Journal of Diabetes & Metabolic Disorders (2020) 19:363–371
https://doi.org/10.1007/s40200-020-00517-0

RESEARCH ARTICLE

Rapid degradation of ABCA1 protein following cAMP withdrawal and treatment with PKA inhibitor suggests ABCA1 is a short-lived protein primarily regulated at the transcriptional level

Neelam Srivastava[1] · Angelo B. Cefalu[1] · Maurizio Averna[1] · Rai Ajit K. Srivastava[2]

Received: 5 April 2019 / Accepted: 12 March 2020 / Published online: 19 March 2020

Abstract

Objectives ATP-binding cassette transporter A1 (ABCA1) is a key player in the reverse cholesterol transport (RCT) and HDL biogenesis. Since RCT is compromised as a result of ABCA1 dysfunction in diabetic state, the objective of this study was to investigate the regulation of ABCA1 in a stably transfected 293 cells expressing ABCA1 under the control of cAMP response element.

Methods To delineate transcriptional and posttranscriptional regulation of ABCA1, 293 cells were stably transfected with the full length ABCA1 cDNA under the control of CMV promoter harboring cAMP response element. cAMP-mediated regulation of ABCA1 and cholesterol efflux were studied in the presence of 8-Br-cAMP and after withdrawal of 8-Br-cAMP. The mechanism of cAMP-mediated transcriptional induction of the ABCA1 gene was studied in protein kinase A (PKA) inhibitors-treated cells.

Results The transfected 293 cells expressed high levels of ABCA1, while non-transfected wild-type 293 cells showed very low levels of ABCA1. Treatments of transfected cells with 8-Br-cAMP increased ABCA1 protein by 10-fold and mRNA by 20-fold. Cholesterol efflux also increased in parallel. Withdrawal of 8-Br-cAMP caused time-dependent rapid diminution of ABCA1 protein and mRNA, suggesting ABCA1 regulation at the transcriptional level. Treatment with PKA inhibitors abolished the cAMP-mediated induction of the ABCA1 mRNA and protein, resulting dampening of ABCA1-dependent cholesterol efflux.

Conclusions These results demonstrate that transfected cell line mimics cAMP response similar to normal cells with natural ABCA1 promoter and suggest that ABCA1 is a short-lived protein primarily regulated at the transcriptional level to maintain cellular cholesterol homeostasis.

Keywords Diabetes · ABCA1 · Regulation · Stably transfected · cAMP · Protein kinase

Abbreviations

ABCA1	ATP-binding cassette transporter A1
cAMP	cyclic AMP
PK	protein kinase
TD	Tangiers Disease
USF	upstream stimulatory factor
DMEM	Dulbecco's minimal essential medium
FBS	fetal bovine serum
LXR	liver x receptor
RXR	retinoid x receptor
apoA1	apolipoprotein A1
HDL	high density lipoprotein
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
8-Br-cAMP	8-bromocyclic adenosine 3′, 5′ monophosphate

✉ Rai Ajit K. Srivastava
ajitsriva@gmail.com

[1] University of Palermo, Palermo, Italy

[2] Integrated Pharma Solution, Philadelphia, USA

Introduction

Eukaryotic cells maintain cholesterol homeostasis by regulated cholesterol synthesis, uptake, efflux, transport, and removal. Imbalance of any of these regulated pathways leads to cholesterol abnormalities and susceptibility to diseases. In addition to apolipoprotein A-I (apoA-I) [1], other important player that determines circulating levels of HDL is ATP-binding cassette transporter A1 (ABCA1) [2]. Loss of

function mutation in *abca1* gene causes a severe HDL deficiency syndrome known as Tangier disease (TD) [3–7]. These TD patients have cholesteryl esters accumulation in tissues, and increased risk of cardiovascular disease. Mice lacking ABCA1 showed similar phenotypes observed in TD patients with low levels of HDL [8, 9]. Further understanding of the roles of ABCA1 in lipid transport and atherosclerosis became clear from studies with ABCA1 transgenic mice [10–12]. These mice showed enhanced cholesterol efflux from macrophages and reduced atherosclerotic lesion formation [13]. Thus, ABCA1 plays a key role in reverse cholesterol transport and atherosclerosis regression [2].

Regulation of ABCA1 occurs by the transcriptional [14–17] as well post-transcriptional [18] mechanisms. Lipoproteins, oxysterols, and oxidized LDL induce ABCA1 mRNA concomitant with increased levels of LXRα expression [15–17]. ABCA1 and diabetes play important roles in maintaining HDL level and function [19]. HDL function is compromised in diabetic conditions [20] through oxidative modification of the main apoprotein of HDL, apolipoprotein A-I [21]. These modifications lead to dampening of cholesterol efflux and increased risks of CVD [19]. Impaired reverse cholesterol transport in diabetic conditions also results from low levels of ABCA1 expression in liver and peritoneal macrophages [22, 23] and advanced glycation end products (AGE)-mediated ABCA1 dysfunction [24]. Studies in type 2 diabetic patients have shown that both ABCA1gene expression and protein are reduced in leukocytes and there was an inverse correlation between ABCA1 expression and HbA1c and blood glucose and positive correlation between ABCA1 expression and plasma HDL concentration [25]. ABCA1 degradation in diabetic condition may be affected since unsaturated fatty acids were found to induce ABCA1 degradation [26]. To have further insights into the degradation mechanism of ABCA1, we investigated transcriptional and post-transcriptional regulation of ABCA1 in a cell-based system expressing human ABCA1 under the control of CMV promoter that harbors cAMP response element. Cyclic AMP inducible cholesterol efflux to apoA1 in macrophages was attributed to the transcriptional up-regulation of the ABCA1 [27–29]. A number of studies in mice have confirmed that LXR is an important transcriptional activator of ABCA1 [15–18, 30]. Among other promoter elements that control ABCA1 transcription, a cAMP response element in the ABCA1 promoter has been identified [31]. ABCA1-mediated cholesterol efflux has also been shown to be dampened by protein kinase A inhibitor [32], suggesting phosphorylation as an essential component in the ABCA1-mediated cholesterol efflux. To understand whether cAMP response element is indeed required for the cAMP-induced upregulation of the ABCA1, and whether rapid normalization of the ABCA1 occurs following cAMP withdrawal, we used a different approach using a transfected cell line expressing ABCA1 under the control of CMV promoter that harbors cAMP response element [33, 34] [35]. In this cell line, we studied the cAMP-mediated regulation of the ABCA1 gene. Our studies demonstrate that the transfected cell line shows similar cAMP-induced upregulation of ABCA1 as in the WT macrophages, and this effect occurs via PKA-mediated pathway. We also showed that the continued transcription of the ABCA1 is required to control the physiological level of intracellular ABCA1 and maintain cholesterol homeostasis.

