Sterol regulatory element binding proteins downregulate LDL receptor-related protein (LRP1) expression and LRP1-mediated aggregated LDL uptake by human macrophages

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Abstract

Objective: In the extracellular intima, extracellular matrix proteoglycans favor LDL retention and aggregation (agLDL). In contrast to native LDL (nLDL), agLDL induces high intracellular cholesteryl ester (CE) accumulation in macrophages. It has been suggested that LDL receptor-related protein (LRP1) is involved in agLDL binding and internalization by macrophages. The aim of this work was to analyze whether sterol regulatory element binding proteins (SREBPs) modulate LRP1 expression and LRP1-mediated agLDL uptake by human monocyte-derived macrophages (HMDM).

Methods and results: The treatment of HMDM with small anti-LRP1 interfering RNA (siRNA-LRP1) led to the specific inhibition of LRP1 mRNA expression and also to the inhibition of LRP1 protein expression in these cells. In siRNA-LRP1-treated HMDM, CE accumulation from agLDL uptake (84.66±5 μg CE/mg protein) was reduced by 95.76±5.22%. This suggests that LRP1 plays a pivotal role in agLDL uptake by HMDM.

N-acetyl-leucyl-leucyl-norleucinal (ALLN), an inhibitor of SREBP catabolism, maintained high levels of active SREBP-2 and SREBP-1 even in the presence of nLDL and agLDL. Therefore, ALLN induced LDL receptor (LDLR) upregulation. Concomitantly, a strong downregulation of LRP1 mRNA and LRP1 protein was observed in ALLN-treated macrophages. By decreasing LRP1 expression levels, ALLN reduced CE accumulation from agLDL at all tested concentrations.

Conclusions: These results suggest that high levels of active SREBPs downregulate LRP1 expression and intracellular CE accumulation in HMDM.

Keywords: Low density lipoprotein receptor-related protein; Human monocyte-derived macrophages; Cholesterol accumulation; Sterol regulatory element binding proteins

1. Introduction

One of the key events in atherosclerosis is the interaction of monocyte/macrophages (MM) with the lipoproteins infiltrated in the subendothelium [1]. In the vascular wall, extracellular matrix (ECM)-bound LDL suffers modifications such as aggregation, fusion and oxidation [2–4]. Most of LDL isolated from the arterial intima is found in aggregated form [5] because aggregation is highly induced by LDL interaction with extracellular matrix proteoglycans [2,6]. Aggregated LDL, similar to LDL found in the arterial wall [7,8] can be obtained by vortexing [9] or by short incubation with versican [10], one of the main proteoglycans interacting with LDL in the arterial intima [2]. Aggregated LDL is a potent inducer of massive intracellular cholesteryl ester (CE) accumulation in macrophages [11–13]. There is considerable controversy...
regarding the mechanism involved in agLDL uptake by macrophages. While some authors suggest a phagocytic process [11], others using experimental systems specifically designed to mimic the in vivo interaction of arterial wall macrophages with subendothelial LDL, have described a new process named patocytosis [12–15]. The lack of involvement of scavenger receptors on agLDL binding and internalization by macrophages has been clearly demonstrated by several authors [11–15].

