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Krüppel-like factor 4 synergizes with CREB to increase the activity of apolipoprotein E gene promoter in macrophages

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ABSTRACT

Krüppel-like factor 4 (KLF4) is a critical regulator of monocyte differentiation and macrophage polarization, and it also plays an important role in several vascular diseases, including atherosclerosis. Apolipoprotein E (apoE) is an essential anti-atherosclerotic glycoprotein involved in lipid metabolism, expressed by the liver, macrophages and other cell types. We hypothesized that KLF4 is involved in apoE gene regulation in macrophages. Our experiments showed that differentiation of THP-1 monocytes to macrophages using PMA was associated with a robust induction of both KLF4 and apoE genes. KLF4 bound to the apoE promoter, as revealed by chromatin immunoprecipitation and DNA pull-down (DNAP) assays, and transactivated the apoE promoter in a dose-dependent manner. Using a series of apoE promoter deletion mutants we revealed the biological activity of multiple KLF4 binding sites located in the [−500/−100] region of apoE promoter. Moreover, overexpression of cAMP-response-element-binding protein (CREB) further increased KLF4 up-regulatory effect on apoE promoter. Despite the fact that no putative CREB binding sites were predicted *in silico*, we found that in macrophages CREB bound to apoE proximal promoter in the region −200/+4 and even more strongly on −350/-274 region. In similar DNAP experiments using cell extracts obtained from monocytes (lacking KLF4), a very weak binding of CREB was detected, indicating that interaction of CREB with apoE promoter takes place indirectly. In conclusion our results show: (*i*) a robust synchronized induction of KLF4 and apoE expression during differentiation of monocytes to macrophages; (*ii*) KLF4 up-regulates apoE gene in a dose-dependent manner; (*iii*) biologically active KLF4 binding sites are present on apoE promoter and (*iv*) the interaction of KLF4 with CREB results in an enhanced up-regulatory effect of KLF4 on apoE promoter. Taken together these data provide novel knowledge on apoE gene regulation mechanism in macrophages, and offer insight into the therapeutic potential of KLF4 in atherosclerosis.

1. Introduction

KLF4, Krüppel-like factor 4, also named gut-enriched Krüppel-like factor or epithelial zinc finger transcription factor [1,2] is a zinc finger-containing transcription factor that modulates gene expression by binding to GC-rich sites or CACCC sequences [3,4]. KLF4 was first identified in the gut and skin epithelia [1,5], but is also expressed by different cell types involved in the development of vascular disease, such as monocyte-derived macrophages, endothelial and vascular smooth muscle cells [6–8]. Moreover, it was shown that KLF4 regulates vascular inflammation by controlling macrophage polarization [7] and monocyte differentiation [6,9]. It was reported that KLF4 deficiency led to enhanced atherosclerotic lesion formation and macrophage accumulation in the plaque [10]. However, the role of KLF4 in atherosclerosis has not been fully determined yet.

CREB, cAMP-response-element-binding protein, a ubiquitously expressed transcription factor was reported to be involved in a series of processes relevant to cardiovascular physio-pathology

Abbreviations: apoE, apolipoprotein E; CREB, cAMP-response-element-binding protein; ChIP, chromatin immunoprecipitation; DMEM, Dulbecco's Modified Eagle Medium; KLF4, Krüppel-like factor 4; DNAP, DNA pull-down assay; luc, luciferase; PMA, phorbol 12-myristate 13-acetate.

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[11]. Moreover, CREB is essential for the survival of monocytes and macrophages, playing anti-inflammatory roles [12].

Apolipoprotein E (apoE) is a glycoprotein of 34 kDa mostly synthesized by the liver [13]. As a part of very low density lipoproteins, chylomicrons and large high density lipoproteins, apoE plays an important role in lipid metabolism [14,15]. ApoE deficiency, due to impeded clearance of atherogenic lipoproteins from the plasma, causes hyperlipidemia and the ensuing development of atherosclerotic lesions [16,17].

