REPORTS Cell Biology International

Cell Biol. Int. Rep. 17(2):art:e00007.doi:10.1042/CBR20100006

online data

Short Communication

Microarray analysis of ox-LDL (oxidized low-density lipoprotein)-regulated genes in human coronary artery smooth muscle cells

Joe Minta[1]*, James Jungwon Yun*† and Rosanne St. Bernard*

* Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto M5S 1A8, Canada

† Applied Molecular Oncology, Ontario Cancer Institute/University Health Network, Toronto, Ontario M5G 2M9, Canada

Cite this article as: Minta J, Yun JJ and St-Bernard R (2010) Microarray analysis of ox-LDL (oxidized low-density lipoprotein)-regulated genes in human coronary artery smooth muscle cells. Cell Biol. Int. Rep. 17(2):art:e00007.doi:10.1042/CBR20100006

Abstract

Recent studies suggest that circulating LDL (low-density lipoproteins) play a central role in the pathogenesis of atherosclerosis, and the oxidized form (ox-LDL) is highly atherogenic. Deposits of ox-LDL have been found in atherosclerotic plaques, and ox-LDL has been shown to promote monocyte recruitment, foam cell formation and the transition of quiescent and contractile vascular SMCs (smooth muscle cells) to the migratory and proliferative phenotype. SMC phenotype transition and hyperplasia are the pivotal events in the pathogenesis of atherosclerosis. To comprehend the complex molecular mechanisms involved in ox-LDL-mediated SMC phenotype transition, we have compared the differential gene expression profiles of cultured quiescent human coronary artery SMCs with cells induced with ox-LDL for 3 and 21 h using Affymetrix HG-133UA cDNA microarray chips. Assignment of the regulated genes into functional groups indicated that several genes involved in metabolism, membrane transport, cell–cell interactions, signal transduction, transcription, translation, cell migration, proliferation and apoptosis were differentially expressed. Our data suggests that the interaction of ox-LDL with its cognate receptors on SMCs modulates the induction of several growth factors and cytokines, which activate a variety of intracellular signalling mechanisms (including PI3K, MAPK, Jak/STAT, sphingosine, Rho kinase pathways) that contribute to SMC transition from the quiescent and contractile phenotype to the proliferative and migratory phenotype. Our study has also identified several genes (including CDC27, cyclin A1, cyclin G2, glypican 1, MINOR, p15 and apolipoprotein) not previously implicated in ox-LDL-induced SMC phenotype transition and substantially extends the list of potential candidate genes involved in atherogenesis.

Keywords: microarray; oxidized low-density lipoprotein; quantitative PCR; transcriptome; vascular smooth muscle cell

1. Introduction

Atherosclerosis and the subsequent development of occlusive vascular disease is the principal cause of coronary heart disease and cerebral stroke, the most common cause of death and morbidity in industrialized and developing nations (http://www.americanheart.org/presenter.jhtml?identifier=4478). Thus, the understanding of the cellular and molecular mechanism of atherogenesis should provide insight into pharmacological strategies for limiting the initiation and progression of atherosclerosis prior to the development of clinical consequences. Atherosclerosis is a chronic inflammatory disease during which endothelial and SMCs (smooth muscle cells) of the arterial vessel wall are activated by proinflammatory stimuli such as IL-1 and TNFα elaborated by activated macrophages and T cells. It is characterized by complex interactions between a variety of lipids, mononuclear phagocytes and their soluble mediators in the intima and by intimal hyperplasia. Multiple local and systemic risk factors including mechanical shear stress due to haemodynamic changes, hypercholesterolaemia, hypertension and high plasma levels of inflammatory markers may initiate atherosclerosis by inducing endothelial dysfunction and vascular injury (Ross, 1999).

The ox-LDL (oxidized form of low-density lipoprotein) is a major component of cholesterol involved in hypercholesterolaemia, which is a major risk factor. ECs (endothelial cells), vascular SMCs and infiltrating immune cells have been reported to produce superoxide anion and/or hydrogen peroxide, which mediate the oxidation of a lipid component of LDL (Morel et al., 1984; Navab et al., 2004). ox-LDL has been detected in atherosclerosis plaques as well as plasma of atherosclerosis patients, and several lines of evidence have suggested that ox-LDL may play important roles in the pathogenesis and progression of atherosclerosis and the destabilization of the atherosclerotic plaque (Steinberg et al., 1989; Ross, 1999). ox-LDL can bind to scavenger receptors and the LOX-1 (lectin-like ox-LDL receptor-1), and the accumulation of excess cholesterol and cholesteryl esters by macrophages and vascular SMCs leads to the formation of foam cells that are the hallmarks of early fatty streak lesions and atheroma development (Witztum and Steinberg, 1991; Sawamura

[1] To whom correspondence should be addressed (email joe.minta@utoronto.ca).

Abbreviations used: hr, human recombinant; LDL, low-density lipoproteins; MDA, malondialdehyde; n-LDL, non-oxidized LDL; ox-LDL, oxidized low-density lipoproteins; SmBM, SMC basal medium; SMCs, smooth muscle cells; TBARS, thiobarbituric acid-reactive substance.

et al., 1997; Kataoka et al., 2001). ox-LDL has been shown to induce a wide range of biological effects such as SMC proliferation, monocyte chemotaxis and apoptosis/necrosis of vascular ECs and SMCs, depending on the degree of oxidation and the extracellular concentration. Particularly, minimally oxidized LDL has been shown to induce SMC proliferation and migration, which are pivotal events in intimal hyperplasia and atherogenesis. ox-LDL and its lipid constituents may also cause EC dysfunction by inducing the transcription of proatherogenic genes (Kume and Gimbrone, 1994).

ox-LDL-induced proliferation of quiescent SMCs has been associated with the ability of ox-LDL to simultaneously (i) increase the expression and nuclear localization of specific cell cycle-activating proteins (e.g. CDC2, Cdk2, cdk4, cyclin B1, D1 and PCNA1) and cell cycle-inhibiting proteins (e.g. p21 and p27), and (ii) augment intracellular signalling pathways (e.g. PI3K and PLC pathways) involved in the mitogenic response (Zettler et al., 2003). At higher concentrations, ox-LDL has also been shown to be cytotoxic, inducing apoptosis in intimal vascular SMCs and to increase plaque instability and rapture in acute coronary syndromes (Thorne et al., 1996; Okura et al., 2000). ox-LDL-induced apoptosis has been reported to involve both Fas and TNF receptors I and II signalling pathways leading to (i) down-regulation of antiapoptotic proteins of the Bcl-2 family, (ii) up-regulation of apoptotic proteins including caspase 3, and (iii) activation of MAP and Jun kinase-dependent transcription factors (e.g. STAT, NFkB, p53, ATF-2, ELK-1, CREB and AP-1), which may promote apoptosis or growth and survival (Napoli et al., 2000). ox-LDL may thus play an important role in the pathogenesis and development of atherosclerosis by its effect on vascular SMC proliferation, phenotype modulation and apoptosis (Zhao et al., 2005).

The ability of vascular SMCs in the media of arteries to undergo phenotype modulation from the quiescent and contractile state to the proliferative, migratory and synthetic state underlies their crucial role in the development and progression of vascular pathology, such as atherosclerosis and restenosis. Phenotype modulation involves a cascade of events in which different genes are turned on or off in a regulated manner. To gain insight into the early molecular events associated with ox-LDL-mediated SMC phenotype modulation, we used microarray analysis to compare the gene expression profiles of quiescent human coronary artery SMC stimulated with ox-LDL with control cells treated with n-LDL (non-oxidized LDL). Our results show that the 3 and 21 h transcriptional effects of ox-LDL on SMCs were particularly far-reaching, and a number of genes that are involved in various biological mechanisms were differentially regulated. The ox-LDL effect appeared to be mediated via the transcriptional induction of proinflammatory cytokines and growth factors, and these, in turn, initiated multiple signal transduction pathways that induced effector genes of cell proliferation, migration and extracellular matrix formation. Of particular interest is the induced expression of several nuclear receptor transcription factors. We believe that such a comprehensive analysis of the early events of SMC phenotype transition may identify novel targets for drug discovery for the intervention of the progression of atherosclerosis and the development of occlusive vascular complications.

2. Materials and methods

2.1. Oxidation of LDL

LDL (SIGMA Chemical Company) was dialysed in the dark at 4°C for 24 h against three changes of 100 volumes of PBS, pH 7.4, and then sterilized by filtration through 0.45 μm Millipore. n-LDL control and ox-LDL (100 μg/ml) were prepared as described by Steinbrecher et al. (1984) (Morel et al., 1984) by incubation with PBS or freshly prepared $CuSO_4$ solution in PBS (at a final concentration of 5 μM) respectively for 3 and 12 h at 37°C. Then, LDL samples were extensively dialysed against PBS containing 0.1 mM EDTA and sterilized by filtration through 0.22-μm Millipore filters. The samples were stored at 4°C and used within 6 h of preparation.

Lipoprotein concentration was expressed as protein content and was determined using a BCA kit (Pierce) with albumin as standard. The extent of LDL oxidation was assessed by measuring TBARS (thiobarbituric acid-reactive substance), lipid peroxides and conjugated dienes as described by Morel et al. (1984) using MDA (malondialdehyde) as standard, and then, the values were expressed as nmol MDA equivalents per mg or μg of LDL protein. ox-LDL preparations with TBARS ⩾20 nmol MDA/μg were used for SMC treatments. The extent of LDL oxidation was also monitored by the increase in electrophoretic mobility on 0.5% agarose gel in barbital buffer, pH 8.6, relative to n-LDL control.