It has recently been reported that low density lipoprotein receptor-related protein (LRP1) participates in the uptake of matrix-retained LDL and LDL degraded by sphingomyelinase, mainly aggregated, in macrophages [16,17] and our group has previously shown that LRP1 is the receptor involved in the uptake of agLDL by human VSMC [18–20]. We have also previously demonstrated that SREBP-2 and SREBP-1 are expressed by VSMC from coronary arteries [21] and that these transcription factors are involved in the regulation of LRP1 by agLDL in vitro and by hypercholesterolemia in vivo [21]. SREBP-2 and SREBP-1 isoforms are mainly involved in the regulation of cholesterol metabolism [22,23]. SREBP transcription factors are synthesized as inactive precursors bound to the endoplasmic reticulum (ER) membranes. When cholesterol accumulates in cells, the SREBP-cleavage-activating protein (SCAP) does not move from ER to Golgi, SREBPs are not proteolyzed and the low levels of nuclear active SREBPs cause a low transcription rate of target genes. Upon activation, the precursor undergoes a sequential two-step cleavage process to release the NH(2)-terminal active domain in the nucleus (nuclear or active SREBP form) [24]. Nuclear SREBPs are rapidly degraded by the ubiquitin–proteasome pathways [25] and N-acetyl-leu-leu-norleucinal (ALLN) an inhibitor of this pathway, blocks the breakdown of the active SREBPs in different cell types [21,26,27]. In HeLa cells, the sterol regulatory element (SRE) sequence, unusually located in the long untranslated 5' region of the LRP1 promoter [28], is involved in the negative regulation of LRP1 promoter by active or nuclear SREBP-2 [29]. The objectives of this work were to investigate the role of LRP1 on agLDL uptake by human monocyte-derived macrophages (HMDM) and to determine whether the maintenance of high levels of SREBPs downregulate LRP1 expression and agLDL-mediated agLDL uptake by HMDM. Our findings show: 1) agLDL are unable to induce CE accumulation in siRNA-LRP1-treated HMDM, 2) ALLN kept high levels of nuclear SREBP-2 and SREBP-1 even in the presence of nLDL and agLDL, and 3) the maintenance of high levels of active SREBPs downregulates LRP1 expression and inhibits LRP1-mediated agLDL internalization by HMDM. These results indicate that LRP1 is a key receptor for CE accumulation in human macrophages and that SREBPs are key transcription factors downregulating LRP1-mediated CE accumulation in HMDM.

2. Methods

2.1. Isolation and differentiation of human monocyte-derived macrophages (HMDM)

Human monocyte-derived macrophages (HMDM) were isolated by standard protocols from buffy coats (35–40 mL) of healthy donors. The study was approved by the Reviewer Institutional Committee on Human Research of the Hospital of Santa Creu i Sant Pau that conforms to the Declaration of Helsinki. Cells were applied on 15 mL of Ficoll–Hypaque and centrifuged at 400× g, 40 min, 22 °C with no brake. Mononuclear cells were obtained from the central white band of the gradient, exhaustively washed in DPBS, and resuspended in RPMI medium (Gibco) supplemented with 20% human serum AB (Immunogenetics). Cells were allowed to differentiate into macrophages by addition of complete medium with 20% human serum AB and M-CSF (R&D Systems) at 100 ng/mL for three days. HMDM were incubated with native LDL (nLDL) or agLDL (100 μg/mL) and N-acetyl-leu-leu-norleucinal (ALLN) (25 μmol/L) for 24 h. HMDM were then exhaustively washed and collected either for mRNA and protein expression or for lipid extraction.

2.2. Treatment of HMDM with siRNA-LRP1 (siRNA-LRP1-HMDM)

To inhibit LRP1 expression, HMDM were transiently transfected with annealed siRNA (50 nmol/L). LRP1 specific siRNA was synthesized by Ambion according to our previously published LRP1 target sequences [19]. Fasta analysis (Genetic Computer Group Package) indicated that these sequences would not hybridize to other receptor sequences (including LDLR) in the GenBank database. In brief, HMDM were transfected with siRNA-GAPDH (as a control) or siRNA-LRP1 (50 nM) using siPORT™ Amine in serum-free DMEM (1% glutamine) according to the kit instructions (SilencerTM siRNA Transfection kit; Ambion no. 1630). This medium with siRNA was maintained for 48 h and it was then replaced by a new medium containing LDL (100 μg/mL). After 18 h, cells were exhaustively washed and harvested to test either LRP1 protein expression or LRP1 function. Extra wells were used in order to test the specificity of siRNA-LRP1 treatment by analyzing LRP1 and LDL receptor mRNA expression by real-time PCR. The cells did not take up trypan blue and their morphology was not altered by the procedure.

2.3. LDL preparation and determination of the free and cholesteryl ester content

Human LDLs (d1.019–d1.063 g/mL) were obtained from the pooled sera of normocholesterolemic volunteers. The model system of agLDL was generated by vortexing LDL (1 mg/mL) for 4 min at room temperature at maximal speed. AgLDL was then centrifuged at 10,000× g for 10 min and the
precipitable fraction composed of 100% aggregated LDL was added to cell cultures [4,19]. LDL preparations were less than 48 h old, non-oxidized (less than 1.2 mmol malonaldehyde/mg protein LDL) and without detectable levels of endotoxin (Limulus Amebocyte Lysate test, Bio Whittaker). HMDM were incubated with nLDL or agLDL for 24 h and cells were then exhaustively washed and harvested into 1 mL of 0.10 mol/L NaOH.

