SPANDIDOS PUBLICATIONS

Identification of lipid droplet-associated proteins in the formation of macrophage-derived foam cells using microarrays

HANG LI[1*], YUE SONG[1*], FANFAN LI[1], LIJUN ZHANG[2], YU GU[1], LIYING ZHANG[1], LINA JIANG[1], WENPENG DONG[3], JING YE[1] and QING LI[1]

[1]State Key Laboratory of Cancer Biology and Department of Pathology, Xijing Hospital, Fourth Military Medical University, Xi'an; [2]Department of Clinical Laboratory, Tangdu Hospital; [3]State Key Laboratory of Cancer Biology and Xijing Gastroenteropathy Hospital, Fourth Military Medical University, Xi'an, P.R. China

Received March 17, 2010; Accepted May 14, 2010

DOI: 10.3892/ijmm_00000457

Abstract. A large number of macrophage-derived foam cells stores excessive neutral lipids in intracellular droplets, and plays a major role during the development of atherosclerosis. The formation and catabolism of intracellular lipid droplets (LDs) are regulated by LD-associated proteins, a group of proteins which are located on the surface of LDs and regulate the formation, morphology and lipolysis of LDs. In order to illustrate the function of LD-associated proteins during the process of atherosclerosis, the foam cell model is induced by oxidized low-density lipoprotein (ox-LDL) in macrophages originated from the THP-1 cell line, and cDNA microarrays are used to monitor the gene expression profiles of LD-associated proteins. Gene expression data show that 2% of changed genes are lipid binding genes during the transformation of foam cells. The major candidate genes, the cell death-inducing DFF45-like effector (CIDE) family and Perilipin, Adipophilin, and TIP47 (PAT) family, have different alterations during the formation of foam cells. CIDEB, CIDEC, Adipophilin, S3-12 and LSDP5 were up-regulated, while TIP47 was down-regulated. There was no significant change in CIDEA and Perilipin. These results were confirmed by real-time PCR and immunoblotting. This study presents a comprehensive analysis of the gene expression of LD-associated proteins during the differentiation of human foam cells, which may play an important role in the process of atherosclerosis.

Correspondence to: Dr Qing Li or Dr Jing Ye, Department of Pathology, Xijing Hospital, Fourth Military Medical University, Xi'an, CN-710032, P.R. China
E-mail: liqing@fmmu.edu.cn
E-mail: yejing@fmmu.edu.cn

*Contributed equally

Key words: lipid droplet associated protein, foam cell, atherosclerosis

Introduction

Abnormal lipid deposition and accumulation in the artery wall lead to the formation of fatty streaks of atherosclerosis. In these fatty streak lesions, uptake of oxidized low-density lipoprotein (ox-LDL) by macrophages leads to foam cell formation (1). THP-1 cells (human monocytic cell line) can be induced by phorbol 12-myristate 13-acetate (PMA) to undergo differentiation into a macrophage-like phenotype, and then be converted to foam cells following the treatment of ox-LDL (2).

Gene expression studies have identified many genes or proteins to be either up- or down-regulated in animal atherosclerosis models (3,4) or human atherosclerosis plaques (5-7). However, the genes reported mainly focus on aspects of regulation of cell apoptosis (8-10), growth (11), mobility (12), inflammation (13), and signal transduction (14,15), while the mechanisms of lipid droplet (LD) formation were largely ignored or underestimated in these studies. Disorders of lipid homeostasis have been seen as the major factors influencing atherosclerotic traits (16), including cholesterol clearance from foam cells, removal of fatty acids and lipoproteins, and lipid transportation in cell organelles. The most recent studies have proven that LD-associated proteins contribute to the regulation of cellular lipid stores, nascent LD biogenesis and lipid metabolism and transport (17-19). LD-associated proteins contain PAT proteins and other proteins which are involved in the turnover of lipids and in the formation and trafficking of LDs (20). The PAT and CIDE families have been identified as the most potential and specific LD-associated proteins to date. The Perilipin, Adipophilin, and TIP47 (PAT) families, include 5 members in mammals: Perilipin, adipose differentiation-related protein (ADRP, also known as ADFP or Adipophilin), tail-interacting protein of 47 kDa (TIP47), S3-12, and lipid storage droplet protein 5 (LSDP5)/myocardial lipid droplet protein (MLDP)/oxidative tissue-enriched PAT protein (OXPAT) (17). The cell death-inducing DNA fragmentation factor (DFF45)-like effector (CIDE) family comprises three members: CIDEA, CIDEB, and the fat-specific protein of 27 kDa (FSP27) (mouse)/CIDEC (human) (18). To date, there have not been any reports concerning the relationship between the CIDE family and atherosclerosis. Research on lipid metabolism centred on LD-associated proteins in foam cells has yet to be conducted. In this study, we identified differentially

Table I. Changes of CIDE and PAT families and associated genes which were critical in the transformation from macrophages to foam cells by microarray analysis.

Name	Family	Genebank accession	Ratio (foam cell vs macrophage)	Description
CIDEA	CIDE family	NM_001279	0.86±0.51	cell death-inducing DFFA-like effector a
CIDEB	CIDE family	NM_014430	1.56±0.37	cell death-inducing DFFA-like effector b
CIDEC	CIDE family	NM_022094	1.91±1.01	cell death-inducing DFFA-like effector b
Perilipin	PAT family	NM_001145311	0.78±0.45	perilipin 1, also known as PERI; PLIN; PLIN1
Adipophilin	PAT family	NM_001122	2.60±0.33	adipose differentiation-related protein, adipophilin, perilipin 2
TIP47	PAT family	NM_001164189.1	0.68±0.10	tail-interacting protein of 47 kDa, perilipin 3, M6PRBP1
S3-12	PAT family	NM_001080400.1	1.22±0.36	perilipin 4, also known as KIAA1881
LSDP5	PAT family	BC131524	2.07±0.82	lipid storage droplet protein 5, perilipin 5, myocardial lipid droplet protein (MLDP); OXPAT
PPARγ	PPAR family	NM_005037	3.02±0.92	peroxisome proliferator-activated receptor γ, also known as GLM1; CIMT1; NR1C3
CD36	macrophage scavenger receptors	NM_001001548	3.36±0.41	thrombospondin receptor, also known as FAT, GP4, GP3B, GPIV, CHDS7, PASIV, SCARB3
SR-AI	macrophage scavenger receptors	NM_138715	1.98±0.54	Class A of macrophage scavenger receptors isoforms types 1
ApoE	apolipoprotein	NM_000041	2.32±0.12	apolipoprotein E
ApoAI	apolipoprotein	NM_000039	0.46±0.09	apolipoprotein A-I
ABCA1	ATP-binding cassette (ABC) transporters family transporter A1	NM_005502	4.29±0.71	ATP binding cassette

expressed genes during the process of foam cell formation, especially those classified as LD-associated proteins and those that proved critical to uptake and transformation of LDs.