Based on the pivotal role of KLF4 in macrophage biology and various processes involved in atherosclerosis, we hypothesized that KLF4 plays a role in apoE gene induction during monocyte differentiation towards macrophages. We present here data that show a robust simultaneous induction of KLF4 and apoE expression during differentiation of monocyte-derived macrophages, the existence of KLF4 binding sites on apoE promoter and the molecular partnership of KLF4-CREB that results in enhanced apoE gene induction. These results aid to the elucidation of the regulatory mechanism of KLF4 on apoE expression, and indicate KLF4 as a potential new target in the therapy of atherosclerosis.

2. Materials and methods

2.1. Materials

DMEM, RPMI-1640, Streptavidin-Dynabeads M−280 and protein A Sepharose beads were from Thermo-Scientific. Fetal bovine serum (FBS) was from EuroClone (Italy). Phorbol 12-myristate 13-acetate (PMA), GoTaq DNA polymerase, proteinase K and Luciferase assay system were from Promega (USA). Chemiluminescence kit was from Pierce (Rockford, USA). Protease Inhibitors cocktail was from Roche. Rabbit anti-KLF4 polyclonal antibody (11880-1-AP) was from Proteintech Group. Mouse anti-β-actin antibody (sc-47778) and rabbit anti-CREB1 antibody (C-21; sc-186) were from Santa Cruz Biotechnology (USA). Anti-human apoE antibody was from Immuno-Biological Laboratories Co., LTD. (Japan). Secondary antibodies labeled with HRP were from Rockland. All other chemicals were from Sigma−Aldrich.

2.2. Cell culture

Human THP-1 monocytes (from ATCC) were grown in RPMI supplemented with 10% heat-inactivated FBS. To obtain monocyte-derived macrophages, THP-1 monocytes were treated with 50 nM PMA for 16 h as previously described in Ref. [18]. HepG2 and HEK-293 cells (from ATTC) were grown in DMEM with 10% FBS, at 37 °C, in a 5% CO_2 incubator.

2.3. Western blot

Cell lysates from monocytes and macrophages were subjected to SDS-PAGE and transferred onto nitrocellulose membrane (Bio-Rad). The blots were incubated with anti-KLF4, anti-apoE and anti-β-actin, followed by HRP-labeled appropriate secondary antibodies. The proteins were revealed as previously described [19].

2.4. Plasmid construction

Plasmids containing apoE proximal promoter ([−500/+73] apoE-luc) or its deletion mutants [-400/+73]apoE-luc, [−300/+73] apoE-luc, [−200/+73]apoE-luc, [−100/+73]apoE-luc, [−55/+73] apoE-luc) were obtained by cloning the respective inserts in front of the luciferase reporter gene in pGL3 basic vector (Promega). Plasmids [−500/+73] apoE-luc, [−100/+73]apoE-luc, [−55/+73]apoE-luc were described in Ref. [20]. The rest of the mutants were constructed similarly, using primer R+73 [20] and the forward primers described in Table 1.

2.5. Transient transfections

HEK-293 or HepG2 cells were transiently transfected as described in Ref. [20] using the plasmids described above and the expression vectors for KLF4 (Clone ID: 5111134 from Open Biosystem, ThermoScientific) and for CREB, described in Ref. [18]. The luciferase activity was detected and normalized to β-galactosidase activity, as previously described [20]. Each experiment was done in triplicate and repeated at least three times.

2.6. Chromatin immunoprecipitation (ChIP)

Monocyte-derived macrophages were subjected to ChIP assay. Briefly, the cells were fixed in 1% paraformaldehyde (10 min) and then the chromatin samples were prepared by sonication, precleared with protein A Sepharose beads and immunoprecipitated using anti-KLF4 antibody for 18 h at 4 °C. From each chromatin sample, 10% was stored as “input”. The DNA-protein complexes were incubated for 4 h at 65 °C, and then digested with proteinase K to reverse the DNA-protein cross-linking. DNA was purified using phenol/chloroform and analyzed by PCR, using primers for apoE promoter: forward primer (F-254) 5′-GGGGTACCTCCACGCTT GGCCCCC and reverse primer R+4, described in Table 2.