Lipid extraction was performed according to the method of Bligh and Dyer [30] with minor modifications [18–20]. One aliquot of the cell suspension was extracted with methanol/dichloromethane (2:1, vol/vol). After solvent removal under an N2 stream, the lipid extract was redissolved in methanol/dichloromethane (2:1, vol/vol). After solvent removal under an N2 stream, the lipid extract was redissolved in a Dounce homogenizer (10 strokes) in 10 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT and 1% Triton X-100. Nuclei were pelleted by 10 min centrifugation at 600× g at 4 °C. The pellets were then resuspended in lysis buffer. Protein expression was analyzed by western blot as previously described [21,29] and blots were incubated with monoclonal antibodies against human SREBP-2 (MBL, clone 1D2, dilution 1:5, 10 μg/mL).

2.6. Coronary artery sampling and preservation

Human coronary arteries were obtained from explanted hearts immediately after surgical excision. All procedures were approved by the Institutional Review and Ethics Committee. Immunohistochemistry specimens were immersed in fixative solution, sectioned into blocks, cryoprotected and frozen. Vessels were cross-sectionally cut and consecutive sections were collected on chrompotassium-gelatin-coated slides for immunohistochemistry or conventional staining and stored at −20 °C until used. Masson Trichromic staining (performed in representative cross-sectional preparations from each block) allowed classification of the lesions into group I (initial lesions, arterial intimal thickening (AIT), n = 8), group II (mild growing lesions: types I–II and III, n = 15), group III (advanced high-risk lesions: types IV–VI, n = 8) and group IV (severe lesions: types VII–VIII, n = 10) [33]. After washing in PBS, sections were incubated with primary antibodies against LRP1 (RDI-PRO61067) or HAMS6 (Dako). The primary antibodies were detected using the avidin–biotin immunoperoxidase technique. The sections were incubated with an appropriate biotinylated secondary antibody (1:200, Vector®). The chromogen used was 3,3′-diaminobenzidine. In situ LRP1 hybridization was performed with an antisense LRP1 riboprobe. For riboprobe synthesis, human LRP1 cDNA corresponding to nucleotides 2221 to 2764 (544 mer) was cloned in plasmid vectors using standard techniques. Antisense and sense riboprobes were synthesized using T3 and T7 RNA polymerase (Promega) respectively, and DIG labeled nucleotides (Boehringer Mannheim). In situ hybridization studies were performed on a set of 15 μm thick sections of type III lesions, as previously described [21]. Some of the in situ hybridization sections were hydrated, blocked and incubated with primary antibody against HAMS6 for 2 h at RT. Bound antibody was detected using alkaline-phosphatase-conjugated antibody.

2.7. Image analysis

Results were evaluated with an Olympus Vanox AHB-T3 microscope (Leica) and digitalized by a Sony 3CCD camera (Leica). The expression of LRP1 and Ham56 proteins was semiquantitatively assessed by scoring the proportion of staining as previously described [33]. First, the entire section was systematically scanned at 200× magnification and five non-overlapping fields were selected to perform the analysis. The percentage of positive staining with each specific antibody was determined, by using the Visilog 4.12 Image Analysis System (Leica), dividing the area of the target protein by the total area of the field. The results were expressed as the percentage of positive areas and are shown as the mean±SEM of semiquantifications from a minimum of five sections each, from coronary lesions processed in independent immunostainings.
2.8. Statistical analysis

Comparisons among groups were performed by parametric (one factor ANOVA) or non-parametric (Kruskal–Wallis, Mann–Whitney U test) analysis as needed. Statistical significance was considered when $P<0.05$.

3. Results

3.1. Evolution of HAM56 positive cells and intimal LRPI expression during atherosclerotic lesion progression in human arteries

Immunohistochemical analysis of macrophages (measured as HAM56 staining) and LRPI was performed in the

