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Over-expression of STARD3 in human monocyte-macrophages induces an anti-atherogenic lipid phenotype

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Abstract

Dysregulated macrophage cholesterol homeostasis lies at the heart of early and developing atheroma, and removal of excess cholesterol from macrophage 'foam cells', by efficient transport mechanisms, is central to stabilisation and regression of atherosclerotic lesions. This study demonstrates that transient over-expression of STARD3 (MLN64), endosomal cholesterol transporter and member of the 'START' family of lipid trafficking proteins, induces significant increases in macrophage ATP binding cassette transporter A1 (ABCA1) mRNA and protein, enhances [³H]cholesterol efflux to apolipoprotein (apo)AI, and reduces biosynthesis of cholesterol, cholesteryl ester, fatty acids, triacylglycerol and phospholipids from [¹⁴C]acetate, compared with controls. Notably, over-expression of STARD3 prevents increases in cholesterol esterification in response to acetylated LDL, blocking cholesteryl ester deposition. Thus, enhanced endosomal trafficking via STARD3 induces an anti-atherogenic macrophage lipid phenotype, positing a potentially therapeutic strategy.

Keywords

Atherosclerosis, macrophage 'foam cell', STARD3/MLN64, lipid transport, cholesterol efflux

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Introduction

Dysregulated macrophage cholesterol homeostasis lies at the heart of early and developing atheroma, principal cause of coronary heart disease. Macrophage 'foam cells', laden with cholesterol and cholesteryl ester, result from unregulated uptake of modified lipoproteins by macrophage scavenger receptors (CD36, CD68, SR-AI/AII), and influence both plaque stability and progression [1]. Removal of excess cholesterol from macrophage 'foam' cells is central to lesion regression and stabilisation, and can be orchestrated, at least *in vitro*, by ATP binding cassette (ABC) lipid transporters such as ABCA1, ABCG1 and ABCG4, and apolipoprotein acceptors, such as apoAI and apoE [2]. Efficient intracellular cholesterol transport is pivotal in marshalling appropriate cholesterol homeostasis mechanisms, regulating sterol-responsive transcription factors, such as Liver X Receptors (LXR α/β) and sterol regulatory element binding proteins (SREBPs), and controlling cholesterol content of organelles, lipid rafts and membranes, and storage as cytosolic droplets of cholesteryl ester [2,3]. Despite this, the proteins involved in non-vesicular cholesterol transport mechanisms remain poorly understood.

The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain is a 210 amino acid, conserved 'helix-grip' fold, providing an adaptable binding site for lipids such as cholesterol, oxysterols, phospholipids and ceramides. In humans, 'START' domains are found in 15 distinct proteins (STARD1-STARD15), implicated in non-vesicular lipid transport, cell signalling and lipid metabolism [4, 5]. The prototypic member of this family, steroidogenic acute regulatory protein (StAR, STARD1) delivers cholesterol from the outer to the inner mitochondrial membrane, to the P450 side chain cleavage enzyme involved in steroidogenesis [6]. Over-expression of StAR can also reduce macrophage lipid content, inflammatory status and arterial cholesterol levels [7, 8], and increase cholesterol efflux to apoAI via a mechanism dependent upon mitochondrial sterol 27-hydroxylase (CYP27A1), LXR activation and increases in ABCA1 mRNA and protein [9]. However, one 'down-side' of StAR over-expression in macrophages is induction of lipogenesis [9], possibly mediated by LXR-dependent increases in SREBP-1c expression, a problem also associated with non-sterol LXR agonists.

The other member of the STARD1 subfamily, STARD3 (Metastatic Lymph Node 64; MLN64) is a 54kDa protein, co-amplified within chromosome band 17q12, a region containing potent oncogene *ERBB2*, in human breast carcinoma [10-12]. STARD3 is a late endosomal protein with two distinct, conserved, cholesterol-binding domains: a region of four transmembrane helices with three short intervening loops, called the 'MENTAL' domain (MLN64 N-terminal domain), and the C-terminal 'START' domain [13]; the MENTAL domain may maintain cholesterol at the late endosomal membrane, prior to its shuttle to cytoplasmic acceptor(s) via the START domain.