Materials and methods

WT HEK293 cells and stably transfected cell line

WT human embryonic kidney (HEK) 293 cells obtained from ATCC (CRL-1573) were maintained in DMEM with 10% FBS, 50 U/ml penicillin-streptomycin. We used HEK293 cells because of the high efficiency of transfection [36]. Additionally, HEK293 cells have been widely used in studies relating to CMV promoter studies [33]. The ABCA1 cDNA was cloned in front of the CMV promoter harboring cAMP response element [35]. ABCA1 expressing stable human embryonic kidney 293 T cell line was generated by using Flip-In system (Invitrogen). Full length human ABCA1 cDNA was isolated from cultured human skin fibroblast cells and cloned into pcDNA5/FRT vector (Invitrogen). To generate ABCA1 monoclonal stable cell line, HEK293 cells were co-transfected with pcDNA5/FRT-ABCA1 (Dr Michael Heyden, University of Vancouver, British Columbia, CA) and pOG44 (Invitrogen) using Fugene-6 (Roche) in the growth medium without zeocin according to the manufacturer's recommendations. After 24 h following transfection, cells were maintained in 50 μg/ml hygromycin in DMEM with 10% FBS. Individual colonies were examined for the expression of ABCA1 by Western blotting.

Transient transfection studies

The same human ABCA1 construct used for making stably transfected 293 cell line were also used for transient transfection using HEK293 cells.

Cholesterol efflux assay

For cholesterol efflux assay, cells (4×10^4 /ml) were seeded in 24 well plates. Next day, labeling media (culture medium plus 1% FBS with 2 mCi/ml [^{3}H] cholesterol) was added to the cells at 0.5 ml/well. After 24 h of incubation, the cells were switched to 0.2% BSA (defatted, Sigma) in serum-free medium for 3 h to equilibrate radiolabeled-free cholesterol. Medium was removed, cells were incubated in serum-free culture medium with or without apoAI (final concentration

10 mg/ml) and with or without 8-Br-cAMP (0.3 mM) for 24 h. One hundred microliter of the medium was removed and counted on a liquid scintillation counter. Cells were solubilized by adding 500 μl of 0.1 N NaOH to each well, 100 μl of supernatant was removed and counted on a liquid scintillation counter. The amount of cholesterol efflux was calculated as follows: Cholesterol efflux = Total radioactivity in the medium/ (Total radioactivity in the medium + Total radioactivity in the cells).

ABCA1 protein quantitation

After each experiment, the cells were collected by spinning, and lysed in lysis buffer (20 mM Hepes, 5 mM KCl, 5 mM $MgCl_2$, 0.5% (*v*/v) Triton X-100 and complete protease inhibitor). Cell debris was removed by centrifugation at 8000 rpm for 10 min at 4 °C. Protein concentration in the supernatant was determined by BioRad protein assay kit. Twenty-five microgram of protein was separated on 8–16% Tris- Glycine gel (Pre- Cast Invitrogen gels) and electrophoretically transferred to nitrocellulose membrane (Invitrogen). Membranes were probed with either ABCA1 antibody or a polyclonal anti-beta actin antibody (Santa Cruz Biotechnology) as a control for equal loading. Immunoreactivity was detected by Super signal (Pierce).

ABCA1 mRNA quantitation

Gene- specific primers and probe were designed using the Primer Express software (Perkin Elmer Life Sciences). The real time RT-PCR reaction was performed essentially following the manufacture's protocol with RT-PCR mix and 200 nM FAM-probe and 600 nM of both forward and reverse primers and analyzed in ABI prism 7900HT sequence detection system (Applied Biosystems). Relative amounts of ABCA1 mRNA levels were plotted as fold-change compared to no treatment. For normalization, 18S ribosomal RNA was used. Taqman RT-PCR was performed in triplicate and experiment was independently repeated three times.

Studies with 8-Br-cAMP and PK inhibitors

Effects of 8-Br-cAMP and protein kinase (PK) inhibitors were studied in nontransfected as well as in the stably transfected 293 cells. Cells were treated with 8-Br-cAMP (Sigma) (0.3 mM final concentration) for 24 h as described [29]. To perform experiment with protein kinase inhibitors, cells were first treated for 3 h with PK inhibitors, H7, H8 or H9 (Sigma Chemical Co, St Louis, MO) at the final concentration of 15 μM followed by the addition cAMP together with PK inhibitors for 24 h. These phosphokinase inhibitors are isoquinoline sulfonamides, a class of synthetic protein kinase inhibitors, namely 1-(5-isoquinoline sulfonyl)-2-methylpiperazine dihydrochloride (H7), N-[2-(methylamino)ethyl]-5-isoquinoline sulfonamide dihydrochloride (H8), N-(2-aminoethyl)-5-isoquinoline sulfonamide dihydrochloride (H9) [37]. Parallel experiments were performed without PK inhibitor and without 8-Br-cAMP. After each treatment, cholesterol efflux, ABCA1 protein, and ABCA1 mRNA were measured.