2.7. DNA pull-down assay

This assay was performed as previously described [19]. Briefly, DNA for apoE promoter or its deletion fragments was amplified by PCR, using 5'-biotinylated RV3 forward primer (5′-CTAGCAAAA-TAGGCTGTCCC, Promega) and the reverse primers and the templates described in Table 2. Biotinylated DNA was coupled to Streptavidin-Dynabeads M−280 and incubated with whole cell extracts obtained from PMA-differentiated macrophages as we described earlier [19]. The complexes formed were washed and subjected to Western blot, using anti-KLF4 antibody diluted 1/1000 or anti-CREB1 antibody used as described in Ref. [21].

2.8. Statistical analysis

The results were statistically analyzed using One-way ANOVA. All data were expressed as means ± standard deviations. The p-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Synchronized induction of KLF4 and apoE gene expression during differentiation of monocytes to macrophages

First we investigated the protein expression of KLF4 and apoE in monocytes and macrophages by Western blot. Interestingly, we detected a robust expression of both KLF4 and apoE (Fig. 1A, panels KLF4 and apoE) in PMA-differentiated macrophages (Fig. 1A, lane

Table 1
Primers used for cloning deletion mutants of apoE promoter.

Primer name	ApoE promoter fragment	Primer sequence
F-400	−400/+73	5′-GGGGTACCTGTCCAGCCCCCTAGCC
F-350	−350/+73	5′-GGGGTACCATCCCCAGCCCCCTCTCC
F-300	−300/+73	5′-GGGGTACCGGGTCAGGAAAGGAGGACTC
F-200	−200/+73	5′-GGGGTACCCCTGGGGACTGTGGGGG

Table 2
Primers and templates used in DNAP experiments.

ApoE promoter fragment	Reverse primer	Sequence of reverse primer	Template plasmid
−500/+4	R+4	5′ -GGGAGCTCGTGGGGCTGAGTAGGAC	[−500/+73]apoE
−500/−330	R-330	5′ -CTGGAGAGGGGGCTGGGGAT	[−500/+73]apoE
−350/−274	R-274	5′ -CCCAGAGTCCTCCTTTCCC	[−350/+73]apoE
−300/−170	R-170	5′ -ACCACCCCCCACAGTCCCC	[−300/+73]apoE
−200/+4	R+4	5′ -GGGAGCTCGTGGGGCTGAGTAGGAC	[−200/+73]apoE

PMA), whereas their expression in THP-1 monocytes was absent or below the limits of detection (Fig. 1A, lane Ctr). The level of β-actin was tested as endogenous control (Fig. 1A, panel β-actin). These results indicated a similarity in the expression pattern of KLF4 and apoE, namely they are expressed only in macrophages, but not in monocytes.

3.2. KLF4 binds to apoE promoter and regulates apoE gene expression

Next, we questioned whether the enhanced KLF4 expression (detected in macrophages) has an effect on apoE promoter activity. To this purpose, we employed HEK-293 cells which do not express KLF4 (data not shown) and induced by transfection different KLF4 levels in these cells. Briefly, the cells were seeded in 12-well plates and were transiently transfected with plasmids containing apoE promoter ([−500/+73]apoE-luc) in the presence of different amounts of KLF4 expression vector (0–2 μg plasmid/triplicate wells). The results shown in Fig. 1B revealed a dose-dependent increase in apoE promoter activity ~3.7-fold for 0.2 μg KLF4 ($p < 0.05$), ~6-fold for 1 μg KLF4 ($p < 0.01$) and ~11-fold for 2 μg KLF4 ($p < 0.001$).

The interaction between KLF4 and the apoE promoter in macrophages was verified also by ChIP assay. Thus, chromatin precipitated with anti-KLF4 antibodies showed a band of 258 bp for apoE promoter (Fig. 1C, lane KLF4), while for the negative control, where anti-KLF4 antibodies were omitted, the non-specific precipitated DNA was almost undetectable (Fig. 1C, lane NoAb). PCR using primers for apoE promoter and the input as template revealed the expected band of 258 bp (Fig. 1C, lane Input). These results clearly indicated that the binding of KLF4 to apoE proximal promoter in macrophages is specific.