Our work shows that cholesterol loading represses STARD3 expression, implicating this protein in dysfunctional cholesterol pathologies [14]. Here, we sought to establish a functional role for this endosomal cholesterol trafficking protein in macrophage lipid homeostasis, testing the hypothesis that enhanced expression of STARD3 may be useful in prevention of 'foam cell' formation.

Materials and Methods

Materials Tissue culture reagents were purchased from Lonza (Wokingham, UK); other sources include pCMV.Script, *STARD4* and *STARD5* clones (Origene, Cambridge Biosciences, UK), *STARD3* clone (Stratagene, UK), Amaxa monocyte-macrophage transfection reagent, NuPAGE gels and buffers (Invitrogen), antibodies (AbCAM, Cambridge, UK), primers and fluorescent probes (Eurogentec, Belgium); low-density lipoprotein (Athens Research, PA, USA), acetylated as previously [14]. Peripheral human monocyte-macrophages were purchased from Lonza (Wokingham) and human heart aorta RNA, derived from four to seven human heart aortae, was purchased from Clontech (USA).

Cellular studies Human (THP-1) monocytes (ECACC 880812101) were maintained in RPMI 1640 medium containing foetal bovine serum (10%, v/v) as previously described [14, 15]. STARD3,

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STARD4, STARD5 and empty vector control (pCMV; 0.5µg DNA) were delivered to THP-1 monocytes (10^7) using Amaxa Human Monocyte Nucleofector® kit (VPA-1007). Transfection efficiency using protocol Y001 (78.5%) was determined using the proprietary pmaxGFP® vector provided by Amaxa, and flow cytometric analysis of 50,000 cells. Transfected human THP-1 monocytes (1.5×10^6 /well) were differentiated into macrophages by addition of phorbol 12-myristate 13-acetate (100nM). Cellular lipids, RNA and cell protein lysates were collected 72h post-transfection. Macrophage 'foam cell' prevention experiments were initiated 48h after transfection: macrophages were radiolabelled with [3 H]oleic acid ($1 \mu\text{Ci ml}^{-1}$; 10µM) in the presence or absence of acetylated LDL ($50 \mu\text{g ml}^{-1}$) and with or without ACAT inhibitor (447C88, 10µM), for 24h. Wild type macrophages were incubated with progesterone (10µM; 24h) or U18666A (25µM; 24h) to inhibit endosomal trafficking, using ethanol vehicle (<0.1%, v/v). Cellular viability was assessed by conversion of dimethylthiazolyl diphenyltetrazolium bromide (MTT) to formazan; the caspase-Glo® 3/7 assay system (Promega) was used to detect apoptosis.

Lipid analyses Flux of [3 H]oleic acid ($1 \mu\text{Ci ml}^{-1}$; 10µM) into the cholesteryl ester pool, and of [14 C]acetate ($1 \mu\text{Ci ml}^{-1}$) into cholesterol, cholesteryl ester, fatty acid, triacylglycerol and phospholipid pools were assessed by t.l.c., as previously [14,15]. Efflux of [3 H]cholesterol ($0.5 \mu\text{Ci ml}^{-1}$) to apoA1 ($10 \mu\text{g ml}^{-1}$) and HDL ($10 \mu\text{g ml}^{-1}$) were measured as previously [13,15]. Mass of macrophage total cholesterol, triacylglycerol and choline-containing phospholipids were measured using Infinity™ and Phospholipids-B colorimetric assays (Alpha Labs, UK) [14,15].

Gene and protein expression Total RNA (Tri-Reagent, Sigma-Aldrich) was isolated from macrophages (above) and reverse transcribed to cDNA utilising MMLV reverse transcriptase (RT) enzyme (Bioline). Expression of *STARD3*, *STARD4*, *STARD5*, *APOE*, *ABCA1*, *ABCG1*, *ABCG4*, *LXRα* and *SREBP-1* and -2 mRNA, relative to the housekeeping gene, *GAPDH*, was performed by quantitative (real-time) PCR, using DNA engine Opticon 2 (MJ Research). The PCR reactions contained cDNA template, Q-PCR mix, molecular biology grade water and 100nM of each forward and reverse primers and fluorescent probe (FAM/TAMRA). Thermal cycling conditions were 15min at 95°C, followed by 40 cycles of 15s at 95°C and 20s at 60°C, with status at 50°C. Primers and probes details are given in **Table 1**. The comparative $2^{-\Delta\text{Ct}}$ method was utilized for quantitation of each gene relative to *GAPDH* mRNA.