Results

Effects of serum on ABCA1 regulation

Expression of ABCA1 was examined in the absence or presence of various concentrations of serum. Effect of 8-Br-cAMP was also examined in serum-deprived and in serum-containing media. As shown in Fig. 1a, absence of serum or presence of 1% or 10% serum in the media had no effect on the expression of ABCA1 protein in the transfected cell line. However, the presence of serum had dramatic effects on the 8-Br-cAMP-mediated induction of ABCA1. 8-Br-cAMP increased ABCA1 expression by 2-fold in serum free media, and 10-fold in the serum-containing media. As a control β-actin protein was also measured on the same cell extract, which did not show any changes in the serum-free or serum-containing media.

cAMP-induced ABCA1 regulation

Next, we studied the expression of ABCA1 by 8-Br-cAMP in the 293 cells and in stably transfected 293 cells in greater details. The non-transfected 293 cells show undetectable amounts of cholesterol efflux in the presence of apoA1 and 8-Br-cAMP. The transfected cells, on the other hand, showed several fold increases in cholesterol efflux to apoA1, which was further magnified following 8-Br-cAMP treatment (Fig. 1b). Measurements of ABCA1 protein in the 8-Br-cAMP-treated transfected cell line for 24 h showed 20-fold increase consistent with increase in cholesterol efflux (Fig. 1c). However, withdrawal of 8-Br-cAMP from the media showed a time-dependent lowering of ABCA1 protein, bringing ABCA1 protein to the basal levels within 6 h. We also measured ABCA1 mRNA in the 8-Br-cAMP-treated transfected cell line. As shown in Fig. 1d, 8-Br-cAMP increased the ABCA1 mRNA, and withdrawal of 8-Br-cAMP decreased the ABCA1 mRNA to the basal level within 6 h. Although the non-transfected 293 cells did not show significant cholesterol efflux in the presence of 8-Br-cAMP, we measured ABCA1 mRNA levels in these cells in the presence of 8-Br-cAMP using a sensitive mRNA detection method, real-time PCR, which can detect even few copies of the ABCA1 mRNA transcripts. As shown in Fig. 2a, treatments by 8-Br-cAMP induced ABCA1 mRNA even in the non-transfected 293

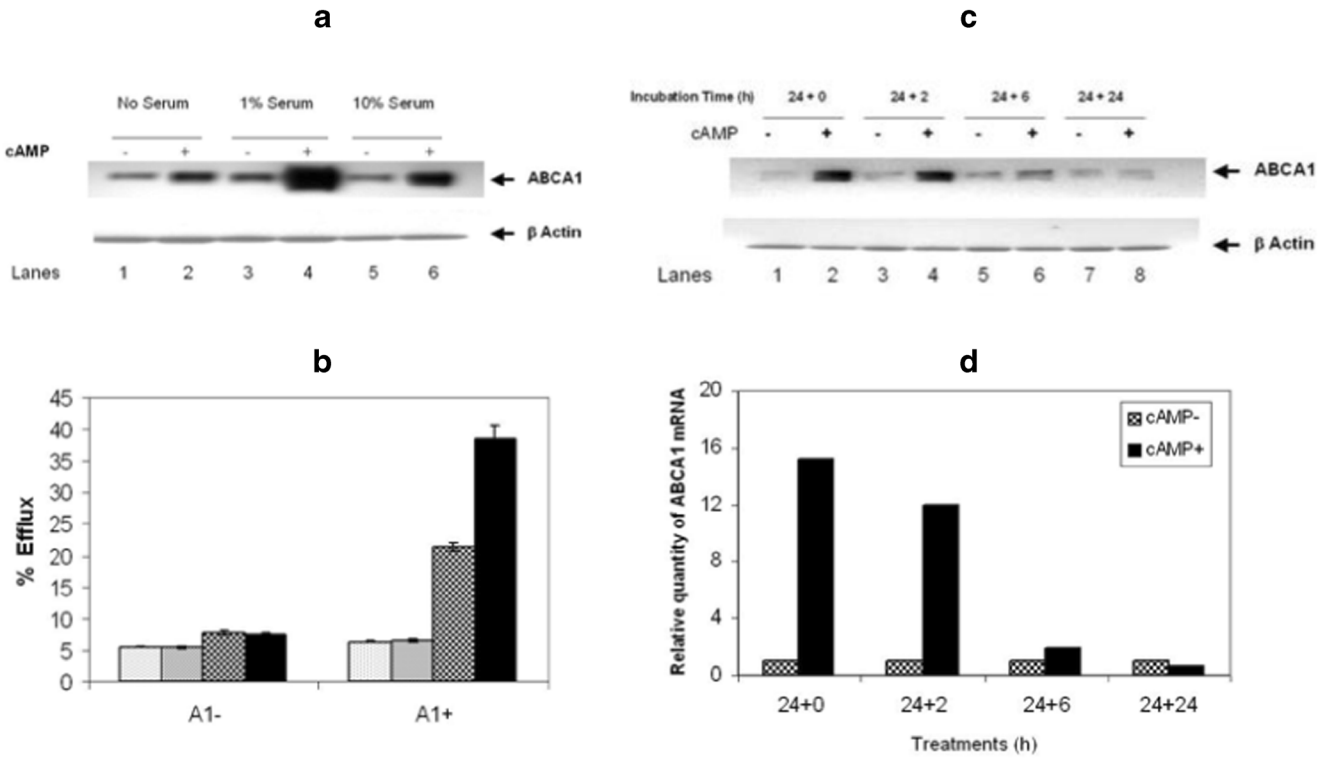

Fig. 1 Panel A, Effect of serum on 8-Br-cAMP-induced regulation of ABCA1. The transfected HEK293 cell line was incubated in the absence or presence of serum with or without 8-Br-cAMP. 8-Br-cAMP treatment was done for 24 h. Cell lysate (25 μg protein) was separated on 8–16% Tris- Glycine gel and transferred to nitrocellulose membrane followed by detection with either ABCA1 antibody or a polyclonal anti-beta actin antibody. The ABCA1 and β-actin proteins are shown by arrows. Panel B, Effect of cAMP on cholesterol efflux in WT 293 cells, and in transfected 293 cells in the presence and absence of apoAI and 8-Br-cAMP. The details of the efflux assay are provided in the methods section. Dotted bars, 293 cells, shaded bars, 293 cells with 8-Br-cAMP, big dotted bars, transfected 293 cells, filled bars, transfected 293 cells with 8-Br-cAMP. Panel C, Effect of 8-Br-cAMP withdrawal on ABCA1 protein levels in the 293 cells stably transfected with hABCA1. The transfected cells were treated with or without cAMP for 24 h, the medium was removed, and cells were further incubated in the same media but without cAMP for 0 h, 2 h, 6 h, and 24 h, as indicated after the + sign. The cell lysates were prepared and ABCA1 and β-actin proteins were measured by Western blotting. The respective protein bands of ABCA1 and β-actin are indicated by arrows. Panel D, Effect of 8-Br-cAMP withdrawal on ABCA1 mRNA levels in the 293 cells stably transfected with hABCA1. Cells were treated with or without 8-Br-cAMP as described in Panel C, and RNA was prepared from the cells. Sixty nanogram of the total RNA were taken for ABCA1 mRNA quantitation by real-time RT-PCR using Taqman 7900HT. As a control 18S RNA was also quantitated. The numbers after the + sign indicates time in hour after cAMP treatment. cAMP- indicates without cAMP, and cAMP + indicates with cAMP