2.2. Smooth muscle cells

Human coronary artery SMCs were purchased from Clonetics and cultured in SmBM (SMC basal medium) containing SmBM-3 growth supplements [FBS (fetal bovine serum) (5%), bovine insulin (50 ng/ml), hr (human recombinant)-EGF (epidermal growth factor) (5.0 ng/ml), hr-FGF-B (20 ng/ml) and GA-1000 (Gentamicin, Amphotericin B)] supplied by BioWhittaker Inc. The culture medium and FBS contained less than 50 pg of LPS per ml, as measured by the Limulus amoebocyte assay (BioWhittaker Inc.). SMCs were characterized by (i) their typical 'hill and valley' growth pattern, (ii) positive staining with anti-SM-α-actin antibody (Dako Diagnostics) and (iii) negative staining of Factor VIII-related antigen, an endothelial cell marker, using anti-factor VIII antibody (Dako Diagnostics). Cells cultures were used between passages 4 and 7 and in accordance with our institutional guidelines for research on human tissues and cells.

2.3. Isolation of total RNA

Confluent SMC cultures in 10 cm diameter Petri dishes were synchronized to quiescence by incubation for 48 h in SmBM+ 0.5% FBS. The cells were washed and incubated in SmBM+0.5% FBS in the absence or presence of n-LDL or ox-LDL (2 μg/ml) for 3 and 21 h. These two time points were used for the analysis of the regulation of early- and late-response genes, respectively. The reactions were set up in quadruplicates. Total RNA was extracted from the cells using TRIzol reagent (Invitrogen Life Technologies Inc.), and RNA samples from corresponding cell cultures were pooled.

2.4. Microarray analysis of differential gene expression in control n-LDL and ox-LDL-treated SMCs

Total RNA samples were treated with RNase-free DNase, and mRNA was isolated using Oligotex according to the manufacturer's instructions (Qiagen Inc.). Biotinylated cRNA (complementary RNA) samples for chip hybridization were prepared according to protocols supplied by Affymetrix (Affymetrix) and then hybridized to HG-U133A oligonucleotide array Gene Chip (Affymetrix) following the manufacturer's protocol. The arrays were washed, stained with streptavidin–phycoerythrin and scanned. Data files were analysed using Affymetrix GeneChip® Operating Software (GCOS) version 1.0 (Affymetrix).

2.5. Real-time PCR

Quantitative real-time PCR was performed with an ABI Prism 7900HT Sequence Analyzer using the manufacturer's recommended protocol (PerkinElmer Applied Biosystems) to validate differential expression of selected genes. Two different primer sets were designed and synthesized for each investigated gene using Primer Express version 2.0 (PerkinElmer Applied Biosystems). Each reaction was run in triplicate in 10 μl volumes containing 4 μl of diluted first-strand cDNA template, 5 μl of SYBR Green PCR Master Mix, 0.1 μl (50 μM) of each forward and reverse primer and 0.8 μl of H_2O. Samples were incubated at 95°C for 3 min to activate Taq polymerase, and 40 cycles were performed at 95°C for 10 s, at 65°C for 15 s and at 70°C for 20 s. Sequences for the primers used in this study are available upon request.

3. Results and discussion

3.1. Effect of n-LDL and ox-LDL on the proliferation of SMC

Confluent cultures of human coronary artery SMC were grown in the presence of 0.5% FBS for 48 h to induce quiescence. The cultures were then exposed to 2 μg/ml n-LDL or ox-LDL. The culture medium was replaced at day 2, and the cells were detached at day 5 to assess proliferation by cell counts. The mean and S.E.M. were determined for three separate experiments, each performed in quadruplicate. The increase in cell number in cultures containing n-LDL was only 1.3-fold greater than in cells cultured in the presence of 0.5% FBS. However, in the presence of ox-LDL, SMC proliferation was increased 3.1-fold relative to cells grown in 0.5% FBS alone (Figure 1).

3.2. Microarray analysis of differential gene expression in ox-LDL-treated SMCs

The differential gene expression responses of SMCs treated with n-LDL and ox-LDL were analysed using Affymetrix oligonucleotide arrays (HG-U133A). Gene regulation in SMCs by ox-LDL was measured relative to n-LDL-treated SMCs and expressed as NC

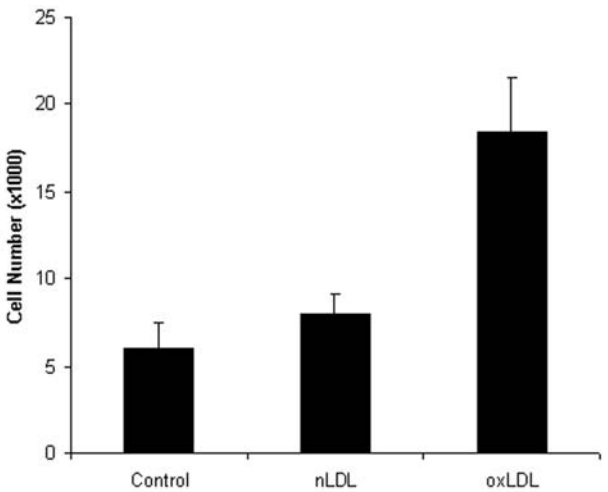

Figure 1 Effect of n (normal) and ox (oxidized) LDL (2 μg/ml) on the proliferation of human coronary artery SMCs

(no change), fold increase or decrease. A total of 1005 genes was found to be differentially regulated by ox-LDL at 3 h (218 genes) and 21 h (833 genes). One hundred and twenty-nine genes were induced, and 89 were suppressed at 3 h, and 311 were induced and 522 were suppressed at 21 h.

3.3. Quantitative real-time PCR validation of microarray analysis

To validate the gene array results, the expression of 24 regulated genes was analysed by quantitative real-time PCR using the expression levels of human GAPDH and beta-2-microglobulin as internal housekeeping gene controls to normalize technical variability between samples (Table 1). These genes were randomly selected from the pool of 80 genes composed of the top 20 genes that were up- or down-regulated at 3 and 21 h. The expression of 16 genes was shown to correlate well in microarray and real-time PCR, whereas the magnitudes of differential expression were somewhat different for eight genes.

3.4. Top 20 SMC genes differentially regulated following 3 and 21 h treatment with ox-LDL

Since it is possible that SMC genes regulated by ox-LDL may be implicated in (i) maintenance of the quiescent phenotype, or (ii) phenotype modulation to the proliferative and synthetic phenotype, we have displayed the top 20 SMC genes regulated by ox-LDL treatments at 3 and 21 h (Table 2). Two genes overexpressed at 3 h, MINOR and NR1D2, are members of nuclear hormone receptor family that function as transcriptional regulators. MINOR is known to exhibit pleiotropic physiological functions including regulation of SMC proliferation (Nomiyama et al., 2006). DLX2 is a transcription regulator that modulates neuron development in ventral embryonic forebrain, and CCL20 is involved in recruitment of activated T cells. RAI3, a G protein-coupled receptor, is implicated in many fundamental cellular processes including

Table 1 Microarray compared with real-time PCR

		Microarray		Real-time PCR		
Accession number	Gene symbol	3 h	21 h	3 h	21 h	Gene title
U12767	NR4A3	37.9	NC	30.6	0.6	Mitogen-induced nuclear orphan receptor (MINOR)
NM_002546	TNFRSF11B	15.1	NC	3.1	0.9	Osteoprotegerin (TNFRSF11B)
NM_004591	SCYA20	14.2	NC	3.8	0.3	Chemokine (cc motif) ligand 20 (CCL20)
N32859	NR1D2	8	NC	3.3	2.2	Nuclear receptor subfamily 1, group D, member 2
U66838	CCNA1	7.6	NC	3.4	1.6	Cyclin A1 (CCNA1)
NM_004405	DLX2	5.1	−1.6	4.8	1.0	Distal-less homoeobox 2 (DLX2)
NM_004904	CREB1	2.9	−1.5	1.0	0.4	cAMP response element-binding protein CRE-Bpa
NM_005544	IRS1	2.8	NC	2.1	0.9	Insulin receptor substrate 1 (IRS1)
NM_002607	PDGFA	2.5	NC	1.8	0.7	Platelet-derived growth factor alpha (PDGFA)
NM_030751	TCF8	−3.3	−4.3	0.9	0.7	Transcription factor 8 (TCF8)
NM_004527	MEOX1	−4.4	−3.2	0.3	0.4	Mesenchyme homoeobox 1 (MEOX1), transcript variant 1
NM_002224	ITPR3	NC	16.8	1.7	2.3	Inositol 1,4,5-triphosphate receptor, type 3 (ITPR3)
NM_005526	HSF1	NC	11	1.6	1.8	Heat shock transcription factor 1 (HSF1)
NM_004672	MAP3K6	NC	8.4	4.5	4.1	MAP 3 kinase 6 (MAP3K6)
NM_003646	DGKZ	NC	6.8	24.8	22.8	Diacylglycerol kinase, zeta (DGKZ)
NM_005483	CHAF1A	NC	5.5	9.2	9.6	Chromatin assembly factor 1, subunit A (CHAF1A)
M64497	NR2F2	NC	3.5	1.3	1.2	Nuclear receptor subfamily 2, group F, member 2
NM_002010	FGF9	NC	2.5	112.5	91.0	Fibroblast growth factor 9 (FGF9)
NM_006166	NFYB	NC	−5.5	1.2	0.9	nuclear transcription factor Y, beta (NFYB)
M68891	GATA2	NC	−7.3	4.3	4.0	GATA-binding protein 2
NM_004622	TSN	NC	−9	1.0	0.5	Translin
NM_001356	DDX3X	NC	−9.5	1.3	1.0	DEAD/H box 3, X-linked (DDX3)
NM_003831	RIOK3	NC	−12.5	0.8	0.5	SudD (suppressor of bimD6 homologue) (SUDD)
NM_000618	IGF1	NC	−12.5	0.4	0.1	Insulin-like growth factor I (somatomedin C)

embryogenesis, cell growth, differentiation and apoptosis (Cheng and Lotan, 1998), and fjx1 is a protein important for growth and differentiation (Ashery-Padan et al., 1999). Growth-promoting genes such as DKK1, CCNA1, HB-EGF (Davis-Fleischer and Besner, 1998), BMP2, BST1 and CDC27 were also up-regulated at 3 h. The majority of the genes up-regulated at 3 h were early response genes, and their induced expression was transient, declining to levels similar to controls in 21 h.