Materials and methods

Isolation and oxidation of LDL. LDL (Density=1.020-1.063 g/ml) was isolated by sequential flotation ultra-centrifugation from human plasma as previously described (21). Hypochlorite modification of LDL was essentially performed according to Vicca *et al* (22). Ox-LDL was dialyzed overnight against 10 mM phosphate-buffered saline (PBS) (pH 7.4). The concentration of proteins was determined by the methods of Lowry (Pierce).

Induction and identification of macrophages. The human monocytic cell THP-1 was donated by Dr Boquan Jin (Department of Immunology, Fourth Military Medical University, Shaanxi, China). Cells were cultured in 5% CO_2 atmosphere in RPMI-1640 medium (Hyclone) supplemented with 10% fetal bovine serum (FBS) (Hangzhou Sijiqing Biological Engineering Materials Co. Ltd.), then induced towards macrophage-like cells by adding 100 ng/ml PMA (Sigma, USA) for 2 days. Induced cells ($1x10^5$) were reaped and incubated with fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies against CD68 and CD11b/CD18 (mac-1), which were the macrophage-specific markers (23,24). Then samples were washed with staining buffer and analyzed by flow cytometry on a FACS Calibur (BD Biosciences). The labelled monoclonal antibodies for cytometry were purchased from BD Biosciences, USA.

Induction and identification of foam cells. After washing with PBS, the induced THP-1-derived macrophages were further induced towards foam cells with ox-LDL (100 μg/ml) for 2 days while the control group was only exposed to PBS. The THP-1-derived macrophages, with or without ox-LDL induction, were then fixed with 4% formaldehyde for 15 min. Cell lipids were stained with Oil Red O (Sigma) (3 mg/ml in 60% isopropanol) for 10 min. Meanwhile, cells were stained by Bodipy 493/503 (neutral lipids) and Hoechst 33258 (nucleus) for further identification as previously described (25). Images were captured by Olympus Type BX51TRF microscope (Olympus) using the accompanied image capture software. The contents of cellular cholesterol and triglyceride (TG) were determined by an enzymatic method as previously described (26).

RNA isolation and quantification. Total RNA from THP-1-derived cells was isolated by using Trizol reagent (Invitrogen) according to the manufacturer's instructions. RNA was

Primer sequences used.

Gene	Primer sequences	Genbank
CIDEA	Forward: CATGTATGAGATGTACTCCGTGTC Reverse: GAGTAGGACAGGAACCGCAG	NM_001279.3
CIDEB	Forward: AGCCAAAGCATTGGAGACCCTACT Reverse: TCTGACCAGACTGCAACACCATCA	NM_014430.2
CIDEC	Forward: TTGATGTGGCCCGTGTAACGTTTG Reverse: AAGCTTCCTTCATGATGCGCTTGG	NM_022094.2
Perilipin/perilipin1	Forward: CCTGCCTTACATGGCTTGTT Reverse: CCTTTGTTGACTGCCATCCT	NM_001145311.1
Adipophilin/perilipin2	Forward: CTGAGCACATCGAGTCACATACTCT Reverse: GGAGCGTCTGGCATGTAGTGT	NM_001122.2
TIP47/perilipin3	Forward: GCTGGACAAGTTGGAGGAGA Reverse: CCGACACCTTAGACGACACA	NM_001164189.1
S3-12/perilipin4	Forward: TGCTGCAGAATGAGTTGGAG Reverse: GACCCAGGTCACCTAAACGA	NM_001080400.1
LSDP5/perilipin5	Forward: AGCTTCCCTTTCTCCAGCAACCTT Reverse: AGTGATCCACCAGCTCCTCTGATT	DQ839131.1
PPARγ	Forward: CACAAGAACAGATCCAGTGGTTGCAG Reverse: AATAATAAGGTGGAGATGCAGGCTCC	NM_005037.5
GADPH	Forward : GAAGGTGAAGGTCGGAGTC Reverse: GAAGATGGTGATGGGATTTC	NM_002046.3
ApoE	Forward: ACCCAGGAACTGAGGGC Reverse: CTCCTTGGACAGCCGTG	NM_000041.2
ABCA1	Forward: GCACTGAGGAAGATGCTGAAA Reverse: AGTTCCTGGAAGGTCTTGTTCAC	NM_005502.2
CD36	Forward: GAGAACTGTTATGGGGCTAT Reverse: TTCAACTGGAGAGGCAAAGG	NM_001001548.2
SR-AI	Forward: CCAGGGACATGGGAATGCAA Reverse: CCAGTGGGACCTCGATCTCC	NM_138715.2
ApoAI	Forward: AAGGACCTGGCCACTGTGTA Reverse: TCTCCTCCTGCCACTTCTTC	NM_000039.1

quantified with the Nanodrop ND-1000 spectrophotometer (Nano-Drop Technologies), and RNA integrity was assessed by the Agilent 2100 Bioanalyzer (Agilent Technologies). Only RNA with an RNA integrity number (RIN) >9.6 was processed for labeling (2100 Expert software, Agilent Technologies). Each total RNA extract constituted a test sample, and an equimolar pool of all RNAs was used as the reference sample. Three independent experiments were performed for each array hybridization experiment.

Agilent hybridization and analysis. Total RNA from foam cells and macrophages (control group) was processed and hybridized to Agilent Whole Human Genome Oligo Microarray 44 k cDNA array following the manufacturer's recommendations. Cell intensity files were analyzed using the Rosetta Resolver Gene Expression Data Analysis System, v3.2. (Agilent Technologies). To identify potential biological relationships among differential expression genes, we used the recently developed PubGene database (http://www.pubgene.org) to identify citation-based gene-network associations.