In silico analysis, using TRANSFAC databases [22], predicted six binding sites for KLF4 transcription factor on apoE promoter, localized at positions −409, −381, −316, −259, −225 and −155, respectively, in the apoE gene promoter (Fig. 2A). To further explore the role of KLF4 on apoE gene modulation, and to confirm that KLF4 binds to apoE promoter, we performed DNA pull-down (DNAP) assay using whole cell extract obtained from PMA-differentiated macrophages. The results showed that KLF4 bound to the full-length apoE promoter sequence (Fig. 2B, lane [−500/+4]). Next we examined the functionality of the KLF4 binding sites predicted on apoE promoter, performing DNAP assay using biotinylated DNA for [−500/−330], [−350/−274], [−300/−170], [−200/+4]

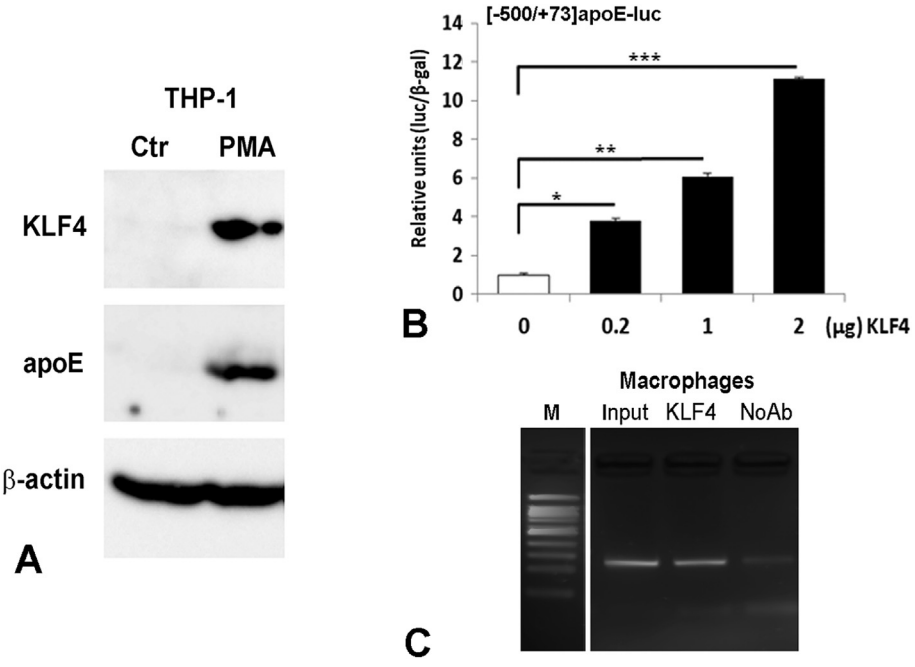

Fig. 1. KLF4 expression and involvement in apoE gene modulation in monocytes/macrophages. *Panel A*. KLF4 and apoE protein expressions were evaluated by Western blot using THP-1 monocytes or PMA-differentiated THP-1 macrophages. Note that KLF4 and apoE are absent in monocytes (lanes Ctr), but are robustly expressed in THP-1 macrophages (lanes PMA). As control for protein loading, β-actin was probed. *Panel B*. HEK-293 cells were transiently transfected with plasmids containing apoE promoter ([−500/+4]apoE-luc) in the presence of different amounts of KLF4 expression vector. The data show a dose-dependent increase in apoE promoter activity (* for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$). *Panel C*. ChIP experiments performed on macrophages using anti-KLF4 antibodies. Note that upon the interaction between KLF4 and apoE promoter (lane KLF4) a band of 258 bp is detected. The negative control (similar experiments except that anti-KLF4 was omitted, lane NoAb) and the input (lane Input) are also shown.