The genes overexpressed at 21 h can be grouped into those involved in metabolism (GBA, PCT2E, SBF1), solute transport (SLC2A6, hTIM44, SLC22A1L), regulation of cell proliferation and differentiation (CDC27, GDF1), transcription regulation (HSF1), inhibition of angiogenesis (GPC1) and signalling (ITPR3, MAP3K6). Several novel genes were also up-regulated at 21 h (KIAA0706, FLJ21935, FLJ14225, KIAA0337).

The transcript with the greatest fold decrease in expression at 3 h was the PBF-1 (papillomavirus regulatory/binding factor), which is a nuclear shuttling transcription factor that mediates inhibition of cell growth (Sichtig et al., 2007). The homoeobox transcription factor, MEOX1, that plays a role in the commitment of mesodermal cells in the developing somite to the skeletal muscle lineage, was also down-regulated (Petropoulos et al., 2004). Genes involved in cell–matrix interactions (liprin-alpha4) and biosynthesis of cholesterol (HMGCS2) were down-regulated. Several metabolic enzymes such as CPE, BCAT1, NUDT4 and PAFAH1B1 were also down-regulated by ox-LDL at 21 h. The

Table 2A Top 20 up-regulated genes at 3 h

Accession number	Gene title	3 h	21 h	Function
U12767	Mitogen-induced nuclear orphan receptor (MINOR)	37.9	NC	Regulation of transcription, DNA-dependent
NM_002546	Osteoprotegerin (TNFRSF11B)	15.1	NC	Regulates bone resorption
NM_004591	Chemokine (cc motif) ligand 20 (CCL20)	14.2	NC	Recruitment of activated T cells
NM_012242	dickkopf (*Xenopus laevis*) homologue 1 (DKK1)	13.8	3.8	Growth factor
NM_003979	Retinoic acid-induced 3 (RAI3)	9.8	NC	Metabotropic glutamate, GABA-B-like receptor
NM_017856	Hypothetical protein FLJ20514	9.3	NC	
N32859	Nuclear receptor subfamily 1, group D, member 2 (NR1D2)	8	NC	Regulation of transcription, DNA-dependent
U66838	Cyclin A1 (CCNA1)	7.6	NC	Regulates cell cycle CDK2 and CDC2
AB007938	KIAA0469	7.5	6.7	
NM_014344	Putative secreted ligand homologous to fjx1	7	7.8	
NM_001945	Heparin-binding EGF-like growth factor	7	−1.8	Binds EGFR; positive regulation of cell proliferation
NM_001200	Bone morphogenetic protein 2 (BMP2)	6.7	NC	Skeletal development
NM_004817	Tight junction protein 2 (zona occludens 2) (TJP2)	6.7	NC	Links junctional membrane proteins to actin
BC000737	Regulator of G-protein signalling 4	6.6	NC	Regulates G-protein-coupled receptor signalling
NM_004334	Bone marrow stromal cell antigen 1 (BST1)	5.4	NC	Facilitates pre-B-cell growth
J00146	Dihydrofolate reductase pseudogene (psi-hd1)	5.4	NC	Converts dihydrofolate into tetrahydrofolate
AA166684	Cell division cycle 27 (CDC27)	5.4	8.2	Mitotic metaphase/anaphase transition
NM_004405	Distal-less homoeobox 2 (DLX2)	5.1	−1.6	Regulation of transcription, DNA-dependent
NM_018039	Hypothetical gene FLJ10251	4.8	NC	
BC001051	ADP-ribosylation factor-like 7	4.7	4.6	Small GTPase-mediated signalling

Table 2B Top 20 down-regulated genes at 3 h

Accession number	Gene title	3 h	21 h	Function
AF263928	Papillomavirus regulatory factor PRF-1 (LOC55893)	−19.7	NC	
NM_017869	BTG3-associated nuclear protein (BANP)	−9.8	NC	
NM_019058	Hypothetical protein (FLJ20500)	−9.8	NC	
AK023365	Liprin-alpha4	−8.6	NC	Regulation of cell–matrix interactions
BC000069	Retinoic acid receptor responder 2	−7.4	NC	Retinoid metabolism
R72286	Microfibrillar-associated protein 4	−6.4	NC	Cell adhesion
NM_001873	Carboxypeptidase E	−6	−10.8	Protein catabolism
NM_000759	Colony-stimulating factor 3 (granulocyte) (CSF3)	−5.8	NC	Positive regulation of cell proliferation
NM_031220	PYK2 N-terminal domain-interacting receptor 1 (NIR1)	−5.6	NC	Receptor PTK; phosphoinositide transporter
M12529	Apolipoprotein E (APOE)	−5.5	−6.2	Lipid metabolism and transport
NM_017606	Hypothetical protein DKFZp434K1210	−5.3	NC	
NM_000312	Protein C (PROC)	−5	NC	Anticoagulant
AF056209	PAM COOH-terminal interactor protein 1 (PCIP1)	−4.9	NC	Neuropeptide signalling pathway
NM_004659	Matrix metalloproteinase 23A (MMP23A)	−4.7	NC	Peptidase
AK023792	Hypothetical protein FLJ13074	−4.5	−3.1	
NM_004527	Mesenchyme homoeobox 1 (MEOX1), transcript variant 1	−4.4	−3.2	Homoeobox; transcription factor activity
NM_005518	3-OH-3-methylglutaryl-CoA synthase 2 (HMGCS2)	−4.3	NC	Cholesterol biosynthesis
NM_030776	Z-DNA-binding protein 1 (ZBP1)	−4.3	NC	Binds left-handed Z-DNA
AL044326	Phosphoribosylformylglycinamidine synthase	−4.2	NC	Purine nucleotide biosynthesis
AA621558	Methionine-tRNA synthetase	−4	NC	Protein biosynthesis
NM_003745	Suppressor of cytokine signalling-1 (SOCS-1)	−4	NC	JAK-STAT cascade inhibitor
NM_001077	UDP glycosyltransferase 2, polypeptide B17 (UGT2B17)	−4	NC	Transferase activity

genes highly repressed at 21 h included a membrane transporter (LMAN1), a transcriptional regulator (TRC8), a translational elongator (EIF2S3), and positive regulators of proliferation (somatomedin C) and cell motility (MSF70, IL8, CXCL5).

3.5. Functional characterization of the ox-LDL-regulated genes

In order to study the effect of ox-LDL treatment on SMC phenotype modulation further, ox-LDL-regulated genes were clustered into functional groups/subgroups using gene annotation information from the Affymetrix database. The functional groups that we believe to be important for SMC phenotype modulation are shown (Table 3). The complete Tables of the functional categories of the regulated SMC genes are available in the online publication (Supplementary Material at http://www.cellbiolintrep.org/cbr/017/cbr0170033add.htm). For most functional groups, it is difficult to speculate on the overall effect of ox-LDL treatment, since a number of genes with various functional effects were modulated at the same time. However, genes categorized under apoptosis (overall inhibition) and cell proliferation (overall induction) predominantly support the proliferative SMC phenotype induced by ox-LDL treatment. Also, the regulation of many cytokines/chemokines (CCL20, CCL7, CSF3, IL6, IL12B, TGFB2, IL11 and CXCL5) and growth factors [PDGFA, GDF1, GDF11, FGF9 and VEGF (vascular endothelial growth factor)] probably also contributes to the induction of proliferation in SMC. Our data show that (i) the majority of the genes were regulated at 21 h compared with 3 h, (ii) very little overlap exists between genes that are differentially regulated at 3 and 21 h, indicating that the early response (3 h) is distinct from the late response (21 h) and subsides by 21 h, and (iii) modulation of genes that are involved in apoptosis, cell proliferation and

Table 2C Top 20 up-regulated genes at 21 h

Accession number	Gene title	3 h	21 h	Function
D13287	Glucosidase, beta; acid (GBA)	NC	27.9	Sphingoglycolipid metabolism
AC005943	Chromosome 19, cosmid R30538	NC	24.3	
BE885926	KIAA0706	NC	17.1	
NM_002224	Inositol 1,4,5-triphosphate receptor, type 3 (ITPR3)	NC	16.8	IP3-sensitive calcium-release channel activity
NM_022772	Hypothetical gene FLJ21935	NC	15.4	
NM_024874	Hypothetical protein FLJ14225	NC	14.7	
NM_017585	Solute carrier family 2 member 6 (SLC2A6)	NC	11.9	Facilitates glucose transport
NM_014786	KIAA0337	NC	11.7	
U93181	Nuclear dual-specificity phosphatase (SBF1)	NC	11.4	Protein dephosphorylation
NM_005526	Heat shock transcription factor 1 (HSF1)	NC	11	Regulation of transcription, DNA-dependent
D64109	Tob family; transducer of ERBB2	NC	10.5	Negative regulation of cell proliferation
AF026030	Mitochondrial inner membrane protein import receptor (hTIM44)	NC	10.1	Import of mitochondrial proteins into mitochondria
BE305165	Phospholipase C, beta 3, neighbour pseudogene	NC	9.5	
NM_002081	Glypican 1 (GPC1)	NC	9.4	Important in endostatin-mediated inhibition of angiogenesis
NM_002861	Phosphate cytidylyltransferase 2, ethanolamine (PCT2E)	NC	8.8	Converts ethanolamine into CDP-ethanolamine
NM_001492	Growth differentiation factor 1 (GDF1)	NC	8.5	Growth factor
NM_004672	MAP 3 kinase 6 (MAP3K6)	NC	8.4	Protein kinase activity
NM_002555	Solute carrier family 22 member 1-like (SLC22A1L)	NC	8.4	Organic cation transporter
N30649	Truncated calcium-binding protein (LOC51149)	2.7	8.4	
AA166684	Cell division cycle 27 (CDC27)	5.4	8.2	Mitotic metaphase/anaphase transition