Macrophage lysates were collected in RIPA buffer plus Complete™ protease cocktail inhibitor (Roche, UK). Protein lysates ($15\text{-}30 \mu\text{g well}^{-1}$) were separated by SDS-PAGE (NuPAGE, 10% w/v gels), transferred to PDVF membrane and probed using anti-STARD3 (1:1000), anti-GAPDH (1:1000) rabbit polyclonal antibodies, and an anti-ABCA1 murine monoclonal antibody (1:1000). Detection was achieved using appropriate secondary antibodies (1:1000) and ECL detection system [14]; densitometry was performed using Scion Image software.

Statistical analyses Numbers of independent experiments are indicated in legends to Figures; values are mean±SEM, with significance ($p < 0.05$) determined by Student's *t*-test, or repeated measures ANOVA, followed by Dunnett's or Tukey-Kramer post *t*-tests, as appropriate; * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ for comparisons indicated.

Results

Comparison of the endogenous expression of *STARD3* mRNA, relative to housekeeping gene *GAPDH*, in human THP-1 monocytes, THP-1 macrophages, human peripheral blood monocyte-macrophages and human heart aortae, is shown in **Table 2**. Transient over-expression of *STARD3* in human THP-1 monocyte-macrophages increased mRNA levels by 87-fold (24h; $p < 0.001$), 20-fold (48h; $p < 0.001$) and 18-fold (72h; $p < 0.05$) (Figure 1a), which translated into 2.2-fold ($p < 0.05$) increases in levels of STARD3 protein after 72h (Figure 2b), by comparison with empty vector control; all values normalised to housekeeping protein, GAPDH. Cell viability was not altered by *STARD3* over-expression after 72h (empty vector control $25.4 \pm 2.4 \mu\text{M}$ formazan *versus* *STARD3* $29.2 \pm 6.4 \mu\text{M}$ formazan; $p = 0.59$; mean±SEM from six independent experiments), and levels of

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macrophage caspase 3/7 activity (empty vector control 6971 ± 270 versus STARD3 7632 ± 1905 ; $p=0.509$; mean \pm SEM from three independent experiments; data expressed as arbitrary fluorescent units) did not change when STARD3 over-expression was compared with the empty vector control. No changes in steady state levels of mRNA encoding sterol-responsive transcription factors *SREBP1* (not detectable), *SREBP2* or *LXR α* were noted, as judged by Q-PCR (Figure 1b). In comparison, over-expression of genes encoding cytosolic cholesterol transporters, STARD4 (Figure 1c) and STARD5 (Figure 1d), exerted distinct influences on gene expression of these transcription factors: STARD4 was associated with reduced ($p<0.05$) expression of *SREBP2*, while STARD5 over-expression was associated with marked increases ($p<0.05$) in both *SREBP2* and *LXR α* mRNA levels.