![Fig. 1. Evolution of HAM56 positive cells and intimal LRPI expression during atherosclerotic lesion progression in human arteries. (A) Image analysis quantification of macrophages content and LRPI staining in group I, group II, group III and group IV lesions. *$P<0.05$, versus group I, †$P<0.05$ versus group II, ‡$P<0.05$ versus group III. (B) Representative Masson Trichromic staining in group I (a) and group III (b) lesions. Representative HAM56 immunostaining in group I (c) and group III (d) lesions. Representative LRPI immunostaining in group I (e) and group III (f) lesions. (C) In situ hybridization of LRPI mRNA in a representative type III lesion human coronary lesion; hybridization with antisense riboprobe (a), with the sense riboprobe (b) and co-localization of LRPI mRNA with HAM56 positive cells (c). Bar: 100 μm. l, lumen; i, intima; m, media; c, lipid core.](image-url)
four groups of plaques previously described in the Methods section. Image analysis quantification showed a significant increase in the percentage of intimal surface occupied by HAM56 with atherosclerotic lesion progression (Fig. 1A). Intimal LRP1 significantly increased in group II, group III and group IV lesions (Fig. 1A). Fig. 1B shows a representative group I (panel a) and group III (advanced high-risk lesions) (panel b) lesions and the increase on intimal macrophage content (Fig. 1B: panel d versus panel c) and LRP1 expression (Fig. 1B: panel f versus panel e) with

Fig. 2. Effect of siRNA-LRP1 treatment on LRP1 expression and function in HMDM; control HMDM (black bars) or siRNA-LRP1-treated HMDM (white bars) were incubated in the absence or presence of nLDL or agLDL (100 μg/mL) for 18 h. (A) Real-time PCR analysis of LRP1, LDLR and SREBP-2 mRNA expression. Data were processed with a specially designed software program based on Ct values of each sample and normalized to 18s rRNA mRNA (n=3). (B) Western blot analysis of LRP1 protein expression. Results are expressed as arbitrary units. Results are shown as mean±SEM of three experiments performed in duplicate. (C) Effect of nLDL and agLDL on intracellular CE and FC content. HMDM were incubated with nLDL or agLDL (100 μg/mL) for 18 h. Results are expressed as microgram cholesterol per milligram protein and are shown as mean of 2 experiments performed in triplicate. P<0.05: * versus siRNA-LRP1-untreated HMDM. (D) Photographs (original magnification ×20) of control and siRNA-treated HMDM.
atherosclerotic lesion progression, in agreement with previous results [34,35]. In situ hybridization analysis with LRP1 antisense riboprobe plus immunohistochemistry with HAM56 antibodies showed a high co-localization (arrows) between LRP1 mRNA and HAM56 positive cells in group II lesions (Fig. 1C).

3.2. Aggregated LDL fails to induce CE accumulation in siRNA-LRP1-HMDM

The involvement of LRP1 in agLDL uptake by HMDM has been demonstrated by specific inhibition of LRP1 expression. Real-time PCR results show that LRP1 mRNA expression is inhibited by 81±1.05% in siRNA-LRP1-treated HMDM (Fig. 2A). No effect of siRNA-LRP1 treatment was observed on LDL receptor (LDLR) or SREBP-2 mRNA expression. In agreement with the effects on LRP1 mRNA expression, LRP1 protein expression was inhibited by more than 90% by siRNA-LRP1 treatment in HMDM incubated in the absence or presence of LDL (nLDL or agLDL) (Fig. 2B).

As shown in Fig. 2C, agLDL induced high levels of intracellular CE accumulation in HMDM (80.37±0.4 μg CE/mg protein at 100 μg/mL) and siRNA-LRP1 treatment inhibited CE accumulation from agLDL by 90±1.96%. In contrast, siRNA-LRP1 treatment did not alter the CE content of nLDL-exposed HMDM. No significant alterations on FC content were observed by any treatment. siRNA-LRP1 treatment did not induce morphological alterations in HMDM (Fig. 2D).

3.3. ALLN maintains high levels of active SREBP-2 levels even in the presence of nLDL or agLDL

As shown in Fig. 3A, western blot analysis demonstrated that nLDL and agLDL reduced the levels of the inactive protein form of SREBP-2 by 48±1.94% and 63±0.29%, respectively. Interestingly, nLDL and agLDL also reduced the nuclear concentration of the active form of SREBP-2 protein by 77±0.4% and 88±0.5%, respectively. ALLN treatment increased the inactive precursor concentration by 1.72-fold in HMDM incubated in the absence of lipoproteins and it completely prevented the downregulatory effects of LDL. ALLN also increased the active nuclear SREBP-2 form by 2.67-fold in HMDM incubated in the absence of LDL. Besides, it not only prevented LDL downregulatory effects on nuclear SREBP-2 concentrations but also increased active SREBP-2 levels by 1.64±0.68% and 1.80±0.54% in nLDL- and agLDL-exposed HMDM, respectively. Results from real-time PCR show that both nLDL and agLDL significantly
reduced SREBP-2 mRNA levels by 43±2.7% and 59±3.9%, respectively (Fig. 3B). In contrast to the ALLN upregulatory effects on SREBP-2 protein (precursor and active forms), ALLN reduced SREBP-2 mRNA expression levels in control HMDM (by 56±4%), and it did not significantly alter the low levels of SREBP-2 mRNA achieved by exposure to nLDL and agLDL.