Real-time PCR. Total RNA was reversely transcribed using Super-Script II (Takara), and then dilutions of cDNA were amplified using PCR primers for transcripts of interest. Primer sequences are listed in Table II. Amplimer quantity was measured at each of 40 cycles using SYBR green (Takara). The specificities of the reaction were assessed by analyzing the melting curves (single peak) and gel electrophoresis (single band). Each group had three to six samples. Transcriptal levels were expressed as a ratio to control gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Western blot analysis. The antibodies of CIDEA (rabbit polyclonal), CIDEC (rabbit polyclonal), LSDP5 (rabbit polyclonal) and CIDEB (mouse monoclonal) were made in our laboratory. Equivalent amount of protein homogenate was resolved by 10 or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE), and immunoblotting was performed as previously described (27). The expression levels of CIDEA, CIDEB, CIDEC, Perilipin (Santa Cruz), Adipophilin (RDI), TIP47 (Santa Cruz), S3-12 (Everest Biotech), LSDP5 and peroxisome proliferator-activated receptors γ (PPARγ) (Santa Cruz) were detected. GAPDH (Santa Cruz) was used as a loading control.

Results

Identification of macrophages and foam cells. The THP-1 cells had a round shape and maintained suspension in cell culture medium. After treated with PMA, they became adherent and

acquired a macrophage-like phenotype. The differentiation of the THP-1-derived macrophages was confirmed by the expression of CD68 and CD11b/CD18 (mac-1), which were generally accepted as specific macrophage surface markers (24,28). The THP-1-derived macrophages showed an increase of ~4.5-fold of CD11b/CD18 (mac-1) expression (45.69±7.18) compared to the non-induced THP-1 cells (10.11±0.60). There was also a 3-fold increase of CD68 expression (75.70±4.96) in macrophages (Fig. 1A).

After treated with ox-LDL, cell lipids were stained with Oil Red O or Bodipy 493/503. The content of cellular lipids after ox-LDL treatment was significantly higher than those treated with PBS (Fig. 1B). Furthermore, the amounts of TG and cholesterol in ox-LDL-treated cells were significantly higher than those in PBS-treated cells (2.1- and 6.7-fold compared to PBS-treated cells, respectively) (Fig. 1C), which have the same effects as foam cells (29).

Microarray analysis. By performing microarray studies, we were able to analyze the overall changes in the gene expression profiles between foam cells and THP-1-derived macrophages. Among the 41,000 genes examined, 516 genes were up-regulated while 369 were down-regulated significantly (Log2 ratio >1 or <-1) in foam cells. The top up-regulated genes by microarray analysis were dopamine receptor D5, La ribonucleoprotein domain family member 6, stanniocalcin 2, interleukin 23 α subunit p19, S100 calcium binding protein P, and the top down-regulated genes were chemokine ligand 5, interleukin 1 receptor type II, serpin peptidase inhibitor clade E (nexin, plasminogen activator inhibitor type 1) member 1, pro-platelet basic protein (chemokine ligand 7), serpin peptidase inhibitor clade B (ovalbumin) member 2.

Differentially expressed genes classified by biological process mainly included 26% for protein binding, 15% for catalytic activity, 12% for ion binding, 8% for nucleic acid binding and 7% for molecular transducer activity (Fig. 2A). Genes classified by biological function are shown in Fig. 2B. Specifically, we noted that the presence of lipid binding genes, which accounted for 2% of the total, differentially expressed genes during the formation of foam cells. Furthermore, we also found interesting changes in LD-associated proteins (the PAT and CIDE families), apolipoprotein family members, and PPAR family members (Table I). We mapped the network of the genes of interest that are involved in fatty acid partitioning and binding, synthesis, lipolysis and monocyte/macrophage recruitment based on information from the NCBI (Fig. 3), which can help in understanding the machinery of lipid accumulation in foam cells and its meaning in the pathological process of atherosclerosis.

Validation of gene expression changes with real-time PCR. To confirm individual gene expression changes, real-time PCR analysis was performed on 15 genes. The genes selected are shown in Table I. GAPDH was used as control. As shown in Fig. 3, in the CIDE family, the mRNA level of *CIDEB* and *CIDEC* in foam cells increased 2.67-fold (P=0.058) and 12.2-fold (P=0.034), respectively, in comparison with the macrophages. However, the ox-LDL treatment did not affect the mRNA level of *CIDEA* (P=0.919). *Adipophilin*, a PAT member, in foam cells was robustly up-regulated 36.5-fold vs.