Table 2D Top 20 down-regulated genes at 21 h

Accession number	Gene title	3 h	21 h	Function
BG257762	Hypothetical protein	NC	−15.6	
NM_005570	Lectin, mannose-binding, 1 (LMAN1)	NC	−15.4	Transport of mannose glycans from ER to Golgi
L19161	Eukaryotic translation initiation factor 2, subunit 3 (EIF2S3)	NC	−13.9	Translational elongation
AK021846	Sec23-interacting protein p125	NC	−13.6	Golgi organization and biogenesis
NM_003831	SudD (suppressor of bimD6 homologue) (SUDD)	NC	−12.5	Chromosome segregation
NM_000618	Insulin-like growth factor I (somatomedin C)	NC	−12.5	RAS signal transduction; regulation of proliferation
NM_004779	Transcription complex, subunit 8 (TRC8)	NC	−12.4	Regulation of transcription, DNA-dependent
NM_001873	Carboxypeptidase E (CPE)	−6	−10.8	Protein catabolism
AI652662	Branched-chain aminotransferase 1, cytosolic (BCAT1)	NC	−10.3	Branched-chain family amino acid biosynthesis
NM_001356	DEAD/H box 3, X-linked (DDX3)	NC	−9.5	ATP-dependent RNA helicase
NM_004622	Translin	NC	−9	A recombination hotspot binding protein
AF130055	Translocating chain-associating membrane protein	NC	−8.7	Protein targeting; co-translational membrane targeting
AJ276395	Migration stimulation factor FN70 (MSF70)	NC	−8.4	Cell motility
NM_003246	Thrombospondin 1	NC	−8.2	Angiogenic activity
AF043337	Interleukin 8 C-terminal variant (IL8)	−1.8	−8.1	Cell motility; intracellular signalling cascade
BG166705	Small inducible cytokine subfamily B (CXC), member 5 (SCYB5/CXCL5)	NC	−8	Chemotaxis; positive regulation of cell proliferation
AF021233	TRAIL-R4-B TNFR superfamily, member 10d	NC	−8	Decoy with truncated death domain; apoptosis
NM_018243	Hypothetical protein FLJ10849	NC	−7.9	
AF191653	Nucleoside diphosphate-linked moiety X-type motif 4 (NUDT4)	NC	−7.9	Cyclic nucleotide metabolism
BE256969	PAF acetylhydrolase, isoform Ib, alpha subunit (PAFAH1B1)	NC	−7.8	Lipid metabolism

cytokines/growth factors could support the induction of proliferation by ox-LDL. The data suggest that the induction of the early response cytokine and growth factor genes may be involved in the induction of the late-response apoptosis, proliferation and structural and ECM (extracellular matrix) genes that are characteristic of the transition of the quiescent SMC to the proliferative and synthetic phenotype.

The profile of the regulated genes observed in this study showed several similarities to the recent report by Deng et al. (2006) on differentially expressed genes in human coronary artery SMCs treated with 40 μg/ml ox-LDL for 24 h. Among the top 50 up- and down-regulated genes, GPC1, DGKZ, DDR2, HMOX1 and FOXD1 were up-regulated in both studies, and thrombospondin 1 and VCAM1 were down-regulated. Whereas COL6A1, PTGF1, CD36, GPR32 and DCN were shown to be down-regulated in our study, these genes were reported to be up-regulated by Deng et al. We believe that the observed differences may be due to the differences in the time of exposure of the cells and the concentrations of ox-LDL used.

Recently Reeve et al. (2007) have reported that treatment of human coronary artery SMCs with ox-LDL induced a gene regulation profile comparable with the gene expression pattern in the aorta of apoE−/− mice. An analysis of expression of antioxidant genes in this study indicated that ox-LDL induced an oxidative stress response in coronary artery SMCs with increased expression of Hsp70, HSF-1, MnSOD, HO-1 (haem oxygenase-1) and ferritin that induced coronary artery SMC death in a caspase-independent manner. In agreement with Reeve et al, our data also document that (i) ox-LDL induced expression of HSF-1, chromobox homologue 6, NQO1 [NAD(P)H dehydrogenase quinone 1], truncated calcium-binding protein and dual-specificity tyrosine-phosphorylation-regulated kinase 2 and (ii) down-regulation of VEGFA (vascular endothelial growth factor A) precursor and API5. This agreement further supports the idea that the effect of ox-LDL on SMC bears relevance to the development of atherosclerosis.

In our study, several transcription and chromatin-remodelling genes were found to be differentially regulated, which have not been reported previously. Thirty-seven genes encoding transcription and chromatin-remodelling factors were induced more than 2-fold (11 at 3 h and 26 at 21 h), whereas 47 genes were down-regulated (12 at 3 h and 35 at 21 h). In particular, NR4A3, NR1D2, NR2F2, Tes1, CREB1 and FOXD1, which were identified as transient immediate early TF genes, were highly up-regulated (3- to 38-fold) within 3 h, whereas HSF1, CHAF1A, TIEG2, GADD153, TRAP95, NR2F2, PURA, SMARCAL1, MNT and SREBF1 were induced 3- to 11-fold at 21 h. Thus, our data are consistent with the notion that the expression of genes that mediate SMC phenotype modulation is regulated by a variety of transcription factors. Furthermore, the rapid, abundant and transient induction of CREB1 and several genes belonging to the nuclear hormone receptor superfamily transcription factors (including NR4A3, NR1D2, NR2F2 and TRAP59) suggest that these transcription factors may play a fundamental role in the induction of genes required for ox-LDL-induced activation and proliferation of quiescent SMC. We believe that our study contributes further to (i) the understanding of the molecular mechanism of ox-LDL-mediated vascular SMC phenotype modulation, and (ii) the identification of several potential biomarker genes for targeted disruption and overexpression for the strategic development of treatment for various vascular diseases.

Author contribution

Joe Minta designed and directed the experiments, discussed the results and wrote the manuscript. James Jungwon Yun performed the quantitative real-time PCR experiments and the classification of regulated genes into functional groups. Rosanne St-Bernard took part in cell growth assays, isolation of total RNA from oxidized

Table 3 **Functional categories**
Numbers in bold indicate increases/decreases greater than 2-fold

Accession number	Gene title	3 h	21 h	Function
Apoptosis				
AF083421	Immediate early response 3 (IER3)	**−2.1**	NC	Apoptosis inhibitor activity
NM_005178	B-cell CLL/lymphoma 3 (BCL3)	**−2.4**	NC	Cell cycle regulation
AF069073	p8 protein homologue (COM1)	NC	**2.9**	Induction of apoptosis
NM_002342	Lymphotoxin beta receptor (LTBR) (TNFRSF3)	NC	**2.8**	TNFR-related protein
NM_005380	Neuroblastoma, suppression of tumourigenicity 1 (NBL1)	NC	**2.2**	Negative regulation of cell cycle
NM_022121	P53-induced protein (PIGPC1)	NC	**−2**	
Z70519	FASApo 1 protein (TNFRSF6)	NC	**−2.2**	Induction of apoptosis
NM_014452	death receptor 6	NC	**−2.4**	Induction of apoptosis
NM_002583	PRKC, apoptosis WT1 regulator (PAWR)	NC	**−2.6**	Negative regulation of proliferation
NM_003842	TNFR superfamily, member 10b (TNFRSF10B)	NC	**−3.4**	Induction of apoptosis
NM_013437	Potential tumour suppressor (ST7)	NC	**−3.6**	Tumour suppressor
NM_021960	Myeloid cell leukaemia sequence 1 (BCL2-related)	NC	**−6**	Apoptotic program
Cell adhesion and cell–cell signalling				
NM_001200	Bone morphogenetic protein 2 (BMP2)	**6.7**	NC	Skeletal development
NM_013372	Cysteine knot superfamily 1 (CKTSF1B1)	**2.8**	NC	Block BMP signalling
AF154054	DRM; cysteine knot superfamily 1	**2.4**	NC	Antagonist of bone morphogenetic protein
NM_016157	Trophinin (TRO)	**−3.7**	NC	Embryo implantation
NM_016223	PKC and casein kinase substrate in neurons 3 (PACSIN3)	NC	**7.3**	Kinesin complex; focal adhesion
NM_004952	Ephrin-A3	NC	**7**	Cell–cell signalling
AI692180	Liprin beta 2	NC	**6.3**	Cell adhesion
NM_002587	Protocadherin 1 (cadherin-like 1) (PCDH1)	NC	**3.9**	Calcium-dependent cell–cell adhesion
NM_002204	Integrin, alpha 3; CD49C (ITGA3)	NC	**2.2**	Cell matrix adhesion
NM_002087	Granulin (GRN)	NC	**2.1**	Cell–cell signalling; signal transduction
BC004542	Plexin B2	NC	**2.1**	Cell adhesion molecule
NM_013231	Fibronectin leucine-rich transmembrane protein 2	NC	**2**	Cell adhesion
NM_001792	Cadherin 2, type 1 (CDH2)	NC	**−2.1**	Cell adhesion
NM_001078	Vascular cell adhesion molecule 1 (VCAM1)	NC	**−2.1**	Adhesion of monocytes and lymphocytes
NM_000885	Alpha 4 subunit of VLA-4 receptor CD49D (ITGA4)	NC	**−2.6**	Cell–matrix adhesion; integrin-mediated signalling pathway
AF152501	Protocadherin beta 8 (PCDHB8)	NC	**−2.6**	Homophilic cell adhesion; cell adhesion
NM_005506	CD36	NC	**−2.8**	Cell adhesion
AF263279	Sialomucin CD164	NC	**−3.3**	Regulation of haematopoiesis; cell adhesion
AU135154	A disintegrin and metalloproteinase domain 10	NC	**−3.4**	Cell–cell signalling
Cell motility and cytoskeleton				
NM_004817	Tight junction protein 2 (zona occludens 2) (TJP2)	**6.7**	NC	Links junctional membrane proteins to actin
NM_012134	Leiomodin 1 (LMOD1)	**3.1**	NC	Tropomyosin binding
AF043337	interleukin 8 C-terminal variant (IL8)	−1.8	**−8.1**	Cell motility; intracellular signalling cascade
D49372	Eotaxin	**−2.2**	**−2.5**	Chemokine; signal transduction
U88321	Beta chemokine Exodus-3	**−3**	**−4.1**	Chemotaxis; cell communication
NM_004999	Myosin VI	NC	**6.1**	Myosin ATPase activity; motor activity
NM_006709	HLA-B-associated transcript 8 (BATS8)	NC	**5**	Histone-lysine *N*-methyltransferase activity
M13452	Lamin A	NC	**4.8**	Interacts with intermediate filaments
BG475299	ems1 (cortactin) p8085 src substrate	NC	**3.1**	Actin-binding protein
NM_020987	Ankyrin 3 (ANK3)	NC	**2.7**	Cytoskeletal anchoring
NM_004395	Drebrin 1 (DBN1)	NC	**2.4**	Actin binding
NM_006848	Hepatitis delta antigen-interacting protein A (DIPA)	NC	**2.4**	Kinesin complex
M86406	Skeletal muscle alpha 2 actinin (ACTN2)	NC	**2.4**	Anchor myofibrillar actin filaments
NM_002373	Microtubule-associated protein	NC	**2.1**	Modulate the assembly of microtubules
NM_002480	Myosin phosphatase, target subunit 1 (MYPT1)	NC	**−2.1**	Regulation of muscle contraction
NM_000366	Tropomyosin 1 (alpha) (TPM1)	NC	**−2.5**	Regulation of muscle contraction
AI214061	Tropomyosin 4	NC	**−2.9**	Constituent of muscle
NM_005722	Actin-related protein 2, yeast homologue (ACTR2)	NC	**−3**	Cell motility
BE675337	Gelsolin	NC	**−6.4**	Actin filament polymerization
BC001352	Tubulin, beta polypeptide	NC	**−6.5**	Microtubule-based movement
AJ276395	Migration stimulation factor FN70	NC	**−8.4**	Cell motility
Cell proliferation				
U66838	Cyclin A1 (CCNA1)	**7.6**	NC	Regulates cell cycle CDK2 and CDC2
AA166684	Cell division cycle 27 (CDC27)	**5.4**	**8.2**	Mitotic metaphase/anaphase transition
NM_021120	Discs, large homologue 3 (DLG3)	**4.6**	NC	Negative regulation of cell proliferation
AI770084	Dihydropyrimidinase-like 2	**3.8**	NC	Regulates axonal growth and branching
AW189518	Piwi (*Drosophila*)-like 1	**3.2**	NC	Oogenesis; spermatogenesis
D84212	Serine/threonine kinase 6 (STK6)	**2.9**	NC	Cell growth
NM_001423	Epithelial membrane protein 1 (EMP1)	**2.3**	NC	Cell proliferation; epidermal differentiation
AF188298	Disabled 2 p93 (DAB2)	**2.2**	NC	Cell proliferation; tumour suppressor
BF514079	Gut-enriched Kruppel-like factor (Gklf)	**2.1**	NC	Inhibition of DNA synthesis