Over-expression of STARD3 was associated with significant ($p<0.05$) increases (20.7-fold) in *ABCA1* mRNA (Figure 2a) and protein (2.6-fold) (Figure 2b); by contrast, no changes in *ABCG1* and *ABCG4* mRNA (Figure 2a) were noted between STARD3 over-expressing macrophages and control cells, following normalisation to GAPDH. These changes in gene expression strongly predicted functional increases in cholesterol efflux to apoAI, rather than to HDL, following STARD3 over-expression, and this proved correct, despite a small reduction in gene expression of the endogenous acceptor *APOE*: [3 H]cholesterol efflux to apoAI ($10 \mu\text{g ml}^{-1}$; 24h) was enhanced by 80% ($p<0.05$) (Figure 2c), whereas efflux to HDL ($10 \mu\text{g ml}^{-1}$; 24h) did not change significantly (% HDL-specific efflux: control $5.27 \pm 0.58\%$ versus STARD3 $6.9 \pm 2.5\%$; $n=3$; NS). By contrast, inhibition of endosomal trafficking using progesterone ($10 \mu\text{M}$), inhibited apoAI-specific cholesterol efflux by 54% ($n=3$; $p<0.01$). Decreases in biosynthesis of cholesterol (25.5%; $p<0.05$) and cholesteryl ester (34.1%; $p<0.01$), fatty acid (27.3%; $p<0.05$), triacylglycerol (41.9%; $p<0.01$) and phospholipids (27.7%; $p<0.05$) from [14 C]acetate, were observed in STARD3 over-expressing macrophages (Figure 2d), compared with empty vector controls; no change in total cholesterol (control $26.1 \pm 3.91 \mu\text{g mg}^{-1}$ protein versus STARD3 $27.2 \pm 3.15 \mu\text{g mg}^{-1}$), triacylglycerol ($83.7 \pm 10.9 \mu\text{g mg}^{-1}$ protein versus STARD3 $86.3 \pm 21.3 \mu\text{g mg}^{-1}$ protein) or phospholipid mass ($42.3 \pm 3.96 \mu\text{g mg}^{-1}$ protein versus STARD3 $47.1 \pm 8.31 \mu\text{g mg}^{-1}$ protein) occurred over the time scale investigated. Again, inhibition of endosomal trafficking using U18666A ($25 \mu\text{M}$) or progesterone ($10 \mu\text{M}$) induced the opposite effect, increasing incorporation of [14 C]acetate into each lipid pool ($p<0.05$) (Figure 2d).

Importantly, when STARD3 over-expressing cells were challenged with AcLDL ($50 \mu\text{g ml}^{-1}$; 24h), no significant increases in cholesterol esterification occurred, as judged by incorporation of [3 H]oleate into the cholesteryl ester pool; in fact, a small but significant ($p<0.05$) decrease was observed. By contrast, in untreated and empty vector control cells, cholesterol [3 H]esterification increased by around 2.2-fold ($p<0.01$) when treated with AcLDL under the same conditions (Figure 3); a change blocked by ACAT inhibitor, 447C88 ($10 \mu\text{M}$). Equally, an apparent reduction in intensity of Oil-Red-O staining was observed in macrophages over-expressing STARD3, compared with empty vector control, after treatment with AcLDL ($50 \mu\text{g ml}^{-1}$; 24h) (Figure 3).

Discussion

The 'START' family of lipid trafficking may be involved in non-vesicular cholesterol transport, regulating sterol-responsive transcription factors, controlling the cholesterol content of organelles, lipid rafts and membranes, and storage of cholesterol as cytosolic droplets of cholesteryl ester, although the mechanisms remain poorly understood at present.

Notably, our work demonstrates that over-expression of distinct cholesterol and oxysterol-binding proteins within the 'START' family of lipid trafficking proteins, STARD1 (StAR) [9], STARD3, STARD4 and STARD5, exert different effects on gene expression of sterol-responsive transcription factors, *SREBP2* and *LXR α* . Over-expression of mitochondrial cholesterol transporter, StAR is associated with induction of lipogenesis [9], possibly via *LXR*-dependent induction of *SREBP-1c*. Transient over-expression of cytosolic STARD4 decreased *SREBP2* mRNA levels by approximately half (Figure 1c), while over-expression of the gene encoding cytosolic STARD5 [16] protein resulted in marked increases in *SREBP2* and *LXR α* message levels, compared with controls (Figure 1d). Over-expression of STARD4 and STARD5 have previously been shown to activate a *LXR* reporter

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plasmid in NIH-3T3 cells [17]. By contrast, STARD3 over-expression did not impact on either *SREBP2* or *LXR α* mRNA levels (Figure 1b) and repressed lipogenesis (Figure 2d). While some of the observed effects may be due to differences in gene expression and translation levels, despite the delivery of equivalent amounts of DNA to the same number of cells, but may also reflect the efficiency and/or directionality of cellular cholesterol transport achieved [3, 4].

