates genes related with transcription, such as SMARCAL1 (a transcription factor), TAF1A (which is involved in the initiation of transcription by RNA polymerase I), and HIRIP3 (which functions as part of a multiprotein complex in chromatin and histone metabolism). Other down-regulated genes act at the translation level, such as MRPL23 (which helps in protein synthesis within the mitochondrion) and TH1L (which interacts with the DSIF protein complex to repress transcriptional elongation by RNA polymerase II). TP down-regulates genes related with the immune system, such as TNFRSF19L (a receptor capable of stimulating T-cell proliferation), ATRN (a secreted protein involved in the initial immune cell clustering during inflammatory responses), and LU (a member of the immunoglobulin superfamily and a receptor for extracellular matrix protein). Other down-regulated genes include cytoskeleton, cell adhesion, and extracellular matrix components such as JPH3 (a component of junctional complexes between the plasma membrane and endoplasmic/sarcoplasmic reticulum), THBS4 (which mediates cell-cell and cell-matrix interactions), and PCDHA6 (which establishes specific cell-cell connections).

Discussion

Prosthetic surgery requires the use of biomaterials with good mechanical and biocompatibility properties. In Europe, individuals receive almost 500,000 hip endoprostheses every year, and demographic data suggest that this figure is to increase in coming years.[16] Moreover, the increase of cementless implants to secure prosthetic hip devices biologically to the surrounding bone has stimulated considerable interest among orthopedic surgeons.[16] Although clinical results

of uncemented implants are good, the findings of histologic studies on retrieved porous implants have revealed that many components were fixed to the skeleton by fibrous tissue ingrowth.[17–19] The actual goal of the study was to reach a better osseointegration of the prosthetic device by improving the materials' biocompatibilities and coating the implants that show osteoconductive properties.[16] Tantalum is a new material showing interesting mechanical properties that allow it to be used in orthopedic applications requiring physiologic load support and bone ingrowth.[6] Specifically, tantalum maintains a relatively high modulus of elasticity, similar to that of subchondral bone, and its fatigue properties and endurance limits are greater than cancellous bone. Moreover, porous tantalum has been shown to support tissue ingrowth in several animal studies,[20–22] and there were no differences in cellular growth rates, gene expression, cell attachment, or cell morphology in human osteoblast-like cells cultured on tantalum disks versus conventional metals disks.[23]

Although tantalum is studied with interest by the scientific community, there are no studies examining the biocompatibility or reactivity of tantalum particulates.[6] In vitro experiments with metal powder could give more detailed information about the eventual direct cell toxicity of the metal studied and about its effect on apoptosis and the cell cycle compared with information from an in vitro study with the same metal in bulk form.[3–7] As a result, the authors decided to analyze the effect of TP on osteoblastlike cells in an in vitro system. Hybridization of mRNA-derived probes to cDNA microarrays allowed the authors to perform a systemic analysis of expression profiles for thousands of genes simultaneously and provide primary information on transcriptional changes related to tantalum debris. Since DNA microarray is a molecular technology that enables the analysis of gene expression in parallel on a large number of genes, spanning a significant fraction of the human genome, it gives a global view of the genetic effect of a substance added to a cell system. In fact, DNA microarray is not only a quantitative analysis (it has the sensitivity to detect a change of expression level in the investigated cells when compared to normal samples), but it is also a qualitative one (it can differentiate a single gene among thousands of sequences).[9–13]

Globally, tantalum produced several up- and down-regulated genes mainly related to transcription, translation, cell cycle control, immunity, and extracellular matrix regulation. Interestingly, down-regulated genes in treated osteoblasts were involved in cell proliferation and differentiation. ERB2 is responsible for the timely progression of chondrocyte maturation and periosteal osteoblast differentiation.[24] ROR2 is a tyrosine kinase that promotes osteoblast differentiation and enhances bone formation in vivo.[25] In addition, ROR2 regulates Wnt signaling, which is known to modulate osteoblast survival and differentiation.[26] Among the up-regulated genes seen in treated osteoblasts, many participate in the negative regulation of the metabolic process, such as VIL1 (a member of a family of calcium-regulated actin-binding proteins) and NRIP2 (nuclear receptor interacting protein 2). Other genes promote cell death (DEDD and ZNF443). DEDD encodes a protein that contains a death effector domain. Overexpression of this gene was shown to induce weak apoptosis.[27,28]

The results seem to support the thesis already reported in previous studies[1,2,8,9] that TP partially inhibits osteoblast activity. In fact, some genes involved in osteoblast differentiation and proliferation are down regulated. In addition, some up-regulated genes are involved in apoptosis and in the negative regulation of cell metabolism.

It is worth noting that MG63 is a cell line, not normal osteoblasts. Notwithstanding, the advantage of using a cell line is related to the fact that the reproducibility of the data is higher because there is no patient variability. Primary cell cultures provide a source of normal cells, but they also contain contaminating cells of different types and cells in variable differentiation states. Moreover, the authors chose to perform the experiment after 24 hours to obtain information on the early stages of stimulation that are critical when a prosthesis is inserted in a bone segment.

Conclusion

TP is able to modulate the expression of several genes that cover a broad range of functional activities, giving a first genetic trait of osteoblast response in the presence of this material. The data from this study demonstrated no major adverse effect of TP on cells and, thus, encourages the clinical use of tantalum implants.

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