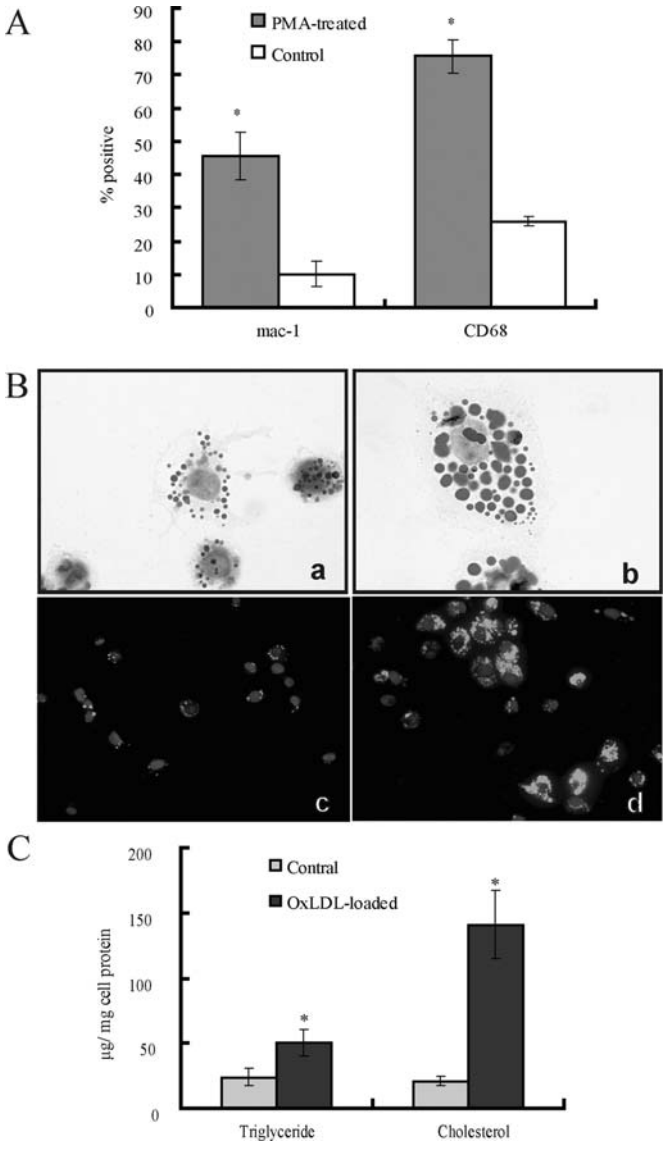


Figure1. Induction and identification of macrophages and foam cells. (A) Expression of CD11b/CD18 (mac-1) and CD68 in non-differentiated and differentiated THP-1 cells. Non-differentiated and differentiated THP-1 cells were labeled with specific antibodies and the expression of surface markers was evaluated by flow cytometry. The data are expressed as mean ± SEM of three independent experiments. (B) Lipid droplets in foam cells and macrophages. THP-1 monocytes were induced to differentiate by treatment with PMA *in vitro*, and then the macrophages were incubated with ox-LDL to form foam cells. ox-LDL-untreated macrophages (a) and ox-LDL-treated macrophages (b) were stained with Oil Red O, in order to observe the morphology of lipid droplets (magnification x400). Furthermore, lipid droplets were stained with Bodipy 493/503 (neural lipids) and nucleus was stained with Hoechst 33258 (nucleus). Cell morphology of ox-LDL-untreated macrophage (c) and ox-LDL-treated macrophages (d) was recorded by fluorescence microscopy (magnification x200). (C) Quantification of the cellular lipids in foam cells and macrophages. Significant difference between samples was determined by Student's t-test (*P<0.05).

macrophages (P=0.017). *S3-12* increased 1.7-fold (P=0.066) and *LSDP5* increased 2.1-fold (P=0.054), respectively. However, a 1.5-fold decrease of *TIP47* was shown in foam cells (P=0.016). The difference expression of *Perilipin* in mRNA level was not statistically significant (P=0.641). The mRNA level of PPARγ, a key element regulating the

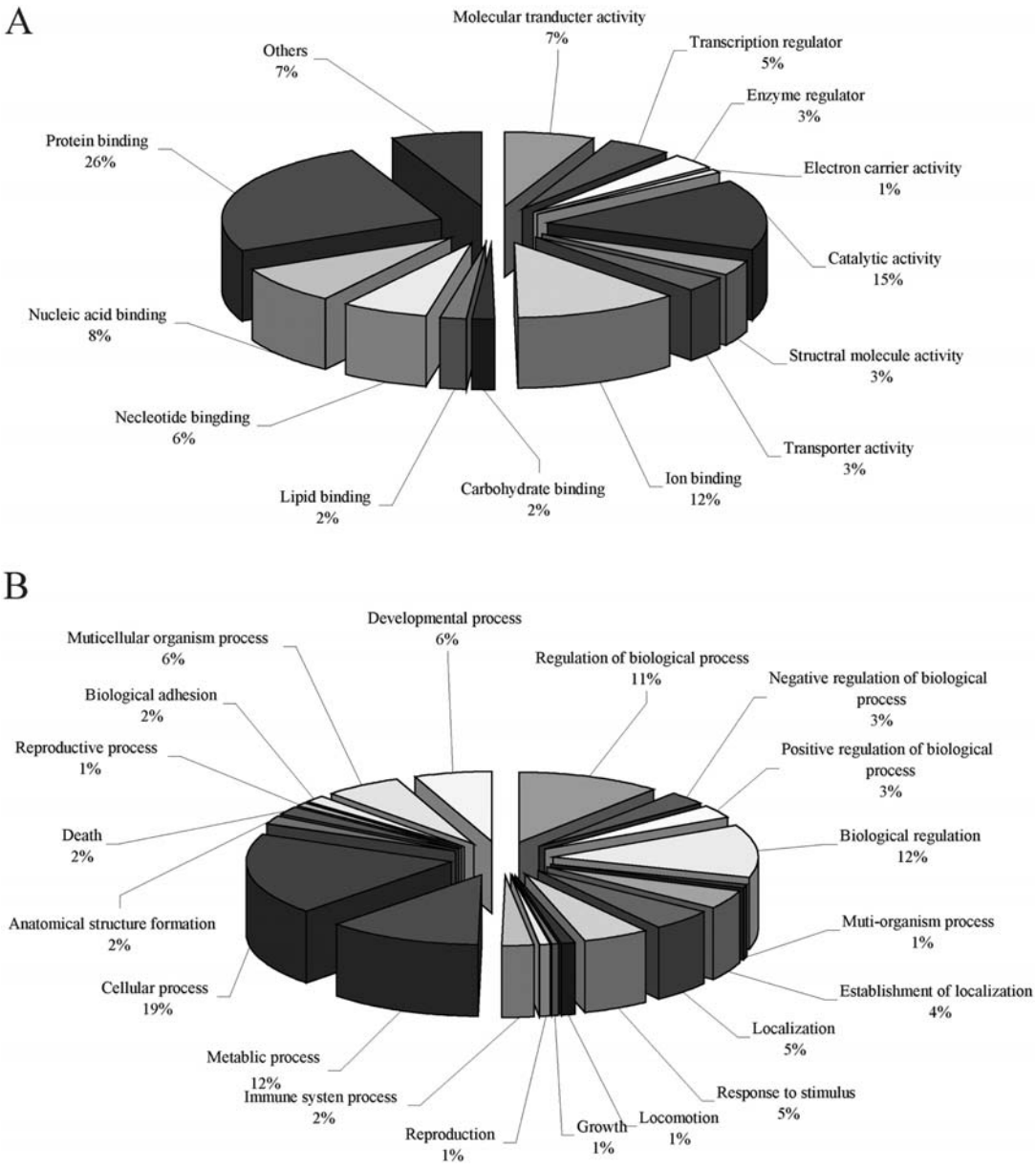


Figure 2. Classification of the differentially expressed genes. Pie charts represented the distribution of the 885 identified genes using microarray analysis according to molecular functions (A) and biological processes (B). Assignments were made based on information from the NCBI (www.ncbi.nlm.nih.gov/PubMed) websites.