However, nLDL and agLDL did not alter the levels of the inactive or active (almost undetectable) protein form of SREBP-1 in HMDM (Fig. 4A). ALLN treatment significantly increased the inactive SREBP-1 precursor levels by approximately 1.4-fold and the active SREBP-1 protein levels by approximately 2.75-fold either in control or LDL-exposed HMDM. In agreement with the lack of LDL effects on SREBP-1 protein, nLDL or agLDL did not exert any significant effect on SREBP-1 mRNA expression in HMDM (Fig. 4B). In contrast, ALLN significantly reduced SREBP-1 levels in control HMDM (by 69±3.9%), in nLDL- (by 71±1.5%) and agLDL- (by 72±2%) exposed HMDM (Fig. 4B). The significant reduction in SREBP-2 and SREBP-1 mRNA levels induced by ALLN might respond to the compensation of the high levels of nuclear SREBP in ALLN-treated macrophages. ALLN treatment did not induce morphological alterations in HMDM.

### 3.4. ALLN downregulates LRP1 expression and LRP1-mediated aggregated LDL uptake

We have previously reported that agLDL upregulates LRP1 expression in human VSMC and HeLa cells, at least in part, by means of SREBP-2 downregulation [20,22]. According to our results, nuclear SREBP-2 decay induced by LDL in HMDM was not able to modulate LRP1 protein (Fig. 5A) or mRNA expression (Fig. 5B). However, as in human VSMC [22], ALLN decreased LRP1 protein expression by approximately 77±2.27% in HMDM incubated in the absence of LDL, by 67±1.3% in HMDM exposed to nLDL and by 64±2.2% in HMDM exposed to agLDL (Fig. 5A). In agreement with the reduction in LRP1 protein expression, ALLN reduced LRP1 mRNA expression by 74±1% in HMDM incubated in the absence of LDL, by 72±2.3% in HMDM exposed to nLDL and by 71±2.6% in HMDM exposed to agLDL (Fig. 5B). On the contrary, ALLN increased LDLR by 1.2-fold in HMDM incubated in the absence of LDL. ALLN almost completely prevented the downregulatory effect of nLDL on LDL receptor mRNA expression and it partially prevented the downregulatory effect of agLDL on LDL receptor mRNA expression (Fig. 5C).

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Fig. 4. Effect of ALLN on SREBP-1 expression in HMDM incubated in the absence or presence of nLDL and agLDL. HMDM were incubated with nLDL or agLDL (100 μg/mL) and in the absence (grey bars) or presence of ALLN (25 μmol/L) (black bars) for 24 h. (A) Representative western blot showing the precursor (125 kDa) and active (68 kDa) forms of SREBP-1. (B) Real-time PCR quantification of SREBP-1 mRNA expression levels. Data were processed with a specially designed software program based on Ct values of each sample and normalized to 18srRNA mRNA (n=3). P<0.05: * versus HMDM incubated in the absence of LDL, # versus HMDM incubated in the absence of ALLN.
In agreement with the pivotal role of LRP1 on agLDL uptake, ALLN decreased CE accumulation from agLDL at any tested concentration (by 46±2.17% at 25 μg/mL; by 45.3±1.52% at 50 μg/mL and by 36±1.15% at 100 μg/mL) (Fig. 6A). In contrast, ALLN increased CE content of nLDL-exposed HMDM (to 1.73-fold at 25 μg/mL; to 1.68-fold at 50 μg/mL and to 1.88-fold at 100 μg/mL) (Fig. 6B). No significant effect of ALLN was observed on the FC content of nLDL- or agLDL-exposed HMDM.