Previously, we speculated that STARD3 may traffick endosomally derived cholesterol to the endoplasmic reticulum and/or the plasma membrane [14]; even in the absence of lipoprotein-derived cholesterol, cholesterol cycles between the plasma membrane and endosomes [18]. Thus, STARD3 could help to increase the cholesterol content of the ER membrane, and retention of SREBPs by the SCAP/Insig-1(-2) complex, in agreement with the generalised repression of cholesterol, fatty acid and triacylglycerol biosynthesis observed here. Cholesterol delivery to the ER might also be expected to expand the substrate pool available for esterification by Acyl CoA: Cholesterol AcylTransferase-1 (ACAT-1; *SOAT1*). Instead, expansion of the cholesteryl [³H]oleate pool following exposure to acetylated LDL, was effectively blocked in cells over-expressing STARD3. One explanation for this data is that delivery of sterol to the endoplasmic reticulum was just sufficient to sequester SREBPs, but does not reach the threshold required to activate ACAT. In turn, this must imply that the bulk of the endosomal cholesterol trafficked by STARD3 be efficiently directed elsewhere, perhaps to the plasma membrane to facilitate cholesterol efflux (*below*), possibly via vesicular transport facilitated by the Rab family of small GTPases. Dissociation between cholesterol transport to membrane-bound SREBP transcription factors and the substrate pool available for cholesterol esterification is documented [19, 20]: Kristiana *et al* (2008) noted clear differences in kinetics between endosomal delivery of LDL-derived cholesterol to SREBPs and ACAT in mutant Chinese Hamster Ovary cells with cholesterol trafficking defects (including Niemann Pick Type C), contending that different cholesterol pools and/or transport pathways supply SREBPs and ACAT within the ER [20].

Our data also agrees well with deletion studies of the START domain of STARD3 in mice *in vivo* [21]. Although relatively modest changes in lipid phenotype were observed, probably due to functional redundancies within the START family of cholesterol transfer proteins [21, 22], significant increases in hepatic sterol ester were noted after feeding a high fat diet, together with reduced conversion of cholesterol to steroid hormones [21]. Use of a dominant negative mutant of STARD3 (Δ START-STARD3) caused extensive cholesterol accumulation in CHO cells and COS-7 cells, accompanied by inhibition of late endosomal trafficking, similar to the phenotype caused by functional loss of Niemann Pick Type C1/2 proteins [23]. Moreover, in cholesterol-laden cells, STARD3 becomes trapped at the periphery of cholesterol-laden lysosomes, reflecting loss of dynamic cholesterol movement [23], and deletion of STARD3 is linked with disrupted actin-mediated dynamics of late endocytic organelles, suggesting that cholesterol binding or sensing by STARD3 in late endosomal membranes may govern actin-dependent fusion and degradative activity of that compartment [24]. Hepatic over-expression of STARD3 *in vivo* is associated with increased conversion to bile acids [25], although in a separate study, apoptosis and hepatic toxicity were also reported, probably resulting from the grossly elevated and highly unphysiological levels of STARD3 utilised [26]; such changes were not observed in this study.

Over-expression of STARD3 in this study was also associated with increased expression of ABCA1 mRNA and protein. The latter suggests that STARD3, like NPC1, may facilitate trafficking of endosomal cholesterol and possibly ABCA1 protein, through this compartment to the plasma membrane, increasing pools of membrane cholesterol and/or transporter available for efflux to apoAI [27]. The mechanism(s) by which STARD3 increases mRNA levels of *ABCA1* are less obvious, as no induction of *LXR α* was observed in our experiments, and *Npc1* inactivation reduces Abca1 protein, but does not alter *Abca1* mRNA levels in murine macrophages [28]. However, SREBPs (1 and 2) can exert repressive effects on ABCA1 expression, decreasing cholesterol efflux [29, 30]. Thus, it is possible that sequestration of SREBPs at the endoplasmic reticulum, suggested by the coordinated loss of lipid biosynthesis observed here, may relieve inhibition of *ABCA1* gene expression and increase cholesterol efflux to apoAI. Alternatively, STARD3 expression could alter levels of ABCA1 mRNA by changing ratios of saturated to unsaturated fatty acids [31], or perhaps via its

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involvement in actin-mediated dynamics of late endosomes [32] trigger changes in actin-dependent gene expression [33].