cells, and the withdrawal of 8-Br-cAMP caused ABCA1 mRNA to drop to the basal levels within 6 h, similar to the observations in stably transfected cell line (Fig. 1d). Withdrawal of 8-Br-cAMP from the media also decreased cholesterol efflux in stably transfected cells (Fig. 2b). Transient transfection of 293 cells with the same construct used for making ABCA1 expressing stable cell lines showed similar 8-Br-cAMP-induced regulation as seen with the transfected cell line (data not shown).

Effect of protein kinase inhibitors on ABCA1 regulation

Since 8-Br-cAMP-mediated induction of gene expression involves phosphorylation events, we tested the hypothesis that cAMP-induced upregulation of the ABCA1 gene expression occurred via protein kinase -mediated pathway. To test this hypothesis, we used several known inhibitors of protein kinases [37], and performed experiments in the presence and absence of 8-Br-cAMP and PK inhibitors. The transfected cells were first treated with PK inhibitors for 3 h followed by addition of 8-Br-cAMP and incubation for 24 h. As shown in Fig. 3, 8-Br-cAMP treatment dramatically increased ABCA1 protein (lane 1) compared to 8-Br-cAMP untreated control (lane 4). Addition of protein kinase inhibitors decreased ABCA1 protein to almost the basal level. This experiment was repeated with several protein kinase inhibitors, and ABCA1 mRNA was quantitated by real-time PCR. The results shown in Fig. 3b clearly demonstrate that 8-Br-cAMP upregulated ABCA1 mRNA by more than 20-fold, and the treatment with protein kinase inhibitors reduced ABCA1 mRNA. PK inhibitor-treated cells were also used to measure cholesterol efflux. As shown in Fig. 3c, PK inhibitors also reduced cholesterol efflux to apoA1, concomitant with reductions in the ABCA1 protein and mRNA levels.

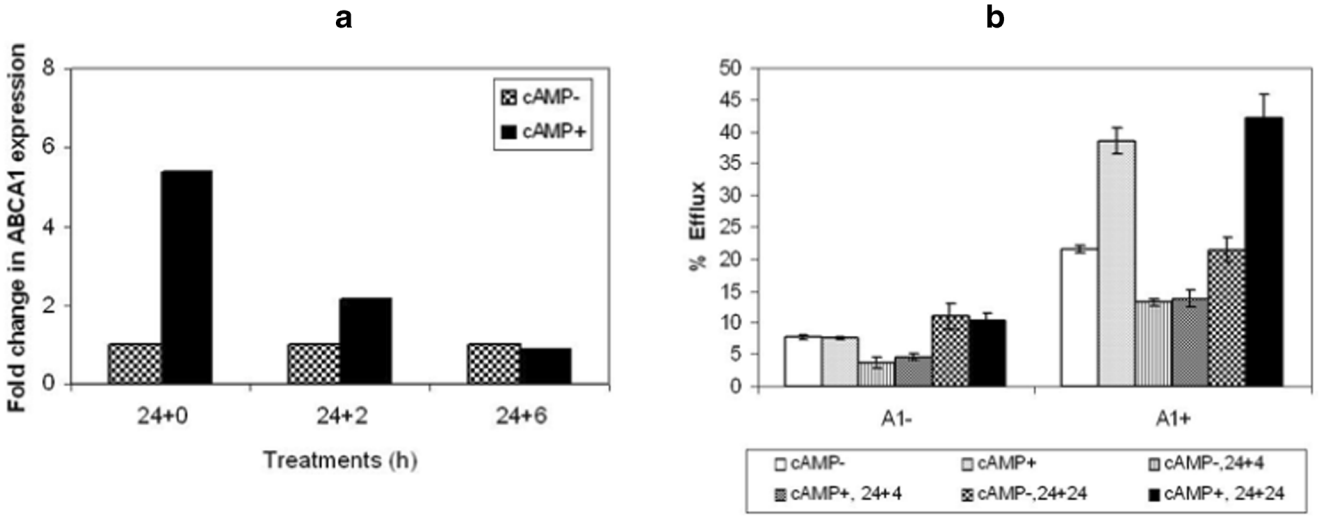

Fig. 2 Panel A, Effect of 8-Br-cAMP withdrawal on ABCA1 mRNA in the wild type 293 cells. Cells were treated with or without 8-Br-cAMP for 24 h, the media was removed and incubated in the media without 8-Br-cAMP for 2 and 6 h, respectively, as indicated. The numbers after the + sign indicates time in h after cAMP treatment. cAMP- indicates without cAMP, and cAMP + indicates with cAMP. Panel B, Effect of cAMP withdrawal on the cholesterol efflux. Stably transfected 293 cells were seeded in triplicate in the 24-well plates and cholesterol assay done in the absence and presence of 8-Br-cAMP as described in the methods section. – indicates without cAMP and + indicates with cyclic AMP. The indicated numbers after the + sign in the caption box indicates the time of incubation after cAMP treatments