expression of the LD-associated proteins (17), was up-regulated 7.0-fold (P=0.082). It is known that, macrophage uptake of ox-LDL mainly depends on the scavenger receptors. The expression levels of *CD36* and *class A macrophage scavenger receptor* (*SR-AI*), the powerful scavenger receptors, increased 19.8-fold (P=0.036) and 4.8-fold (P=0.046), respectively. *Apolipoprotein E* (*ApoE*), a main apolipoprotein of the chylomicron and very low-density lipoprotein (VLDL), was up-regulated 21.9-fold (P=0.093) in the mRNA level. *Apolipoprotein A1* (*Apo AI*) is the major protein component of high density lipoprotein (HDL) which promotes cholesterol efflux from tissues to liver for excretion and its mRNA level was down-regulated ~6.2-fold (P=0.009). ATP binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux pump in the cellular lipid removal pathway and its mRNA level increased 3.9-fold in foam cells (P=0.079) (Fig. 4).

Confirmation of the selected proteins by immunoblotting. In order to screen changes of the LD-associated proteins during the process of lipid accumulation in macrophages, CIDEA, CIDEB, CIDEC, Adipophilin, Perilipin, TIP47, S3-12, LSDP5 and PPARγ were selected on the basis of the results from microarray and real-time PCR. Western blot analysis confirmed that the expression levels of CIDEB, CIDEC, Adipophilin, S3-12 and LSDP5 proteins were higher in foam cells compared with those in macrophages (0.53- vs 0.25-, 0.91- vs 0.4-, 1.52- vs 0.78-, 0.51- vs 0.29-, 0.3- vs 0.17-fold, respectively). However, the expression level of TIP47 showed a significant decrease (1.27- vs 0.71-fold, P=0.001). There were not obvious changes in the expression of CIDEA and Perilipin (0.21- vs 0.17-, 0.07- vs 0.09-fold, respectively). PPARγ had a significant increase during the formation of foam cells (1.62- vs 0.85-fold, P=0.006) (Fig. 5).

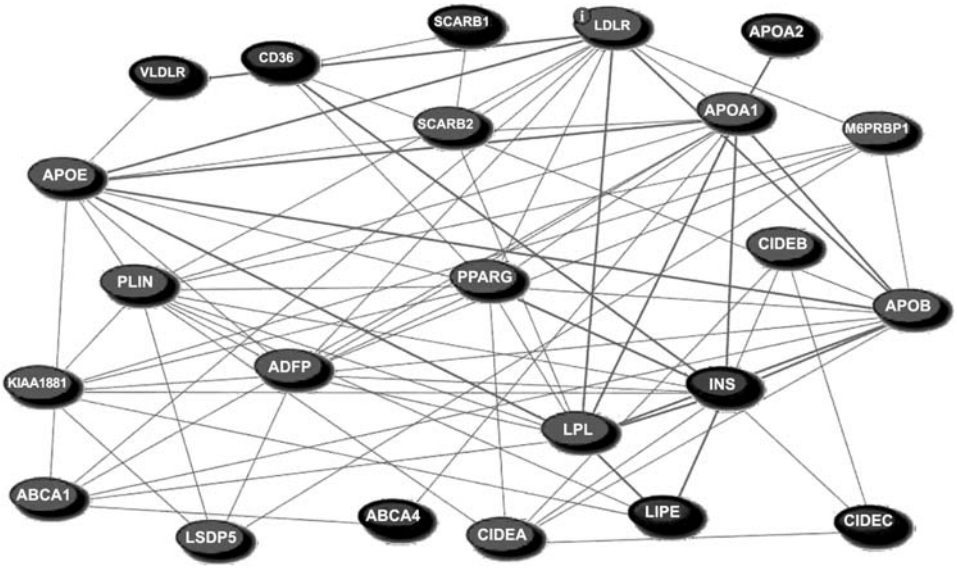
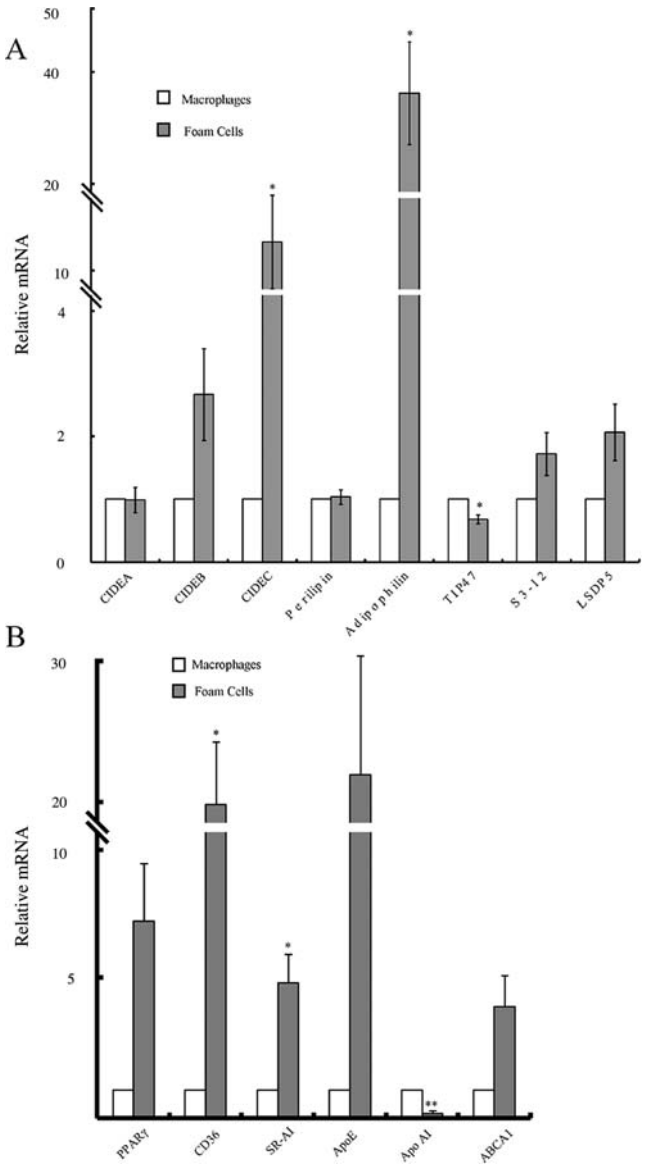