In conclusion, STARD3 over-expression may be useful in limiting atherogenesis, by upregulating cholesterol efflux mechanisms, reducing cholesterol synthesis and blockade of cholesterol esterification: *in vivo* studies are now needed to establish this contention, using murine models of atheroma.

Acknowledgments

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Table 1

Sequence of primers and probes used to quantify mRNA by polymerase chain reaction

Gene	Forward Primer	Reverse Primer	Probe Sequence
<i>STARD3</i>	5'-cctgccccgggtacctcat-3'	5'-gcgctgtcgcaggtgaa-3'	5'-agagcctcgcggccaccatgt-3'
<i>STARD4</i>	5'-aacatcagacaggcctcca-3'	5'-gagagcgggtgaggtgctt-3'	5'-tagcgcgcctagctcca-3'
<i>STARD5</i>	5'-gtggacttggtgctagcaagaga-3'	5'-gacataacggatgctccacatg-3'	5'-atgtggaccatcagttccaaccca-3'
<i>ABCA1</i>	5'tgtccagtcagtaagtgtctgt-3'	5'-aagcgagatattggtcggatt-3'	5'acacctggagagaagcttcaacgagactaacc-3'
<i>ABCG1</i>	5'-acgtgccttcagatcaatgt-3'	5'-gacggctgcgacgtccatc-3'	5'-cccagtgccctactgcagcatcgt-3'
<i>ABCG4</i>	5'-ggttcattgccacgtggtt-3'	5'-gccgggtgttgaagacctt-3'	5'-tctacctgcatattggcgacgatgcc-3'
<i>APOE</i>	5'-tgggtcgtctttgggattac-3'	5'-ccatcagcgcctcagtt-3'	5'-ctgctcagctcccaggtcaccca-3'
<i>LXRalpha</i>	5'-gaacaactggcagatgacga-3'	5'-aaggagcgccttacact-3'	5'-aagctcgtcgtgcccagcaa-3'
<i>SREBP1</i>	5'-gctcctccatcaatgacaaaac-3'	5'-gcttgagtttctggtgctgtgt-3'	5'-aaggccatcgactacattcgtttctgc-3'
<i>SREBP2</i>	5'-cggtaatgatcacgccaaca-3'	5'-tggtatatcaaggctgctggat-3'	5'-tcagcaccactccgagcagagg-3'
<i>GAPDH</i>	5'-cacatggcctccaaggagtaa-3'	5'-tgagggtctctctctctctgt-3'	5'-ctggaccaccagccccagcaag-3'

Table 2 Endogenous gene expression of *STARD3*, relative to *GAPDH*, in human (THP-1) monocytes and macrophages, human peripheral blood monocyte-macrophages and human heart aorta

Source of RNA	Expression of <i>STARD3</i> mRNA to <i>GAPDH</i> mRNA (x1000)
Human THP-1 monocytes	1.2±0.2
Human THP-1 macrophages	0.8±0.1
Human peripheral blood monocyte-macrophages	1.5±0.4
Human heart aorta	0.4±0.1

Data are from three individual cDNA preparations, derived from 4×10^7 human peripheral blood monocyte-macrophages (Lonza, Wokingham), the equivalent number of THP-1 monocytes and macrophages, and from a sample (50µg) of four to seven pooled human heart aortae (Clontech, USA)

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Submission for accelerated publication

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Figure Legends

Figure 1 a) Transient over-expression of *STARD3* compared with empty vector control (24-72h), in human THP-1 monocyte-macrophages and b) effect of *STARD3* over-expression (72h) on levels of *SREBP2* and *LXRα* mRNA compared with over-expression of *STARD4* (c) and *STARD5* (d) on gene expression of the same transcription factors. Values are mean±SEM of three independent experiments; *p<0.05, **p<0.01 and ***p<0.001 compared with empty vector control.