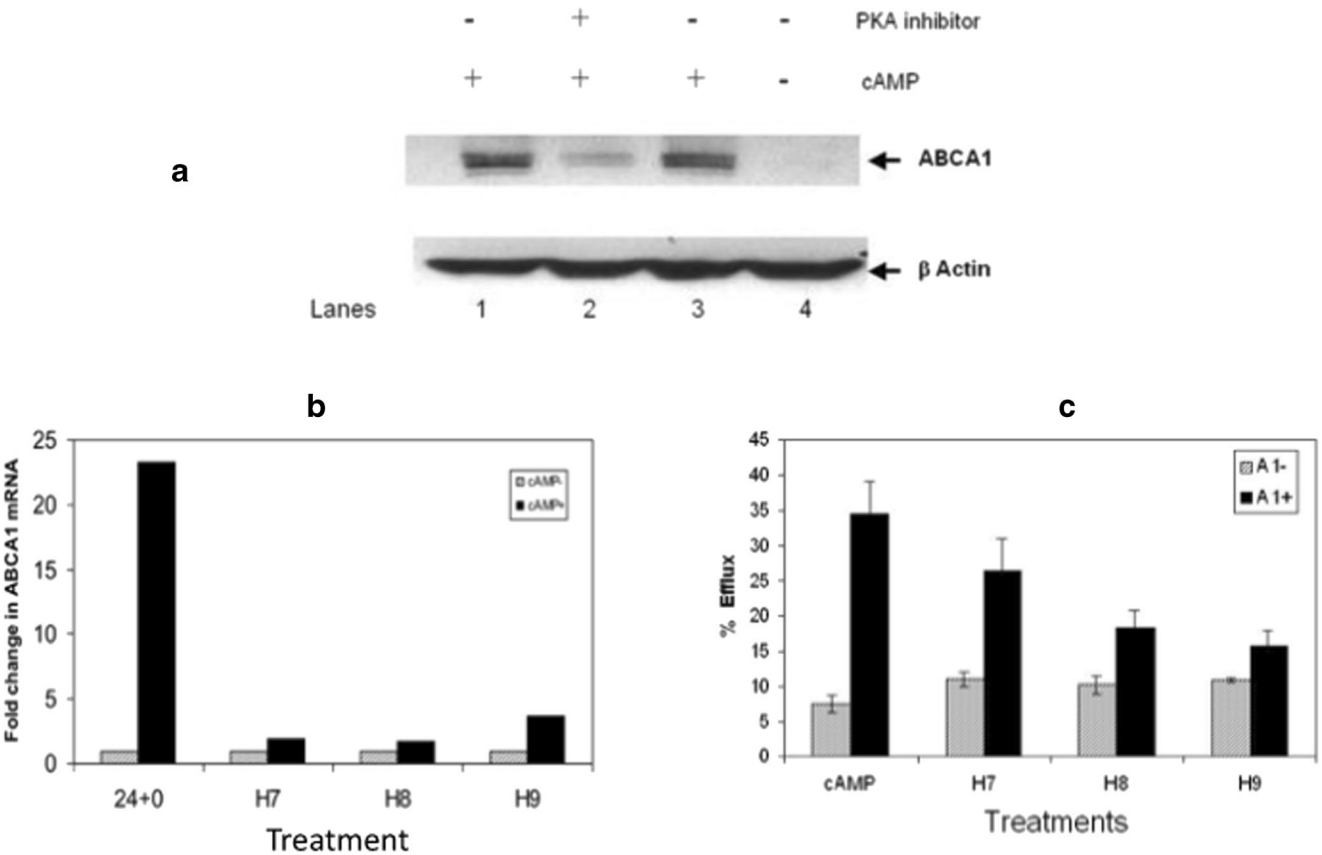

Fig. 3 Effect of PKA inhibitor on cAMP-induced regulation of ABCA1 in stably transfected 293 cells. Stably transfected cells were treated with PKA inhibitor as described in the methods section. Cells were washed after the treatments and cell lysates were prepared. An aliquot of the cell lysates was separated on SDS-PAGE to detect ABCA1 protein and β-actin protein as an internal standard. Panel B, Effect of protein kinase inhibitors on the cAMP-induced ABCA1 mRNA. The stably transfected cells were treated with three different protein kinase inhibitors, followed by washing and RNA preparation. In each RNA samples, ABCA1 mRNA were quantitated. Values shown represent relative to 18S RNA. Panel C, Effect of protein kinase inhibitors on the cholesterol efflux in the 293 cells stably transfected with hABCA1. Stably transfected cells were treated with protein kinase inhibitors as indicated and cholesterol efflux measured following the method described in the methods section

Discussion

The aim of the present study was a) to examine the suitability of 293 cell stably transfected with human full length ABCA1 cDNA to study cAMP-mediated ABCA1 regulation; and b) to study if cAMP-induced regulation of the ABCA1 occurs via protein kinase-mediated pathway. First, we established the levels of ABCA1 expression in the presence of various concentrations of serum. Although the presence of serum appeared to have minimal effect on ABCA1 expression in the transfected cells, it certainly influenced cAMP-induced elevation of ABCA1 protein, suggesting that complete media is needed for the enhanced synthesis of ABCA1 in the presence of cAMP. The functionality of the overexpressed ABCA1 was confirmed by performing cholesterol efflux assay. Increased ABCA1 mRNA in the 8-Br-cAMP-treated transfected cells suggested that 8-Br-cAMP induced increases of ABCA1 occurred via transcriptional mechanism similar to earlier studies with macrophages [27–29]. Thus, stably transfected cells behave similarly at least with regard to the cAMP-induced regulation of the ABCA1.

To further establish that the transfected cell line mimics similar regulation by cAMP, we performed cAMP withdrawal experiments. Earlier studies have shown that cAMP induced ABCA1 expression and withdrawal of cAMP caused time-dependent reduction in ABCA1 protein and mRNA [29]. To ascertain if withdrawal of cAMP- mediated reduction in ABCA1 mRNA and protein is driven by a cAMP response element, we used a human ABCA1 construct in which the expression of the cloned cDNA is driven by a CMV promoter harboring cAMP response element [35]. By transfection of 293 cells with this construct, a stable cell line was produced and the hypothesis that the presence of cAMP is required for continued ABCA1 mRNA transcription and ABCA1 protein production was tested. We also tested the hypothesis that the withdrawal of cAMP would rapidly diminish ABCA1 protein to the basal levels similar to the observations in macrophages [29]. Indeed, the treatments with 8-Br-cAMP induced ABCA1 protein and mRNA, suggesting that this response could have occurred via a cAMP response element in the CMV promoter. These results were further corroborated by the results obtained with the transient transfection studies in two cell lines, 293 and Hela, with the same ABCA1 construct (not shown), suggesting that the cAMP-mediated induction of ABCA1 upregulation occurred via the cAMP response element in the construct. If the induction of ABCA1 expression occurred via cAMP response elements, then the withdrawal of cAMP should cause reduction in the ABCA1 mRNA. Indeed, we found that cAMP increased ABCA1 mRNA by almost 20-fold, and withdrawal of cAMP caused reductions in the ABCA1 mRNA, protein, and cholesterol efflux, suggesting that in order for cAMP to mediate upregulation of ABCA1, it must be present in the media at all times. These results also suggest that ABCA1 protein is a short-lived protein, and cellular ABCA1 levels are highly dependent on the transcription, impying that ABCA1 regulation occurs primarily via transcriptional mechanism, and the rates of transcription are possibly modulated depending upon the need of the cells for lipids. Other studies have also suggested short half-life of ABCA1 [38, 39]. This may be the reason why rates of ABCA1 transcription are accelerated when the cells are loaded with cholesterol, lipoprotein, and oxidized lipoprotein [14–17]. Since lipid loading to cells are detrimental to normal physiology of the cells, higher cellular cholesterol content induces ABCA1 expression at the transcriptional levels to remove the excess cholesterol in order to maintain the normal functioning of the cells. From the present studies as well as from the studies reported earlier, it appears that ABCA1 regulation occurs primarily by transcriptional mechanism in order for cells to quickly adjust to the rapidly changing lipid environment as a result of physiological, pharmacological, or nutritional stimuli.