Table 3 **Continued.**

Accession number	Gene title	3 h	21 h	Function
NM_002510	Glycoprotein transmembrane nmb (GPNMB)	**2**	**4.9**	Negative regulation of cell proliferation
NM_002048	Growth arrest-specific 1 (GAS1)	**−3.3**	NC	Cell cycle arrest
D64109	Tob family; transducer of ERBB2	NC	**10.5**	Negative regulation of cell proliferation
NM_003308	Testis specific protein, Y-linked (TSPY)	NC	**5.5**	Spermatogenesis
NM_016195	M-phase phosphoprotein 1 (MPHOSPH1)	NC	**3.4**	Microtubule disassembly at G2- to M-phase
NM_000820	Growth arrest-specific 6 (GAS6), mRNA.	NC	**2.7**	Negative regulation of cell proliferation
M73554	Bcl-1; cyclin D1 (PRAD1)	NC	**2.5**	G1/S transition of mitosis
L13720	Growth-arrest-specific protein (gas)	NC	**2.5**	Negative regulation of cell proliferation
NM_021873	Cell division cycle 25B (CDC25B)	NC	**2.3**	Positive regulation of cell cycle
BC000076	Cyclin D1 (PRAD1)	NC	**2.3**	Activates cdc2 (p34)
NM_004864	Prostate differentiation factor	NC	**2.2**	
AK023348	Clone 24720 epithelin 1 and 2; granulin	NC	**2**	Growth modulatory activity
NM_015392	Neural proliferation differentiation and control 1 (NPDC1)	NC	**2**	
L49506	Cyclin G2	NC	**−2**	Regulates specific cell cycle CDKs
AV700514	Ceroid-lipofuscinosis, neuronal 5	NC	**−2.4**	Cell growth and/or maintenance
NM_012325	Microtubule-associated protein, RPEB family, member 1	NC	**−2.5**	Regulation of cell cycle
NM_004404	Neural precursor cell expressed, developmentally down-regulated 5	NC	**−2.8**	Cell cycle; cytokinesis
NM_006431	Chaperonin-containing TCP1, subunit 2 (beta)	NC	**−3.2**	Cyclin E maturation
M27281	Vascular endothelial growth factor (VEGF)	NC	**−3.9**	Mitogen that specifically acts on endothelial cells
NM_078487	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4) (CDKN2B)	NC	**−4.1**	Inhibits CDK4 and induces G1-phase cell cycle arrest
Extracellular matrix				
NM_004659	Matrix metalloproteinase 23A (MMP23A)	**−4.7**	NC	Peptidase
R72286	Microfibrillar-associated protein 4	**−6.4**	NC	Cell adhesion
AK023365	Liprin-alpha4	**−8.6**	NC	Regulation of cell–matrix interactions
U48734	Non-muscle alpha-actinin	NC	**2.3**	Attachment of microfilament bundles to adherens-type junctions
NM_002421	Matrix metalloproteinase 1 (MMP1)	NC	**2.1**	Collagen I, II and III catabolism
NM_000362	Tissue inhibitor of metalloproteinase-3	NC	**−2**	Metalloendopeptidase inhibitor
BE350145	Collagen, type VI, alpha 1	NC	**−2.6**	Component of microfibrillar structures
NM_000138	Fibrillin 1	NC	**−3**	Component of extracellular microfibrils
U77706	Laminin alpha 4 chain (LAMA4)	NC	**−4**	Non-collagenous constituent of basement membranes
AV721177	Phosphatidylinositol-binding clathrin assembly protein	NC	**−4.5**	Protein complex assembly; vesicle-mediated transport
NM_003246	Thrombospondin 1	NC	**−8.2**	Angiogenic activity
Receptors and membrane proteins				
NM_004334	Bone marrow stromal cell antigen 1 (BST1)	**5.4**	NC	Facilitates pre-B-cell growth
U01157	Glucagon-like peptide-1 receptor	**3.4**	NC	Stimulator of glucose-induced insulin secretion
M90657	Transmembrane 4 superfamily member 1 (TM4SF1)	**2.8**	**2.1**	Protein complex assembly; tumour metastasis
AF043498	Prostate stem cell antigen (PSCA)	**2.7**	NC	Prostrate cancer progression
NM_031220	PYK2 N-terminal domain-interacting receptor 1 (NIR1)	**−5.6**	NC	Receptor PTK; phosphoinositide transporter
BC000069	Retinoic acid receptor responder 2	**−7.4**	NC	Retinoid metabolism
NM_002081	Glypican 1 (GPC1)	NC	**9.4**	Important in endostatin mediated inhibition of angiogenesis
AF020314	Leucocyte membrane antigen (CMRF-35H)	NC	**4.7**	May play a regulatory role in leukocyte function
U72069	Karyopherin (importin) beta 2	NC	**3.7**	Targets cytoplasmic proteins to the nucleus
NM_000319	Peroxisome receptor 1 (PXR1)	NC	**3.4**	Protein-peroxisome targeting
AK022910	Nuclear transport receptor; transportin-SR	NC	**2.8**	Nucleocytoplasmic transport
NM_004616	Transmembrane 4 superfamily member 3 (TM4SF3)	NC	**2.5**	Protein complex assembly
AI859060	Cholinergic receptor, epsilon polypeptide	NC	**2.1**	Synaptic transmission
NM_003801	GPI anchor attachment protein 1 (GPAA1)	NC	**2.1**	Links proteins to cell membrane
NM_014045	Low-density lipoprotein receptor-related protein 10 (LRP10)	NC	**2**	Lipoprotein metabolism
BC000389	Transmembrane 4 superfamily member 7 (TM4SF7)	NC	**2**	Protein complex assembly
NM_003999	Oncostatin M receptor (OSMR)	NC	**−2.4**	IL6 cell surface receptor linked signal transduction
NM_003144	Signal sequence receptor, alpha (SSR1)	NC	**−2.7**	Co-translational membrane targeting
U50748	Leptin receptor short form (db)	NC	**−2.8**	Gene transcription via activation of STAT
U52914	Leptin receptor	NC	**−5.3**	Gene transcription via activation of STAT
NM_002888	Retinoic acid receptor responder 1 (RARRES1)	NC	**−5.3**	Negative regulation of cell proliferation