Figure 3. Network of the LD associated proteins during the formation of foam cells. The interaction map showed the genes involved in the lipid accumulation and PPARγ pathways. Nodes in a lighter shade represent the primary correlation in the network and nodes in a darker shade represent the secondary correlation. Thicker lines that connect nodes represent a higher number of records that linked the co-cited proteins in Medline.



Discussion

Proteins that coat LDs play important roles in regulating LD formation, morphology and lipolysis. Herein, we concentrated on the expression of LD-associated proteins during the formation of foam cells, in order to illustrate the potential roles of LD-associated proteins in the process of atherosclerosis. In this study, the macrophages derived from THP-1 were incubated for 2 days with ox-LDL to induce foam cells, and then the differentially expressed genes were identified by microarray analysis and comformed by real-time PCR and Western blot analysis.

We used THP-1-derived foam cells in our research for two reasons: (i) THP-1, the human monocytic cell line, is a well-characterized model system for studying the transformation of macrophages into foam cells. Although this cell line has its limitations, it is uniform in its components and suitable for completing research on foam cells; (ii) genes identified by using atherosclerotic vessel tissues seem to be more illustrative. However, the differences in gene expression patterns obtained from atherosclerotic vessel tissues were frequently mixed with the deviation resulting from the difference of cell types between the atherosclerotic and control vessels that we chose.

An increasing number of studies have demonstrated that LDL oxidized by hypochlorite *in vitro* is able to mimic fundamental atherogenic processes including adhesion to endothelial cells, activation of phagocytes with an increase of reactive oxygen species (ROS) and formation of foam cells as a result of phagocytosis of ox-LDL (30). However, Cu^{2+}-mediated LDL oxidation *in vitro* did not reflect the real

Figure 4. Real-time PCR analysis of 15 selected genes in the macrophages and foam cells. The mRNA level of object genes in foam cells normalized to GAPDH and expression was relative to macrophages. Each bar represents the mean ± SEM of the results from 3-6 samples, and data were analyzed with Student's t-test ($^{*}P<0.05$, $^{**}P<0.01$).

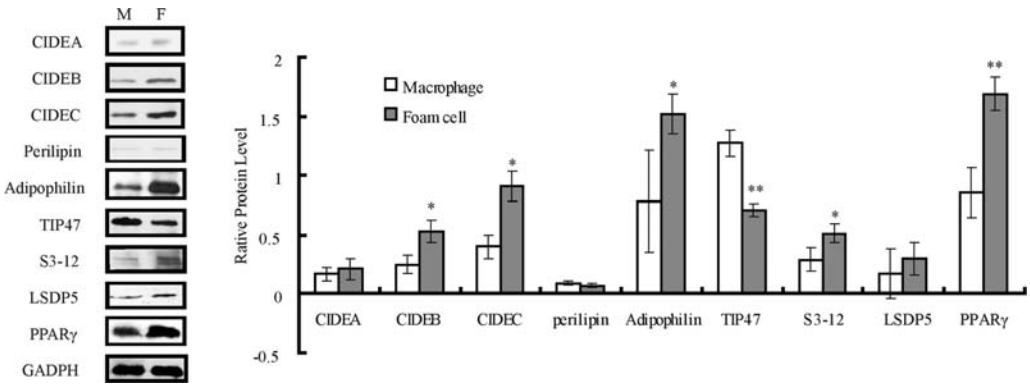


Figure 5. Immunoblot analysis of selected proteins. Left panel, expression levels of LD-associated proteins and PPARγ in ox-LDL-treated and -untreated macrophages. Each blot was probed with a specific antibody and GAPDH was used as control. M, macrophage; F, foam cells. Right panel, grey levels of corresponding bands measured with the Quantity One 1-D analysis software. Data were analyzed with Student's t-test ($^{*}P<0.05$, $^{**}P<0.01$), and represent the mean ± SEM from three independent experiments performed in triplicate.

physiological oxidation modification of LDL *in vivo* (25). In this study, we chose hypochlorite instead of copper ions (Cu^{2+}) in the oxidative modification of LDL.

In our study, 885 genes were differentially expressed during the formation of foam cells. Based on the molecular function, the largest two groups identified were genes of protein binding and catalytic activity. Based on the biological process, the largest three gene groups were cellular and metabolic process and biological regulation. The top changed genes were mainly classified into metabolic process, cell death and proliferation and immune system process, which were in accordance with previous reports (8). Of the 885 differentially expressed genes identified in this study, 2% were lipid binding proteins, which were the proteins of interest, LD-associated proteins.

We detected up-regulation of CIDEB (2.67-fold in mRNA level, 2.12-fold in protein level) and CIDEC (12.2-fold in mRNA levels, 2.28-fold in protein levels) in foam cells, but there was no significant change in CIDEA. The diversity of different CIDE family members in expression level maybe owed to the fact that CIDEs were expressed in a tissue-specific manner. CIDE family members including CIDEA, CIDEB and CIDEC, were expressed at high levels in brown adipose tissue (BAT) and liver and white adipose tissue (WAT), respectively. Similar to uncoupling protein 1 (UCP1), CIDEA was a BAT-specific marker. CIDEA can negatively regulate UCP1 activity, and the loss of UCP1 inhibition by CIDEA would result in enhanced uncoupling activity which leads to decreased levels of plasma-free fatty acids and triglycerides, and less lipid accumulation in WAT. Given these facts, we believe that CIDEA directly plays a role only in BAT, but has a smaller effect on other tissues such as the formation of foam cells in atherosclerotic vessels. CIDEB was detected in various tissues with high expression levels in the liver, and to a lesser extent in the kidney, small intestine, and colon. We have demonstrated that CIDEB promotes the formation of triacylglyceride-enriched VLDL particles in hepatocytes (25), and might play a potential role in atherosclerosis. CIDEC was most highly expressed in WAT. It increased in abundance by >50-fold during adipogenesis and promoted the formation of the large LDs in primary fat cells (31). In the formation of foam cells, our study demonstrated that CIDEB and CIDEC were both up-regulated at the level of mRNA by microarray analysis, the increase in their mRNA levels was significantly greater as shown by real-time PCR. Moreover, the increased tendency was also confirmed in their protein level.