Figure 2 a) Levels of *ABCA1*, *ABCG1*, *ABCG4* and *APOE* mRNA in monocyte-macrophages over-expressing *STARD3* (72h), compared with empty vector control, in three independent experiments. b) Levels of *STARD3* and *ABCA1* protein, compared with housekeeping protein, *GAPDH*, in THP-1 monocyte-macrophages (72h): values representative of three independent experiments. The effect of *STARD3* over-expression on c) cholesterol efflux to apoAI (10 μg ml⁻¹), in five independent experiments, and d) biosynthesis of lipids from [¹⁴C]acetate (1 μCi ml⁻¹), compared with empty vector controls, in four independent experiments; endosomal inhibitors U18666A (25 μM) and/or progesterone (10 μM) are included as positive controls. All values are mean±SEM; significant differences (p<0.05) from the control incubation for each lipid are indicated as **a**: free cholesterol, **b**: cholesteryl esters, **c**: fatty acids, **d**: triacylglycerol and **e**: phospholipids.

Figure 3 Incorporation of [³H]oleate (10 μM; 1 μCi ml⁻¹) into the cholesteryl ester pool, following incubation with acetylated LDL (50 μg ml⁻¹; 24h) in the presence or absence of ACAT inhibitor 447C88 (10 μM) in wild type cells, and in cells transfected with either empty vector or *STARD3*. Values are mean±SEM of four independent experiments; *p<0.05, **p<0.01 compared with controls; †p<0.05 compared with acetylated LDL alone, and ‡‡p<0.01 compared with empty vector control treated with acetylated LDL. The bottom panel (A-D) illustrates Oil-Red-O staining in empty vector (A, C) and *STARD3* over-expressing (B, D), incubated in the absence (A, B) or presence (C, D) of AcLDL (50 μg ml⁻¹; 24h).

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Figure 1a)

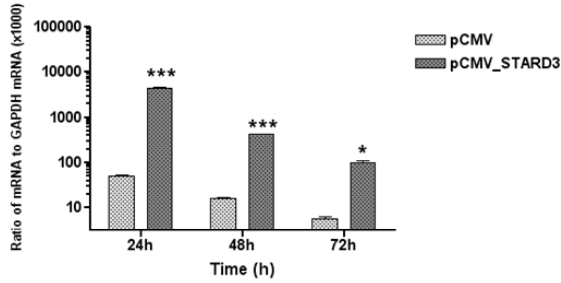


Figure 1c)

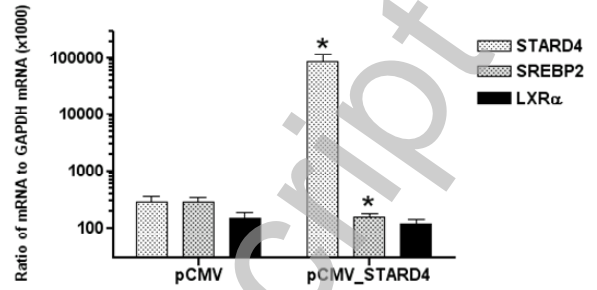


Figure 1b)

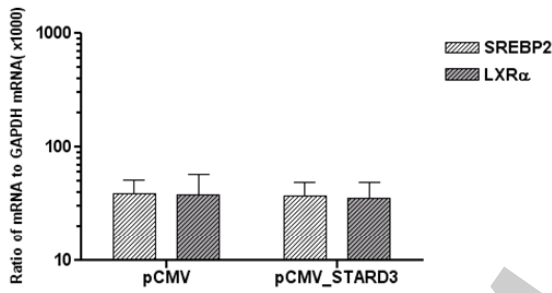
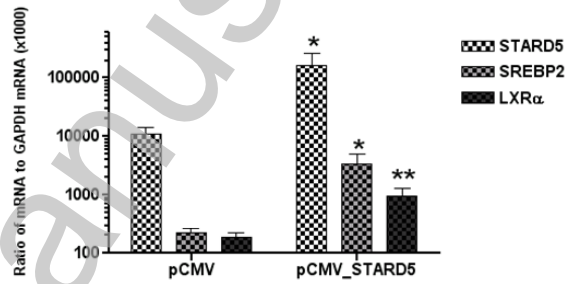