Table 3 **Continued.**

Accession number	Gene title	3 h	21 h	Function
Signal transduction				
NM_003979	Retinoic acid induced 3 (RAI3)	**9.8**	NC	Metabotropic glutamate, GABA-B-like receptor
BC000737	Regulator of G-protein signalling 4	**6.6**	NC	Regulates G-protein-coupled receptor signalling
BC001051	ADP-ribosylation factor-like 7	**4.7**	**4.6**	Small GTPase-mediated signalling
AF091395	Triple functional domain (PTPRF interacting)	**3.2**	NC	Receptor protein tyrosine phosphatase signalling
AY00716	EH domain-containing 1 (EHD1)	**3**	NC	Endocytosis of IGF1 receptors
M16591	Haemopoietic cell kinase (HCK)	**2.9**	NC	Protein tyrosine kinase activity
BE737620	Myosin phosphatase, target subunit 1	**2.9**	NC	Regulates phosphatidylinositol signalling system
BE466525	Ecotropic viral integration site 1(EVI1)	**2.8**	NC	JUN kinase binding; protein kinase inhibitor
NM_005544	Insulin receptor substrate 1 (IRS1)	**2.8**	NC	Stimulates mitogenesis
NM_006482	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	**2.7**	NC	Protein phosphorylation
AF238083	Sphingosine kinase 1 (SPHK1)	**2.7**	NC	Sphingosine metabolism
AB026436	Dual-specificity phosphatase 10	**2.3**	NC	Inactivate MAPKs
AA780381	MAP2 kinase 3 (ERK kinase 3)	**2.3**	NC	Protein phosphorylation
NM_003749	Insulin receptor substrate-2 (IRS2)	**2.2**	NC	Stimulates mitogenesis
AB023137	A kinase (PRKA) anchor protein 2 (AKAP2)	**2.2**	1.8	Activates adenylate cyclase
AF015043	SH3-domain-binding protein 4 (SH3BP4)	**2.1**	NC	Signal transducer activity
NM_005104	Bromodomain-containing 2 (BRD2)	**2**	NC	Protein serine/threonine kinase activity
AB003476	Gravin; A kinase (PRKA) anchor protein 12	**2**	NC	G-protein-coupled receptor protein signalling
U77914	Jagged 1 (JAG1)	**2**	NC	G-protein-coupled receptor protein signalling, ligand in the Notch signalling pathway
NM_002648	Protein kinase-related oncogene (PIM1)	**−2.9**	NC	Haematopoietic development
L37882	Frizzled homology 2 (FZD2)	**−2.9**	**2.2**	Signal transduction; intracellular Ca release
NM_005204	MAP3 kinase 8 (MAP3K8)	**−3**	NC	Ser/thr kinase; regulation of cellular transformation
NM_004842	A kinase (PRKA) anchor protein 7 (AKAP7)	**−3.9**	NC	Protein kinase A anchor protein activity
NM_003745	Suppressor of cytokine signalling-1 (SOCS-1)	**−4**	NC	JAK-STAT cascade inhibitor
NM_002224	Inositol 1,4,5-triphosphate receptor, type 3 (ITPR3)	NC	**16.8**	IP3-sensitive calcium-release channel activity
U93181	Nuclear dual-specificity phosphatase (SBF1)	NC	**11.4**	Protein dephosphorylation
NM_004672	MAP 3 kinase 6 (MAP3K6)	NC	**8.4**	Protein kinase activity
NM_003646	Diacylglycerol kinase, zeta (DGKZ)	NC	**6.8**	Phosphatidylinositol signalling
NM_030981	Small GTP-binding protein (RAB1B)	NC	**4.3**	Ras; small monomeric GTPase activity
AF272035	Rag C protein (GTR2)	NC	**3.4**	Small monomeric GTPase activity
U96922	Inositol polyphosphate-4-phosphatase, type II alpha	NC	**3.3**	Regulates phosphatidylinositol signalling system
BF062886	Vaccinia-related kinase 3 (VRK3)	NC	**3.3**	Protein phosphorylation
AV700224	Casein kinase 1, delta	NC	**3.1**	Protein phosphorylation
NM_017572	G-protein-coupled receptor kinase 7 (GPRK7)	NC	**2.7**	Protein phosphorylation
BE138888	GTP-binding protein Rac2	NC	**2.6**	Small GTPase-mediated signal transduction
AF022212	Rho GTPase-activating protein 6 isoform 2	NC	**2.6**	Actin filament polymerization
NM_002547	Oligophrenin 1 (OPHN1)	NC	**2.5**	Rho GTPase-activating protein; cell migration
M55268	Casein kinase 2, alpha prime polypeptide (CSNK2A2)	NC	**2.3**	Protein phosphorylation
NM_016602	CC chemokine receptor 10 (CCR10)	NC	**2.3**	G-protein-coupled receptor protein signalling
NM_006182	Discoidin domain receptor family, member 2 (DDR2)	NC	**2.3**	Receptor protein tyrosine kinase
NM_002712	Protein phosphatase 1, regulatory subunit 7 (PPP1R7)	NC	**2.2**	Protein dephosphorylation
AB009358	MAP2 kinase 7; JNK-activating kinase 2	NC	**−2**	Specific activator of JNK1 and JNK2
NM_002356	Myristoylated alanine-rich protein kinase C substrate (MARCKS)	NC	**−2**	A filamentous actin cross-linking protein
NM_012250	Oncogene TC21 (TC21)	NC	**−2**	Small GTPase-mediated signal transduction
NM_000291	Phosphoglycerate kinase 1 (PGK1)	NC	**−2**	Phosphoglycerate kinase activity
NM_005607	PTK2 protein tyrosine kinase 2 (PTK2)	NC	**−2**	Protein kinase activity
NM_003022	SH3 domain-binding glutamic acid-rich protein like (SH3BGRL)	NC	**−2**	SH3/SH2 adaptor protein activity
NM_022650	GTPase-activating protein (GAP)	NC	**−2.1**	Bind activated Rho GTPases and stimulate GTP hydrolysis
NM_005261	GTP-binding protein overexpressed in skeletal muscle (GEM)	NC	**−2.1**	Small GTPase-mediated signal transduction
NM_004578	Ras-associated protein (RAB4)	NC	**−2.1**	Rho small monomeric GTPase activity
AI571798	Rho GDP dissociation inhibitor (GDI) alpha	NC	**−2.1**	Negative regulation of cell adhesion
NM_006241	Protein phosphatase 1, regulatory (inhibitor) subunit 2 (PPP1R2)	NC	**−2.2**	Ser-/thr-specific protein phosphatase inhibitor

Table 3 **Continued.**

Accession number	Gene title	3 h	21 h	Function
AL136139	Enhancer of filamentation 1 (HEF1)	NC	**−2.3**	Integrin-initiated cytoskeleton-linked signalling
NM_016322	GTPase Rab14	NC	**−2.3**	Ras; small monomeric GTPase activity
BC005122	ADP-ribosylation factor GTPase activating protein 1	NC	**−2.4**	Regulation of signalling, growth by hydrolysis of GTP
AF218074	MAP3 kinase 7	NC	**−2.4**	Phosphorylates MKK6 to stimulate JNK; NFKB translocation
AF001362	Jak2 kinase (JAK2)	NC	**−2.5**	Protein tyrosine kinase activity; JAK-STAT cascade
NM_002731	Protein kinase, cAMP-dependent, catalytic, beta (PRKACB)	NC	**−2.6**	Protein kinase activity
NM_002716	Protein phosphatase 2 regulatory subunit A beta isoform	NC	**−2.6**	Regulation of proliferation, contraction, transcription
AF002280	Alpha-actinin-2 associated LIM protein alternatively spliced	NC	**−2.7**	Interacts with alpha-actinin-2 in cytoskeletal assembly
NM_003507	Frizzled (*Drosophila*) homologue 7 (FZD7)	NC	**−2.7**	Fz7-mediated signalling controls cell sorting in mesoderm
NM_004161	RAB1, member RAS oncogene family	NC	**−2.7**	Small GTPase-mediated signalling; vesicle-mediated transport
J03005	G-protein alpha-inhibiting activity polypeptide 3 (GNAI3)	NC	**−2.8**	G-protein-coupled receptor protein signalling pathway
NM_003463	Protein tyrosine phosphatase type IVA, member 1	NC	**−2.9**	Protein dephosphorylation; oncogenesis
NM_006575	MAP 4 kinase 5	NC	**−3**	Activates JNK but not ERK1
M18468	Protein kinase, cAMP-dependent, regulatory, type I, alpha	NC	**−3.1**	cAMP-dependent protein kinase
NM_001506	G-protein-coupled receptor 32 (GPR32)	NC	**−3.2**	G-protein-coupled receptor protein signalling
NM_005242	Coagulation factor II (thrombin) receptor-like 1 (F2RL1)	NC	**−3.3**	G-protein-coupled receptor protein signalling pathway
AF092132	p21 (CDKN1A)-activated kinase 2 (PAK2)	NC	**−3.5**	Negative regulation of protein kinase activity
NM_000945	Calcineurin B, type I (CNB1)	NC	**−3.6**	Ca-dependent ser/thr phosphatase activity; calcium binding
NM_004162	RAB5A, member RAS oncogene family	NC	**−3.6**	Bind GTP and exhibits GTPase activity; regulation of endocytosis
X75208	HEK2 protein tyrosine kinase receptor	NC	**−3.7**	Receptor tyrosine kinase signalling
NM_006654	FGF receptor substrate 2 (FRS2)	NC	**−3.8**	FGF signalling; cell growth and differentiation
NM_003688	Calcium Calmodulin-dependent serine protein kinase (CASK)	NC	**−4.2**	Cytoskeletal membrane scaffold; cortical cytoskeleton signalling
AF127481	Dual-specificity phosphatase 1 (DUSP1)	NC	**−4.4**	Dephosphorylate and inactivates p44MAPK (ERK1)
AW665024	Protein tyrosine kinase 9	NC	**−4.7**	Protein phosphorylation
NM_016277	RAB23, member RAS oncogene family	NC	**−4.9**	Small GTPase-mediated signalling; intracellular protein transport
S69182	Protein tyrosine phosphatase (PTPG1); non-receptor type 12	NC	**−5.5**	Protein dephosphorylation
NM_001346	Diacylglycerol kinase, gamma (DGKG)	NC	**−5.6**	PKC activation
Z25435	Protein-ser/thr kinase gene	NC	**−5.8**	Protein phosphorylation
AF051311	Ras-GTPase activating protein SH3 domain-binding protein 2	NC	**−6.1**	RAS protein signal transduction
NM_002184	gp130, oncostatin M receptor	NC	**−7**	Cell surface receptor-linked signal transduction
NM_002869	RAB6, member RAS oncogene family	NC	**−7.4**	Small GTPase-mediated signalling; non-selective vesicle transport
AF021233	TRAIL-R4-B TNFR superfamily, member 10d	NC	**−8**	Decoy with truncated death domain; apoptosis
Transcription and translation				
U12767	Mitogen-induced nuclear orphan receptor (MINOR)	**37.9**	NC	Regulation of transcription, DNA-dependent
N32859	Nuclear receptor subfamily 1, group D, member 2	**8**	NC	Regulation of transcription, DNA-dependent
NM_004405	Distal-less homoeobox 2 (DLX2)	**5.1**	−1.6	Regulation of transcription, DNA-dependent
S77154	Beta-type transcription factor homologue human	**3.2**	NC	Regulation of transcription, DNA-dependent
NM_004904	cAMP response element-binding protein CRE-Bpa	**2.9**	−1.5	Transcription factor activated by translocation of PKC
NM_006981	Nuclear receptor subfamily 4, group A, member 3 (NR4A3)	**2.8**	NC	Regulation of transcription, DNA-dependent
NM_004472	Forkhead box D1 (FOXD1)	**2.6**	NC	Regulation of transcription, DNA-dependent
AL021977	V-maf musculoaponeurotic fibrosarcoma oncogene homologue F	**2.2**	**2.2**	Regulation of transcription, DNA-dependent
NM_003201	Transcription factor 6-like 1 (TCF6L1)	**2.1**	NC	Mitochondrial DNA transcriptional activator
NM_022898	B-cell lymphoma/leukaemia 11B (BCL11B)	**2**	NC	Regulation of transcription, zinc finger protein
M83667	CCAAT-enhancer-binding protein (CEBP), delta (CEBPD)	**−2.1**	NC	Regulation of transcription, DNA-dependent