Since it became evident that ABCA1 regulation by cAMP in the transfected 293 cell lines occurred via cAMP response element in the construct, we next asked the question that if it did occur via cAMP response element, does it involve protein kinase pathway? Earlier reports have shown that cAMP-mediated regulation of target genes occurs via protein kinase pathway [40]. We tested this hypothesis in the stably transfected 293 cell line. We employed three different PK inhibitors [37], and all of them inhibited cAMP-induced cholesterol efflux, concomitant with the reduction in ABCA1 protein, suggesting that cAMP-mediated regulation of the ABCA1 induction involves protein kinase (s). To further have an insight into the loci of PK inhibitors, we measured ABCA1 mRNA as shown in Fig. 3b. It is evident that PK inhibitors reduced cAMP-induced increases of ABCA1 mRNA, suggesting that cAMP-mediated upregulation of ABCA1 mRNA transcription does occur via protein kinase-mediated pathway. These studies further suggested that the transcriptional regulation of the ABCA1 is important in maintaining ABCA1 protein and cellular lipid homeostasis.

PPARs (α, γ, β) are also reported to induce ABCA1 transcription [41–43] via LXR-mediated pathway. Since the stably transfected cell line used in the present study lacks an LXR element, it is highly unlikely that the cAMP-mediated induction of ABCA1 occurred via LXR-mediated pathway. Based on the results in the present study, and the results from other studies [15–18, 30, 41–43] it appears that the regulation of the ABCA1 transcription occurs at least via two different mechanisms, one involving LXR-mediated pathway, and the other involving protein kinase-mediated pathway. Mixed results on the regulation of ABCA1 have been reported by cytokines. For instance, while TGF-β [44] and TLR2 [45] increase ABCA1 expression, interferon-γ [46] and TNF-α [47] decrease ABCA1 expression. These findings are important in view of the anti-inflammatory function of ABCA1 in macrophages [48]. Geranylgeranyl pyrophosphate is also reported to down-regulate ABCA1 [49], but it appears to reduce ABCA1 expression via two different mechanisms, by acting directly as

an antagonist of LXR and also by activation of the Rho GTP-binding proteins.

These findings have implications in diabetes-induced dysfunction of HDL in the process of reverse cholesterol transport, which is one of the important pathways of cholesterol removal from the body [2]. While the major pathway of ABCA1 regulation appears to be the transcription depending upon the cellular cholesterol level, some studies do suggest posttranslational regulation of ABCA1 protein [26]. Diabetic individuals have higher oxidative stress and low-grade inflammation that result in impaired reverse cholesterol transport due to low levels of ABCA1 expression in liver and peritoneal macrophages [22, 23]. Additionally, in diabetic conditions, advanced glycation end products (AGE) are reported to make ABCA1 dysfunctional [24]. The ABCA1 dysfunction may occur indirectly by oxidative modification of HDL/apoA-I protein [50–54] as well as by direct effect on ABCA1 [24, 25, 55]. This was confirmed in type 2 diabetic patients showing reduced ABCA1 mRNA and protein in leukocytes with an inverse correlation between ABCA1 expression and HbA1c and blood glucose, and positive correlation between ABCA1 expression and plasma HDL concentration [25]. These earlier findings together with the present finding suggest the transcriptional regulation of ABCA1 as the major pathway in the regulation of ABCA-1-mediated cholesterol efflux.

In summary, we showed that the stably transfected HEK293 cells with human ABCA1 mimic the same cAMP-mediated regulation as seen with the normal macrophages, and therefore, could be used for studies on the ABCA1 regulation. We also show that the cAMP-mediated transcriptional upregulation of the ABCA1 occurs via protein kinase-mediated pathway.

Author's Contribution Rai Ajit K Srivastava evaluated data from the studies and has contributed to the interpretation and analyses of data as well as writing of the manuscript. Neelam Srivastava and Angelo Cefalu designed the studies, carried out some of the experiments, and wrote part of the manuscript. Maurizio Averna contributed to the review and interpretation of data and wrote part of the manuscript.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

Ethical approval and informed consent statement Studies carried out in this report did not use any animal species. The cell-based assays were carried out according to the institutional guidelines. All authors agree to the publication of the results contained in this manuscript.