4. Discussion

Most of LDL isolated from the arterial intima is found in aggregated form [5] and LDL aggregation is a process highly facilitated in the intima by LDL interaction with extracellular matrix proteoglycans [2,6]. Our results demonstrate that agLDL uptake by macrophages depends on LRP1 since there is no CE accumulation derived from agLDL in siRNA-LRP1-treated HMDM. These results are in agreement with the role of LRP1 on matrix-retained LDL and SMase-aggregated LDL uptake by macrophages [16,17]. Both macrophage content and intimal LRP1 expression increase with atherosclerotic lesion progression. LRP1-mediated agLDL uptake by macrophages might thus be crucial for lipid deposition during atherosclerotic lesion progression. In the present study, we demonstrated that ALLN, a cysteine protease inhibitor that maintains high levels of nuclear SREBP-2 and SREBP-1, strongly reduces LRP1 expression in macrophages either in control or exposed to LDL. It is known that ALLN increases LDL receptor expression in macrophages because of its ability to maintain high levels of both SREBP-2 and SREBP-1 since both isoforms upregulate LDL receptor promoter transcriptional activity [36,37]. However, further studies are required to analyze the contribution of SREBP-2 and SREBP-1 isoforms to the downregulation of LRP1 promoter transcription.
According to our results, LDLs (either nLDL or agLDL) have the ability to modulate SREBP-2 but not SREBP-1 mRNA or protein levels in HMDM, in agreement with the results obtained in human VSMC [21]. It has been previously demonstrated that agLDL, different from nLDL, strongly induced cholesterol esterification in macrophages [11]. The extent of cholesterol esterification by the (ER)-resident protein, acyl-coenzyme A:cholesterol acyl-transferase (ACAT) is considered to reflect the regulatory pool of ER cholesterol [38], which seems to modulate the processing of SREBPs by acting directly on SCAP activity [39]. However, several experimental evidences suggest that cholesterol transport to ACAT in the ER might be different from cholesterol transport to the homeostatic machinery where SREBP/SCAP resides [40,41]. The capacity of agLDL to modulate ACAT and SCAP activities depending on induced alterations in the ER-cholesterol content of control and siRNA-treated macrophages is a highly relevant mechanism that requires further investigation.

In contrast to that observed in human VSMC and HeLa cells [21,29], the active SREBP-2 decay induced by LDL was insufficient to upregulate LR1P1 expression in HMDM. The lack of LR1P1 upregulation by LDL-induced SREBP decay in macrophages might be explained by the use of M-CSF to differentiate monocytes into macrophages [42–44]. The lack of LR1P1 upregulation by LDL might thus be due to the fact that LR1P1 is fully upregulated in the differentiated HMDM used in our experiments.

According to our results SREBP-2 seems to modulate LR1P1 expression and function in two of the most important human cells in atherosclerosis, macrophages and vascular smooth muscle cells. Additionally, we demonstrate that reduction of LR1P1 levels induced by high levels of nuclear SREBPs leads to a functional change in HMDM; that is, to a decrease on CE accumulation from agLDL uptake. Our results support the concept of LR1P1 playing a central role on EC-bound LDL uptake in the vascular wall. Since both LR1P1 expression and macrophage content increase with atherosclerotic lesion progression [33–35], SREBP by modulating LR1P1 expression levels, could play a major role in the control of intracellular lipid accumulation associated to atherosclerotic lesion progression. In a tissue-specific knockout mouse line that lacks LR1P1 only in VSMC [45], LR1P1 seems to play an essential role in controlling VSMC proliferation and vascular wall integrity. Indeed, the absence of LR1P1 in the vascular wall led to elastic membrane disturbances and aneurysms. In mice, the protective role of LR1P1 in maintaining vascular wall structure might be related to the crucial requirement for LR1P1 in embryonic development [46], and it is not opposed to a proatherogenic role for LR1P1 overexpression in adult cells and human lipid-enriched atherosclerotic plaques [47]. LR1P1 is a putative receptor for many different ligands and their respective roles in vascular homeostasis and pathology require a thoughtful investigation.

Our results suggest that the maintenance of high levels of SREBP-2 in the vascular wall might prevent intracellular lipid deposition in both macrophages and VSMC. Recently, different SREBP-2 gene polymorphisms have been associated to a higher incidence of atherosclerosis due to defective processing of SREBPs and a consequent lack of LDLR upregulation [48]. Additionally, our results suggest that the higher incidence of atherosclerosis might also be due to a defective regulation of LR1P1 transcription in a situation in which SREBP-2 is not efficiently processed.

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