Table 3 **Continued.**

Accession number	Gene title	3 h	21 h	Function
L07648	MAX-interacting protein 1 (MXI1)	**−2.1**	NC	Transcription factor
NM_005384	Nuclear factor, interleukin 3 regulated (NFIL3)	**−2.2**	NC	Transcriptional co-repressor
BE542323	TONDU	**−2.2**	NC	Regulation of transcription, DNA-dependent
NM_014112	Trichorhinophalangeal syndrome I gene (TRPS1)	**−2.2**	NC	Zn finger transcription factor
NM_003670	Basic helix-loop-helix domain containing, class B, 2	**−2.4**	**−3.9**	Regulation of transcription, DNA-dependent
BG250310	Zinc finger protein 36, C3H type-like 1 (ZFP36L1)	**−2.9**	NC	Transcription factor
AF055993	Sin3-associated polypeptide (SAP30)	**−3.2**	NC	Transcription co-repressor activity
NM_020529	NFk light polypeptide gene enhancer in B-cells inhibitor, alpha	**−3.3**	NC	Cytoplasmic sequestering of NF-kappaB
NM_030751	Transcription factor 8 (TCF8)	**−3.3**	**−4.3**	Represses interleukin 2 expression
NM_004527	Mesenchyme homoeobox 1 (MEOX1), transcript variant 1	**−4.4**	**−3.2**	Homoeobox; transcription factor activity
NM_005526	Heat shock transcription factor 1 (HSF1)	NC	**11**	Regulation of transcription, DNA-dependent
AB015332	Neighbour of A-kinase-anchoring protein 95	NC	**6.1**	DEAD/H-box RNA helicase binding
NM_005483	Chromatin assembly factor 1, subunit A (CHAF1A)	NC	**5.5**	Assembles histone octamers onto replicating DNA
NM_003597	TGFB-inducible early growth response 2	NC	**5.5**	Transcriptional regulator
NM_004083	DNA-damage-inducible transcript 3	NC	**4.3**	A dominant-negative inhibitor of C/EBP and LAP
AL161985	Transcription factor binding to IGHM enhancer 3	NC	**4.2**	Regulation of transcription, DNA-dependent
AF106934	Thyroid hormone receptor-associated protein (TRAP95)	NC	**3.7**	Transcription co-activator
M64497	Nuclear receptor subfamily 2, group F, member 2	NC	**3.5**	Transcription co-repressor activity
AB019219	Similar to yeast pre-mRNA splicing factors, Prp1Zer1 and Prp6	NC	**3.5**	Spliceosome assembly
U67734	HIV-1 Tat interactive protein	NC	**3.2**	Transcriptional co-activator
NM_005859	Purine-rich element-binding protein A (PURA)	NC	**3.2**	Regulation of transcription, binds to GAGA boxes
X72631	Rev-ErbA alpha	NC	**3**	Regulation of transcription, DNA-dependent
U19769	Centromere protein F (mitosin) (CENPF)	NC	**2.8**	Regulation of mitosis
NM_014140	HepA-related protein (HARP)	NC	**2.8**	ATP-dependent helicase
AC004908	Ribosomal protein L23a	NC	**2.8**	Protein biosynthesis
NM_006943	SRY (sex-determining region Y)-box 22 (SOX22)	NC	**2.8**	Regulation of transcription from Pol II promoter
NM_020310	MAX-binding protein (MNT)	NC	**2.7**	Regulation of transcription; negative regulation of cell proliferation
BC002704	Signal transducer and activator of transcription 1 (STAT1)	NC	**2.7**	Transcription from Pol II promoter
AF295773	Ral guanine nucleotide dissociation stimulator (RALGDS)	NC	**2.6**	RAS protein signal transduction
AF055078	Zinc finger protein 42 (ZNF42)	NC	**2.6**	Regulation of transcription, DNA-dependent
U79283	Albumin D-box-binding protein	NC	**2.5**	Regulation of transcription
AI884867	Ribosomal protein L26	NC	**2.4**	Protein biosynthesis
NM_006736	Hsp, neuronal DNAJ-like 1 (HSJ1) subfamily B, member 2	NC	**2.2**	Co-chaperone activity
NM_004176	Sterol regulatory element binding transcription factor 1 (SREBF1)	NC	**2.2**	Regulation of transcription; LDL metabolism regulation
NM_014292	Chromobox homologue 6	NC	**2.1**	Regulation of transcription; chromatin modification
AW517464	Ribosomal protein L3	NC	**2.1**	Protein biosynthesis
NM_003925	Methyl-CpG binding endonuclease (MED1)	NC	**−2**	DNA repair
NM_000176.1	Nuclear receptor subfamily 3, group C, member 1 (NR3C1)	NC	**−2**	Regulation of transcription, DNA-dependent
AF098483	PC4- and SFRS1-interacting protein 2 (PSIP2)	NC	**−2**	Transcriptional co-activators
AK026426.1	SWI-/SNF-related, matrix associated, subfamily a, member 1	NC	**−2**	Chromatin modelling
NM_004500	Heterogeneous nuclear ribonucleoprotein C (C1C2)	NC	**−2.1**	mRNA Splicing
NM_014319	Integral inner nuclear membrane protein (MAN1)	NC	**−2.1**	Nuclear membrane localization
NM_005324	H3 histone, family 3B (H3.3B)	NC	**−2.2**	Nucleosome assembly
D13889	Inhibitor of DNA-binding 1 (ID1)	NC	**−2.2**	Regulation of transcription from Pol II promoter
AK021418	Putative RNA helicase	NC	**−2.2**	rRNA processing
BC000451	Splicing factor, arginine-/serine-rich 10	NC	**−2.2**	mRNA splicing
AL136621	Zinc finger protein 198	NC	**−2.2**	Protein binding
NM_004379	cAMP-responsive element-binding protein 1	NC	**−2.3**	Regulation of transcription, DNA-dependent
M62829	Early growth response 1 (EGR1)	NC	**−2.3**	Regulation of transcription
AA679988	Polypyrimidine tract-binding protein 1(PTPB1)	NC	**−2.3**	mRNA splicing
BC000627	Signal transducer and activator of transcription 3 (STAT 3)	NC	**−2.3**	JAK-STAT cascade; transcription factor activity
NM_012266	DnaJ (Hsp40) homologue, subfamily B, member 5	NC	**−2.4**	Response to stress