References

1. Srivastava RA, Srivastava N. High density lipoprotein, apolipoprotein A-I, and coronary artery disease. Mol Cell Biochem. 2000;209:131–44.
2. Srivastava N. ATP binding cassette transporter A1–key roles in cellular lipid transport and atherosclerosis. Mol Cell Biochem. 2002;237:155–64.
3. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet. 1999;22:336–45. https://doi.org/10.1038/11905.
4. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet. 1999;22:347–51. https://doi.org/10.1038/11914.
5. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet. 1999;22:352–5. https://doi.org/10.1038/11921.
6. Lawn RM, Wade DP, Garvin MR, Wang X, Schwartz K, Porter JG, et al. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. J Clin Invest. 1999;104:R25–31. https://doi.org/10.1172/jci8119.
7. Remaley AT, Rust S, Rosier M, Knapper C, Naudin L, Broccardo C, et al. Human ATP-binding cassette transporter 1 (ABC1): genomic organization and identification of the genetic defect in the original Tangier disease kindred. Proc Natl Acad Sci U S A. 1999;96:12685–90.
8. Orso E, Broccardo C, Kaminski WE, Bottcher A, Liebisch G, Drobnik W, et al. Transport of lipids from golgi to plasma membrane is defective in tangier disease patients and Abc1-deficient mice. Nat Genet. 2000;24:192–6. https://doi.org/10.1038/72869.
9. McNeish J, Aiello RJ, Guyot D, Turi T, Gabel C, Aldinger C, et al. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. Proc Natl Acad Sci U S A. 2000;97:4245–50.
10. Cavelier C, Rohrer L, von Eckardstein A. ATP-binding cassette transporter A1 modulates apolipoprotein A-I transcytosis through aortic endothelial cells. Circ Res. 2006;99:1060–6. https://doi.org/10.1161/01.RES.0000250567.17569.b3.
11. Singaraja RR, Bocher V, James ER, Clee SM, Zhang LH, Leavitt BR, et al. Human ABCA1 BAC transgenic mice show increased high density lipoprotein cholesterol and ApoAI-dependent efflux stimulated by an internal promoter containing liver X receptor response elements in intron 1. J Biol Chem. 2001;276:33969–79. https://doi.org/10.1074/jbc.M102503200.
12. Vaisman BL, Lambert G, Amar M, Joyce C, Ito T, Shamburek RD, et al. ABCA1 overexpression leads to hyperalphalipoproteinemia and increased biliary cholesterol excretion in transgenic mice. J Clin Invest. 2001;108:303–9. https://doi.org/10.1172/jci12517.
13. Joyce CW, Amar MJ, Lambert G, Vaisman BL, Paigen B, Najib-Fruchart J, et al. The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. Proc Natl Acad Sci U S A. 2002;99:407–12. https://doi.org/10.1073/pnas.012587699.
14. Langmann T, Klucken J, Reil M, Liebisch G, Luciani MF, Chimini G, et al. Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. Biochem Biophys Res Commun. 1999;257:29–33. https://doi.org/10.1006/bbrc.1999.0406.
15. Costet P, Lalanne F, Gerbod-Giannone MC, Molina JR, Fu X, Lund EG, et al. Retinoic acid receptor-mediated induction of ABCA1 in macrophages. Mol Cell Biol. 2003;23:7756–66.
16. Fu X, Menke JG, Chen Y, Zhou G, MacNaul KL, Wright SD, et al. 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. J Biol Chem. 2001;276:38378–87. https://doi.org/10.1074/jbc.M105805200.
17. Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, et al. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. Proc Natl Acad Sci U S A. 2000;97:12097–102. https://doi.org/10.1073/pnas.200367697.
18. Wang Y, Oram JF. Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding

cassette transporter A1. J Biol Chem. 2002;277:5692–7. https://doi.org/10.1074/jbc.M109977200.
19. Srivastava RAK. Dysfunctional HDL in diabetes mellitus and its role in the pathogenesis of cardiovascular disease. Mol Cell Biochem. 2018;440:167–87. https://doi.org/10.1007/s11010-017-3165-z.
20. Morgantini C, Natali A, Boldrini B, Imaizumi S, Navab M, Fogelman AM, et al. Anti-inflammatory and antioxidant properties of HDLs are impaired in type 2 diabetes. Diabetes. 2011;60:2617–23. https://doi.org/10.2337/db11-0378.
21. Shao B, Oda MN, Oram JF, Heinecke JW. Myeloperoxidase: an oxidative pathway for generating dysfunctional high-density lipoprotein. Chem Res Toxicol. 2010;23:447–54. https://doi.org/10.1021/tx9003775.
22. Uehara Y, Engel T, Li Z, Goepfert C, Rust S, Zhou X, et al. Polyunsaturated fatty acids and acetoacetate downregulate the expression of the ATP-binding cassette transporter A1. Diabetes. 2002;51:2922–8.
23. Tang C, Kanter JE, Bornfeldt KE, Leboeuf RC, Oram JF. Diabetes reduces the cholesterol exporter ABCA1 in mouse macrophages and kidneys. J Lipid Res. 2010;51:1719–28. https://doi.org/10.1194/jlr.M003525.
24. Passarelli M, Tang C, McDonald TO, O'Brien KD, Gerrity RG, Heinecke JW, et al. Advanced glycation end product precursors impair ABCA1-dependent cholesterol removal from cells. Diabetes. 2005;54:2198–205.
25. Patel DC, Albrecht C, Pavitt D, Paul V, Pourreyron C, Newman SP, et al. Type 2 diabetes is associated with reduced ATP-binding cassette transporter A1 gene expression, protein and function. PLoS One. 2011;6:e22142. https://doi.org/10.1371/journal.pone.0022142.
26. Wang Y, Oram JF. Unsaturated fatty acids phosphorylate and destabilize ABCA1 through a phospholipase D2 pathway. J Biol Chem. 2005;280:35896–903. https://doi.org/10.1074/jbc.M506210200.
27. Smith JD, Miyata M, Ginsberg M, Grigaux C, Shmookler E, Plump AS. Cyclic AMP induces apolipoprotein E binding activity and promotes cholesterol efflux from a macrophage cell line to apolipoprotein acceptors. J Biol Chem. 1996;271:30647–55.
28. Takahashi Y, Miyata M, Zheng P, Imazato T, Horwitz A, Smith JD. Identification of cAMP analogue inducible genes in RAW264 macrophages. Biochim Biophys Acta. 2000;1492:385–94.
29. Oram JF, Lawn RM, Garvin MR, Wade DP. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. J Biol Chem. 2000;275:34508–11. https://doi.org/10.1074/jbc.M006738200.
30. Repa JJ, Berge KE, Pomajzl C, Richardson JA, Hobbs H, Mangelsdorf DJ. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. J Biol Chem. 2002;277:18793–800. https://doi.org/10.1074/jbc.M109927200.
31. Le Goff W, Zheng P, Brubaker G, Smith JD. Identification of the cAMP-responsive enhancer of the murine ABCA1 gene: requirement for CREB1 and STAT3/4 elements. Arterioscler Thromb Vasc Biol. 2006;26:527–33. https://doi.org/10.1161/01.Atv.0000201042.00725.84.
32. See RH, Caday-Malcolm RA, Singaraja RR, Zhou S, Silverston A, Huber MT, et al. Protein kinase A site-specific phosphorylation regulates ATP-binding cassette A1 (ABCA1)-mediated phospholipid efflux. J Biol Chem. 2002;277:41835–42. https://doi.org/10.1074/jbc.M204923200.
33. Meier JL, Stinski MF. Regulation of human cytomegalovirus immediate-early gene expression. Intervirology. 1996;39:331–42. https://doi.org/10.1159/000150504.
34. Lashmit P, Wang S, Li H, Isomura H, Stinski MF. The CREB site in the proximal enhancer is critical for cooperative interaction with the other transcription factor binding sites to enhance transcription of the major intermediate-early genes in human cytomegalovirus-infected cells. J Virol. 2009;83:8893–904. https://doi.org/10.1128/jvi.02239-08.
35. Back S, Dossat A, Parkkinen I, Koivula P, Airavaara M, Richie CT, et al. Neuronal activation stimulates cytomegalovirus promoter-driven transgene expression. Mol Ther Methods Clin Dev. 2019;14:180–8. https://doi.org/10.1016/j.omtm.2019.06.006.
36. Thomas P, Smart TG. HEK293 cell line: a vehicle for the expression of recombinant proteins. J Pharmacol Toxicol Methods. 2005;51:187–200. https://doi.org/10.1016/j.vascn.2004.08.014.
37. Juszczak RJ, Russell JH. Inhibition of cytotoxic T lymphocyte-mediated lysis and cellular proliferation by isoquinoline sulfonamide protein kinase inhibitors. Evidence for the involvement of protein kinase C in lymphocyte function. J Biol Chem. 1989;264:810–5.
38. Munehira Y, Ohnishi T, Kawamoto S, Furuya A, Shitara K, Imamura M, et al. Alpha1-syntrophin modulates turnover of ABCA1. J Biol Chem. 2004;279:15091–5. https://doi.org/10.1074/jbc.M313436200.
39. Bowden K, Ridgway ND. OSBP negatively regulates ABCA1 protein stability. J Biol Chem. 2008;283:18210–7. https://doi.org/10.1074/jbc.M800918200.
40. Hansen TV, Rehfeld JF, Nielsen FC. Mitogen-activated protein kinase and protein kinase A signaling pathways stimulate cholecystokinin transcription via activation of cyclic adenosine 3′,5′-monophosphate response element-binding protein. Mol Endocrinol. 1999;13:466–75. https://doi.org/10.1210/mend.13.3.0257.
41. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, et al. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med. 2001;7:53–8. https://doi.org/10.1038/83348.
42. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, et al. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell. 2001;7:161–71.
43. Oliver WR Jr, Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, et al. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc Natl Acad Sci U S A. 2001;98:5306–11. https://doi.org/10.1073/pnas.091021198.
44. Panousis CG, Zuckerman SH. Interferon-gamma induces downregulation of Tangier disease gene (ATP-binding-cassette transporter 1) in macrophage-derived foam cells. Arterioscler Thromb Vasc Biol. 2000;20:1565–71.
45. Park DW, Lee HK, Lyu JH, Chin H, Kang SW, Kim YJ, et al. TLR2 stimulates ABCA1 expression via PKC-eta and PLD2 pathway. Biochem Biophys Res Commun. 2013;430:933–7. https://doi.org/10.1016/j.bbrc.2012.11.135.
46. Hao XR, Cao DL, Hu YW, Li XX, Liu XH, Xiao J, et al. IFN-gamma down-regulates ABCA1 expression by inhibiting LXRalpha in a JAK/STAT signaling pathway-dependent manner. Atherosclerosis. 2009;203:417–28. https://doi.org/10.1016/j.atherosclerosis.2008.07.029.
47. Field FJ, Watt K, Mathur SN. TNF-alpha decreases ABCA1 expression and attenuates HDL cholesterol efflux in the human intestinal cell line Caco-2. J Lipid Res. 2010;51:1407–15. https://doi.org/10.1194/jlr.M002410.
48. Tang C, Liu Y, Kessler PS, Vaughan AM, Oram JF. The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor. J Biol Chem. 2009;284:32336–43. https://doi.org/10.1074/jbc.M109.047472.
49. Gan X, Kaplan R, Menke JG, MacNaul K, Chen Y, Sparrow CP, et al. Dual mechanisms of ABCA1 regulation by geranylgeranyl

pyrophosphate. J Biol Chem. 2001;276:48702–8. https://doi.org/10.1074/jbc.M109402200.

50. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, et al. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. J Clin Invest. 2004;114:529–41. https://doi.org/10.1172/jci21109.
51. Nicholls SJ, Zheng L, Hazen SL. Formation of dysfunctional high-density lipoprotein by myeloperoxidase. Trends Cardiovasc Med. 2005;15:212–9. https://doi.org/10.1016/j.tcm.2005.06.004.
52. Shao B, Cavigiolio G, Brot N, Oda MN, Heinecke JW. Methionine oxidation impairs reverse cholesterol transport by apolipoprotein A-I. Proc Natl Acad Sci U S A. 2008;105:12224–9. https://doi.org/10.1073/pnas.0802025105.
53. Ory DS, Schaffer JE. ApoA-1 in diabetes: damaged goods. Diabetes. 2010;59:2358–9. https://doi.org/10.2337/db10-1040.
54. Kotani K, Sakane N, Ueda M, Mashiba S, Hayase Y, Tsuzaki K, et al. Oxidized high-density lipoprotein is associated with increased plasma glucose in non-diabetic dyslipidemic subjects. Clin Chim Acta. 2012;414:125–9. https://doi.org/10.1016/j.cca.2012.08.021.
55. Iborra RT, Machado-Lima A, Okuda LS, Pinto PR, Nakandakare ER, Machado UF, et al. AGE-albumin enhances ABCA1 degradation by ubiquitin-proteasome and lysosomal pathways in macrophages. J Diabetes Complicat. 2018;32:1–10. https://doi.org/10.1016/j.jdiacomp.2017.09.012.