Figure 1d)



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Figure 2a

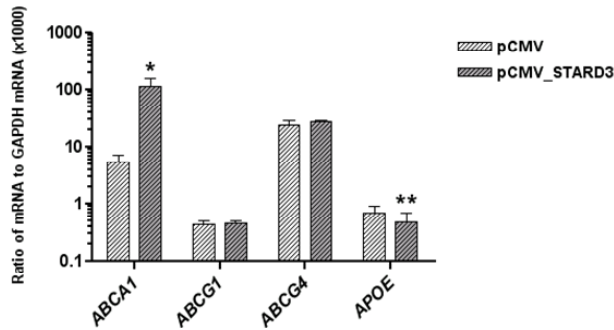


Figure 2c

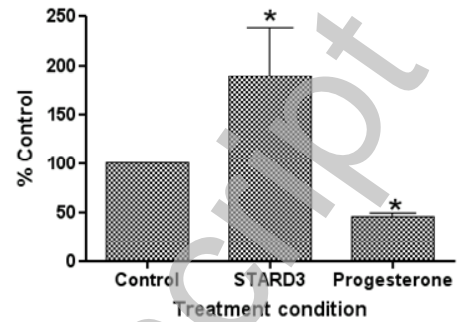


Figure 2b

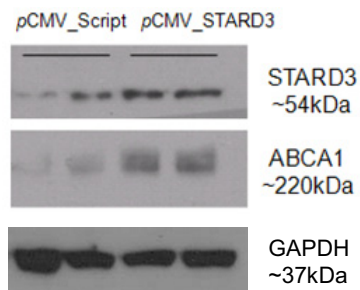
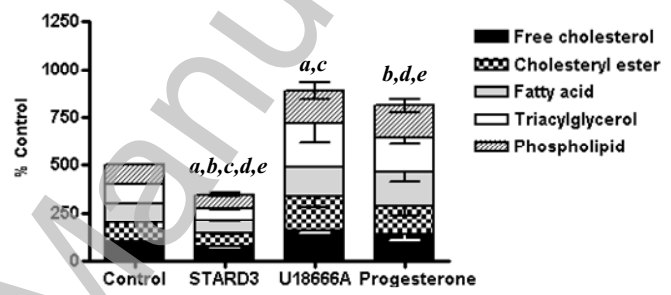


Figure 2d

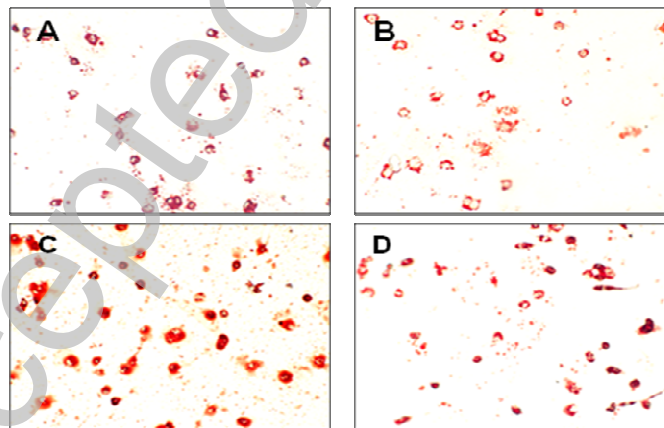
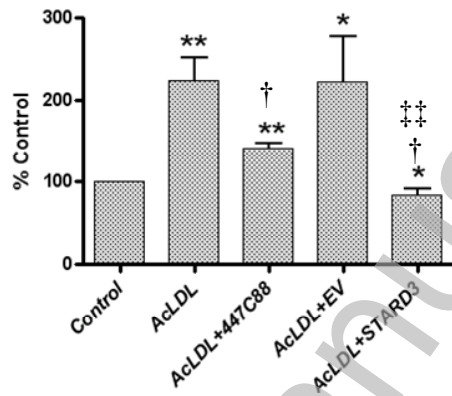


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Figure 3



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