Table 3 **Continued.**

Accession number	Gene title	3 h	21 h	Function
BC000806	Polymerase (RNA) II (DNA directed) polypeptide K (POLR2K)	NC	**−2.4**	Transcription from Pol III promoter
NM_003016	Splicing factor, arginine-/serine-rich 2	NC	**−2.4**	mRNA splicing
BC000997	Splicing factor, arginine-/serine-rich 7 (SFRS7)	NC	**−2.5**	mRNA processing
AF309553	Meiotic recombination protein REC14	NC	**−2.6**	Meiotic recombination
NM_006902	Paired mesoderm homoeobox 1a (PMX1a)	NC	**−2.6**	Regulation of transcription, DNA-dependent
AL117487	Transcriptional adaptor 3-like (ADA3)	NC	**−2.6**	Transcriptional activity
M94630	Heterogeneous nuclear ribonucleoprotein D (HNRPD)	NC	**−2.9**	RNA processing
D13891	Inhibitor of DNA-binding 2 (ID2)	NC	**−2.9**	Transcriptional repressor
AL553320	Stress-induced phosphoprotein 1 (STIP1)	NC	**−2.9**	Association of molecular chaperones HSP70 and HSP90
L23959	Transcription factor Dp-1 (TFDP1)	NC	**−2.9**	Regulation of transcription from Pol II promoter
NM_006265	RAD21 (*S. pombe*) homologue	NC	**−3**	Chromosome segregation
NM_006924	Splicing factor, arginine-/serine-rich 1 (SFRS1)	NC	**−3**	mRNA splice site selection
NM_004779	CCR4-NOT transcription complex, subunit 8 (CNOT8)	NC	**−3.1**	Regulation of transcription, DNA-dependent
AF039942	HCF-binding transcription factor Zhangfei (ZF)	NC	**−3.2**	Regulation of transcription, DNA-dependent
AB009023	RNA guanylyltransferase and 5-phosphatase (RNGTT)	NC	**−3.2**	mRNA capping
NM_003017	Splicing factor, arginine-/serine-rich 3 (SFRS3)	NC	**−3.3**	mRNA splicing
M97935	Transcription factor ISGF-3	NC	**−3.3**	IRF; transcription factor activity
BC001255	Nuclear cap-binding protein subunit 2 (NCBP2)	NC	**−3.5**	snRNA-nucleus export
U12170	Zinc finger homoeodomain protein; transcription factor 8	NC	**−3.7**	Represses interleukin 2 expression
AF061261	Muscleblind-like 2 (*Drosophila*) (MBNL2)	NC	**−4.2**	Transcription factor activity
AF072814	PHD finger DNA-binding protein isoform 1 (M96)	NC	**−4.3**	regulation of transcription, DNA-dependent
BF983406	heterogeneous nuclear ribonucleoprotein H1	NC	**−4.4**	RNA processing
NM_021038	Muscleblind (*Drosophila*)-like	NC	**−4.8**	Nucleic acid-binding activity
AI217362	Trinucleotide repeat containing 11 (THR-associated protein)	NC	**−5**	Regulation of transcription, DNA-dependent
NM_006166	Nuclear transcription factor Y, beta (NFYB)	NC	**−5.5**	Regulation of transcription, DNA-dependent
U71300	snRNA activating protein complex subunit (SNAP50)	NC	**−5.5**	snRNA transcription
NM_007034	DnaJ (Hsp40) homologue, subfamily B, member 4	NC	**−6.3**	Heat shock protein activity
M68891	GATA-binding protein 2	NC	**−7.3**	Regulation of transcription, DNA-dependent
NM_004622	Translin	NC	**−9**	A recombination hotspot-binding protein
NM_001356	DEAD/H box 3, X-linked (DDX3)	NC	**−9.5**	ATP-dependent RNA helicase
NM_004779	Transcription complex, subunit 8	NC	**−12.4**	Regulation of transcription, DNA-dependent
NM_003831	SudD (suppressor of bimD6 homologue) (SUDD)	NC	**−12.5**	Chromosome segregation
Cytokine and growth factor				
NM_002546	Osteoprotegerin (TNFRSF11B)	**15.1**	NC	Regulates bone resorption
NM_004591	Chemokine (cc motif) ligand 20 (CCL20)	**14.2**	NC	Recruitment of activated T cells
NM_012242	dickkopf (*Xenopus laevis*) homologue 1 (DKK1)	**13.8**	**3.8**	Growth factor
NM_001945	Heparin-binding EGF-like growth factor	**7**	−1.8	Binds EGFR; positive regulation of cell proliferation
NM_000417	Interleukin 2 receptor, alpha (IL2RA)	**3.8**	NC	T-cell proliferation
NM_002506	Nerve growth factor, beta (NGFB)	**2.8**	1.6	Survival of nerve cells
NM_002607	Platelet-derived growth factor alpha (PDGFA)	**2.5**	NC	Cell proliferation
NM_013246	Cardiotrophin-like cytokine (CLC)	**2**	NC	IL-6 family of cytokines
AF229253	FGF2-interacting factor (API5)	−1.5	**−2.9**	Apoptosis inhibitor
S69738	Monocyte chemotactic protein human (MCP-1)	−1.8	**−2**	Recruitment of monocytes
AF125392	Insulin-induced protein 2	**−2**	**−2.3**	
NM_006273	Chemokine (C–C motif) ligand 7 (CCL7)	**−2**	**−2.6**	Monocytes/macrophages recruitment
X16323	Hepatocyte growth factor (HGF)	**−2.3**	**−4.1**	Cell proliferation
M59465	TNF alpha-induced protein 3 (TNFAIP3)	**−2.7**	−1.9	Inhibits NFk-B and TNF-mediated apoptosis
M57731	Gro-beta; GRO2 oncogene	**−3.1**	NC	G-protein-coupled receptor protein signalling pathway
NM_000759	Colony-stimulating factor 3 (granulocyte) (CSF3)	**−5.8**	NC	Positive regulation of cell proliferation
NM_001492	Growth differentiation factor 1 (GDF1)	NC	**8.5**	Growth factor
AF028333	Growth differentiation factor-11 (GDF11)	NC	**4.8**	Neurogenesis; skeletal development
NM_021805	Single Ig IL-1R-related molecule (SIGIRR)	NC	**4.7**	Subtype of the IL-1R superfamily
NM_002010	Fibroblast growth factor 9 (FGF9)	NC	**2.5**	Cell proliferation
NM_000599	Insulin-like growth factor-binding protein 5 (IGFBP5)	NC	**2.1**	IGF binding; regulation of cell growth
NM_000600	Interleukin 6 (IL6)	NC	**−2**	Acute-phase response; cell proliferation
AF214570	Vascular endothelial growth factor (VEGF)	NC	**−2.4**	Positive regulation of cell proliferation; angiogenesis
NM_000598	Insulin-like growth factor binding protein 3	NC	**−2.5**	Regulation of cell growth

Table 3 **Continued.**

Accession number	Gene title	3 h	21 h	Function
U81380	Interleukin-13 receptor soluble form	NC	−**2.6**	IL-13 regulation
NM_002187	Interleukin 12B (IL12B)	NC	−**2.7**	Positive regulation of activated T-cell proliferation
BC001281	TNF receptor superfamily, member 10b	NC	−**2.8**	Induction of apoptosis via death domain receptors
D78132	Ras homologue enriched in brain 2 (RHEB2)	NC	−**2.9**	Ras-related growth factor
U19495	Intercrine-alpha stromal cell-derived factor 1	NC	−**3.1**	Regulation of actin polymerization; cell–cell signalling
AW770896	Insulin-like growth factor-binding protein 7	NC	−**3.7**	Negative regulation of cell proliferation
M19154	Transforming growth factor, beta 2 (TGFB2)	NC	−**4**	Regulation of proliferation
NM_005711	EGF-like repeats and discoidin I-like domains 3	NC	−**5.3**	Integrin binding; cell adhesion
NM_000641	Interleukin 11 (IL11)	NC	−**6.4**	Positive regulation of cell proliferation
BG166705	Small inducible cytokine subfamily B (CXC), member 5 (SCYB5)	NC	−**8**	Chemotaxis; positive regulation of cell proliferation
NM_000618	Insulin-like growth factor I (somatomedin C)	NC	−**12.5**	RAS signal transduction; regulation of proliferation

and non-oxidized-LDL-treated cells and the initial assignment of biological functions to genes regulated in the microarray data.

Funding

This work was supported by a grant from the University of Toronto, Faculty of Medicine Dean's Research Fund.

References

Ashery-Padan R, Alvarez-Bolado G, Klamt B, Gessler M, Gruss P. Fjx1, the murine homologue of the *Drosophila* four-jointed gene, codes for a putative secreted protein expressed in restricted domains of the developing and adult brain. Mech Dev 1999;80:213–7.

Cheng Y, Lotan R. Molecular cloning and characterization of a novel retinoic acid-inducible gene that encodes a putative G protein-coupled receptor. J Biol Chem 1998;273:35008–15.

Davis-Fleischer KM, Besner GE. Structure and function of heparin-binding EGF-like growth factor (HB-EGF). Front Biosci 1998;3:d288–99.

Deng DX, Spin JM, Tsalenko A, Vailaya A, Ben-Dor A, Yakhini Z et al. Molecular signatures determining coronary artery and saphenous vein smooth muscle cell phenotypes: distinct responses to stimuli. Arterioscler Thromb Vasc Biol 2006;26:1058–65.

Kataoka H, Kume N, Miyamoto S, Minami M, Morimoto M, Hayashida K et al. Oxidized LDL modulates Bax/Bcl-2 through the lectinlike Ox-LDL receptor-1 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2001;21:955–60.

Kume N, Gimbrone MA, Jr. Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. J Clin Invest 1994;93:907–11.

Morel DW, DiCorleto PE, Chisolm GM. Endothelial and smooth muscle cells alter low density lipoprotein *in vitro* by free radical oxidation. Arteriosclerosis 1984;4:357–64.

Napoli C, Quehenberger O, De Nigris F, Abete P, Glass CK, Palinski W. Mildly oxidized low density lipoprotein activates multiple apoptotic signaling pathways in human coronary cells. FASEB J 2000;14:1996–2007.

Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC et al. The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J Lipid Res 2004;45:993–1007.

Nomiyama T, Nakamachi T, Gizard F, Heywood EB, Jones KL, Ohkura N et al. The NR4A orphan nuclear receptor NOR1 is induced by platelet-derived growth factor and mediates vascular smooth muscle cell proliferation. J Biol Chem 2006;281:33467–76.

Okura Y, Brink M, Itabe H, Scheidegger KJ, Kalangos A, Delafontaine P. Oxidized low-density lipoprotein is associated with apoptosis of vascular smooth muscle cells in human atherosclerotic plaques. Circulation 2000;102:2680–6.

Petropoulos H, Gianakopoulos PJ, Ridgeway AG, Skerjanc IS. Disruption of Meox or Gli activity ablates skeletal myogenesis in P19 cells. J Biol Chem 2004;279:23874–81.

Reeve JL, Stenson-Cox C, O'Doherty A, Porn-Ares I, Ares M, O'Brien T et al. OxLDL-induced gene expression patterns in CASMC are mimicked in apoE−/− mice aortas. Biochem Biophys Res Commun 2007;356:681–6.

Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999;138:S419–20.

Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y et al. An endothelial receptor for oxidized low-density lipoprotein. Nature 1997;386:73–7.

Sichtig N, Silling S, Steger G. Papillomavirus binding factor (PBF)-mediated inhibition of cell growth is regulated by 14-3-3beta. Arch Biochem Biophys 2007;464:90–9.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915–24.

Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci U S A 1984;81:3883–7.

Thorne SA, Abbot SE, Winyard PG, Blake DR, Mills PG. Extent of oxidative modification of low density lipoprotein determines the degree of cytotoxicity to human coronary artery cells. Heart 1996;75:11–6.

Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1991;88:1785–92.

Zettler ME, Prociuk MA, Austria JA, Massaeli H, Zhong G, Pierce GN. OxLDL stimulates cell proliferation through a general induction of cell cycle proteins. Am J Physiol Heart Circ Physiol 2003;284:H644–53.

Zhao GF, Seng JJ, Zhang H, She MP. Effects of oxidized low density lipoprotein on the growth of human artery smooth muscle cells. Chin Med J (Engl) 2005;118:1973–8.

Received 21 October 2010/accepted 27 October 2010

Published as Immediate Publication 1 November 2010, doi 10.1042/CBR20100006