In the PAT family, Adipophilin which was found to be associated with intracellular LDs in a variety of cells and tissues (17) has been well studied in atherosclerotic lesions. Larigauderie *et al* observed a 3.5-fold increase of *Adipophilin* expression in atherosclerotic lesions and found that most of the *Adipophilin* mRNA expression was presented within lipid-rich macrophages. The expression of Adipophilin in macrophages was enhanced by the treatment of modified LDL (32). In this study, we also found a great increase of Adipophilin at both mRNA and protein levels in foam cells. Unlike Adipophilin, the change of Perilipin seems to be incongruous. The microarray analysis revealed a tenuous decrease of Perilipin mRNA, but the result from real-time PCR and immunoblotting did not show any significant difference. Similar conflicting results have been reported in recent studies (33-35). We believe that Perilipin, which was specific in adipose tissue and critical in regulation of lipolysis, plays an important role in the process of atherosclerosis but has not been studied in depth to date. In contrast to Perilipin, TIP47 was expressed in most, if not all tissues. Robenek *et al* studied the distribution of TIP47 in lipid bodies of THP-1 cell-derived macrophages and found that TIP47 was a component of many LDs in structure (36). However, we observed a decrease of TIP47 not only in mRNA but also in protein levels. S3-12 was expressed primarily in white adipose tissue and to a lesser degree in skeletal muscle and heart (37). LSDP5 was expressed prominently in tissues that exhibited a high capacity of fatty acid oxidation, including heart, BAT, liver, and skeletal muscle (38). In our study, the above two PAT members increased during the transformation of foam cells. Apart from tissue distribution, we believe that the change in composition of PAT proteins in LD biogenesis should also be taken into account. It is reasonable to speculate that after laded with ox-LDL, the LD biogenesis in foam cells was similar to that in adipocytes (37), during which there was a dynamic change of PAT family members. Therefore, the

monitoring site we chose was just accompanied with the phenotype of high level of Adipophilin, medium level of S3-12 and LSDP5, but it was too early to detect the peak of perilipin, whereas, TIP47 which coated the LDs at the early stage had left the LDs and began to diminish. The mechanism speculated here may also explain the reason why the results of perilipin reported by different researchers varied greatly.

Overexpression of lipid binding protein in macrophage-derived foam cells caused the increase of SR-AI expression which was resistant to the accumulation of cholesterol and triglycerides in foam cells, while ABCA1 was down-regulated which facilitated cholesterol efflux (39). Similarly, we also observed genes involved in the accumulation and trafficking of LDs during the formation of foam cells. CD36, macrophage scavenger receptor, was up-regulated. In addition, ApoE, a component of VLDL, was up-regulated, and ApoAI, a component of HDL, was down-regulated. As a major regulator of lipid homeostasis, PPARγ has been proven to be upstream the regulation element of many LD-associated proteins (17). Consistent with previous reports, our study indicated that the PPARγ signaling pathway is involved in the expression of LD-associated proteins in the formation of foam cells.

In conclusion, our study puts forward a detailed report tracking the changes of LD-associated proteins in the formation of foam cells. The data contribute to the understanding of the gene profiling of cellular lipid formation and its influence in atherosclerosis which may help provide novel approaches to this pressing issue in human health. However, further experiments are still necessary to illustrate the mechanisms and the biological meaning of the changes of lipid binding proteins in lipid accumulation during the foam cell formation.

Acknowledgements

We thank members in Qing Li's laboratory in the Fourth Military Medical University for technical assistance and Dr Peng Li (Department of Biological Sciences and Biotechnology, Tsinghua University) for critical editing of the manuscript. This work was supported by grants (30671087 and 30700268) from National Natural Science Foundation of China and Doctoral Assistant Fund of the Fourth Military Medical University (2008D03).

References

1. Berliner JA and Heinecke JW: The role of oxidized lipoproteins in atherogenesis. Free Radic Biol Med 20: 707-727, 1996.
2. Jessup W and Kritharides L: Metabolism of oxidized LDL by macrophages. Curr Opin Lipidol 11: 473-481, 2000.
3. Yuan Z, Miyoshi T, Bao Y, Sheehan JP, Matsumoto AH and Shi W: Microarray analysis of gene expression in mouse aorta reveals role of the calcium signaling pathway in control of atherosclerosis susceptibility. Am J Physiol Heart Circ Physiol 296: H1336-H1343, 2009.
4. Smith JD, Peng DQ, Dansky HM, *et al*: Transcriptome profile of macrophages from atherosclerosis-sensitive and atherosclerosis-resistant mice. Mamm Genome 17: 220-229, 2006.
5. Sung HJ, Ryang YS, Jang SW, Lee CW, Han KH and Ko J: Proteomic analysis of differential protein expression in atherosclerosis. Biomarkers 11: 279-290, 2006.
6. Andersson T, Borang S, Larsson M, *et al*: Novel candidate genes for atherosclerosis are identified by representational difference analysis-based transcript profiling of cholesterol-loaded macrophages. Pathobiology 69: 304-314, 2001.
7. Zhang QJ, Goddard M, Shanahan C, Shapiro L and Bennett M: Differential gene expression in vascular smooth muscle cells in primary atherosclerosis and in stent stenosis in humans. Arterioscler Thromb Vasc Biol 22: 2030-2036, 2002.
8. Lim WC and Chow VT: Gene expression profiles of u937 human macrophages exposed to *Chlamydophila pneumoniae* and/or low density lipoprotein in five study models using differential display and real-time RT-PCR-pcr. Biochimie 88: 367-377, 2006.
9. Martinet W, Schrijvers DM, De Meyer GR, *et al*: Gene expression profiling of apoptosis-related genes in human atherosclerosis: Upregulation of death-associated protein kinase. Arterioscler Thromb Vasc Biol 22: 2023-2029, 2002.
10. Hegyi L, Skepper JN, Cary NR and Mitchinson MJ: Foam cell apoptosis and the development of the lipid core of human atherosclerosis. J Pathol 180: 423-429, 1996.
11. Lu Y, Guo J, Di Y, Zong Y, Qu S and Tian J: Proteomic analysis of the triglyceride-rich lipoprotein-laden foam cells. Mol Cells (In press).
12. O'Brien KD, Vuletic S, McDonald TO, *et al*: Cell-associated and extracellular phospholipid transfer protein in human coronary atherosclerosis. Circulation 108: 270-274, 2003.
13. Hansson GK, Robertson AK and Soderberg-Naucler C: Inflammation and atherosclerosis. Annu Rev Pathol 1: 297-329, 2006.
14. Rosenfeld ME: Cellular mechanisms in the development of atherosclerosis. Diabetes Res Clin Pract 30: S1-S11, 1996.
15. Hamilton JA, Byrne R, Jessup W, Kanagasundaram V and Whitty G: Comparison of macrophage responses to oxidized low-density lipoprotein and macrophage colony-stimulating factor (M-CSF or CSF-1). Biochem J 354: 179-187, 2001.
16. Cagnin S, Biscuola M, Patuzzo C, *et al*: Reconstruction and functional analysis of altered molecular pathways in human atherosclerotic arteries. BMC Genomics 10: 13, 2009.
17. Bickel PE, Tansey JT and Welte MA: PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. Biochim Biophys Acta 1791: 419-440, 2009.
18. Gong J, Sun Z and Li P: Cide proteins and metabolic disorders. Curr Opin Lipidol 20: 121-126, 2009.
19. Ducharme NA and Bickel PE: Lipid droplets in lipogenesis and lipolysis. Endocrinology 149: 942-949, 2008.
20. Olofsson SO, Bostrom P, Andersson L, Rutberg M, Perman J and Boren J: Lipid droplets as dynamic organelles connecting storage and efflux of lipids. Biochim Biophys Acta 1791: 448-458, 2009.
21. Redgrave TG, Roberts DC and West CE: Separation of plasma lipoproteins by density-gradient ultracentrifugation. Anal Biochem 65: 42-49, 1975.
22. Vicca S, Hennequin C, Nguyen-Khoa T, *et al*: Caspase-dependent apoptosis in THP-1 cells exposed to oxidized low-density lipoproteins. Biochem Biophys Res Commun 273: 948-954, 2000.
23. Holness CL and Simmons DL: Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. Blood 81: 1607-1613, 1993.
24. Rehman J, Li J, Orschell CM and March KL: Peripheral blood 'Endothelial progenitor cells' Are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation 107: 1164-1169, 2003.
25. Ye J, Li JZ, Liu Y, *et al*: CIDEB, an er- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with Apolipoprotein B. Cell Metab 9: 177-190, 2009.
26. Carr TP, Andresen CJ and Rudel LL: Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 26: 39-42, 1993.
27. Zhang LY, Ye J, Zhang F, *et al*: Axin induces cell death and reduces cell proliferation in astrocytoma by activating the p53 pathway. Int J Oncol 35: 25-32, 2009.
28. Ramprasad MP, Terpstra V, Kondratenko N, Quehenberger O and Steinberg D: Cell surface expression of mouse macrosialin and human CD68 and their role as macrophage receptors for oxidized low density lipoprotein. Proc Natl Acad Sci USA 93: 14833-14838, 1996.
29. Palmer AM, Murphy N and Graham A: Triglyceride-rich lipoproteins inhibit cholesterol efflux to Apolipoprotein (Apo) A1 from human macrophage foam cells. Atherosclerosis 173: 27-38, 2004.
30. Kopprasch S, Leonhardt W, Pietzsch J and Kuhne H: Hypochlorite-modified low-density lipoprotein stimulates human polymorphonuclear leukocytes for enhanced production of reactive oxygen metabolites, enzyme secretion, and adhesion to endothelial cells. Atherosclerosis 136: 315-324, 1998.

M and Jessup W: Lipid droplets and adipose metabolism: l role for FSP27/CIDEC. Curr Opin Lipidol 20: 147-149, 2009.
32. Larigauderie G, Furman C, Jaye M, *et al*: Adipophilin enhances lipid accumulation and prevents lipid efflux from THP-1 macrophages: Potential role in atherogenesis. Arterioscler Thromb Vasc Biol 24: 504-510, 2004.
33. Forcheron F, Legedz L, Chinetti G, *et al*: Genes of cholesterol metabolism in human atheroma: Overexpression of perilipin and genes promoting cholesterol storage and repression of ABCA1 expression. Arterioscler Thromb Vasc Biol 25: 1711-1717, 2005.
34. Larigauderie G, Bouhlel MA, Furman C, Jaye M, Fruchart JC and Rouis M: Perilipin, a potential substitute for adipophilin in triglyceride storage in human macrophages. Atherosclerosis 189: 142-148, 2006.
35. Persson J, Degerman E, Nilsson J and Lindholm MW: Perilipin and adipophilin expression in lipid loaded macrophages. Biochem Biophys Res Commun 363: 1020-1026, 2007.
36. Robenek H, Robenek MJ and Troyer D: PAT family proteins pervade lipid droplet cores. J Lipid Res 46: 1331-1338, 2005.
37. Wolins NE, Quaynor BK, Skinner JR, Schoenfish MJ, Tzekov A and Bickel PE: S3-12, Adipophilin, and TIP47 package lipid in adipocytes. J Biol Chem 280: 19146-19155, 2005.
38. Dalen KT, Dahl T, Holter E, *et al*: LSDP5 is a PAT protein specifically expressed in fatty acid oxidizing tissues. Biochim Biophys Acta 1771: 210-227, 2007.
39. Fu Y, Luo L, Luo N and Garvey WT: Lipid metabolism mediated by adipocyte lipid binding protein (ALBP/AP2) gene expression in human THP-1 macrophages. Atherosclerosis 188: 102-111, 2006.