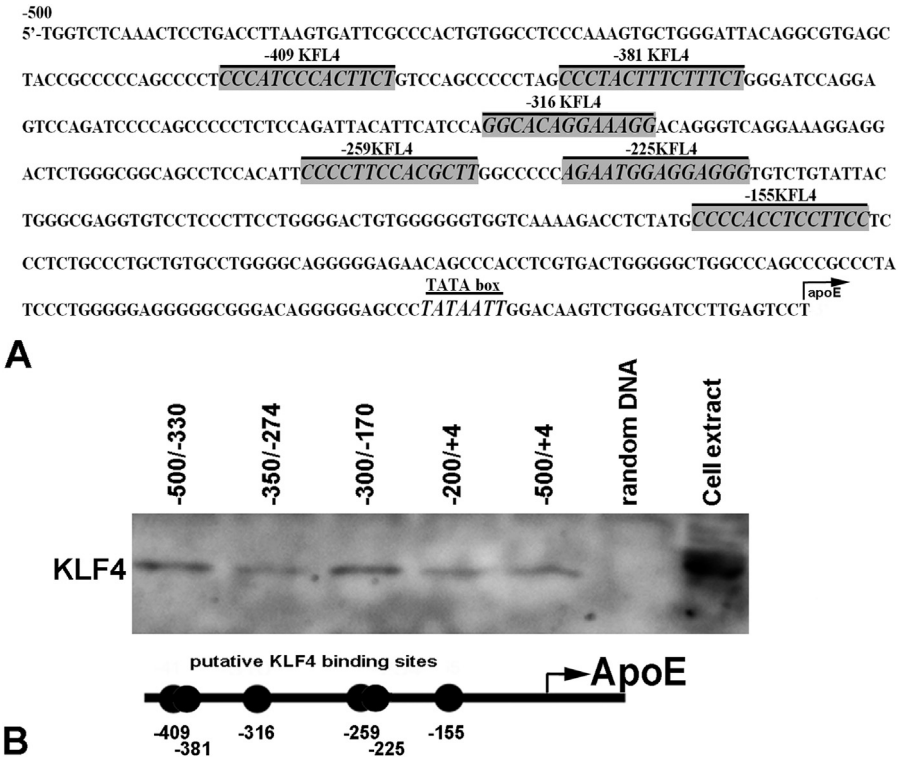

Fig. 2. KLF4 interacts with apoE promoter. *Panel A*. The putative binding sites of KLF4 located on apoE promoter sequence as determined by TRANSFAC analysis. *Panel B*. DNA pull-down assays were performed using biotinylated DNA for proximal apoE promoter or its fragments and cell extracts obtained from THP-1 differentiated macrophages. Note that KLF4 binds to multiple active binding sites of apoE promoter, as schematically illustrated above. No bands are detected in negative controls experiments in which random DNA sequence was used (lane random DNA). KLF4 expression (Western blot) in the cells used in the experiment is shown (lane cell extract).

fragments of apoE promoter. The results showed that all apoE promoter fragments bound KLF4 (Fig. 2B, lanes −500/−330, −350/-274, −300/−170 and −200/+4, respectively). No band was detected when DNAP assay was performed with a random DNA sequence (Fig. 2B, lane Random DNA). These results corroborate with the above ChIP data and provide additional evidence for the specific binding of KLF4 to apoE promoter sequence.

3.3. KLF4 enhances the activity of apoE promoter

To test whether KLF4 binding sites on apoE promoter are functionally active, HepG2 cells were transiently transfected with plasmids containing full-length apoE promoter ([−500/+73]apoE-luc) or its deletion mutants ([-400/+73]apoE-luc, [−300/+73]apoE-luc, [−200/+73]apoE-luc, [−100/+73]apoE-luc, [−55/+73]apoE-luc) in the presence or in the absence (control) of KLF4 expression vector. We found that KLF4 overexpression increased the activity of apoE promoter and its deletion mutants, as follows: ~11-fold for full-length promoter [−500/+73], ~11-fold for [−400/+73], ~6-fold for [−300/+73] and ~4-fold for [−200/+73], all with $p < 0.001$ (Fig. 3, columns −500apoE, −400apoE, −300apoE and −200apoE, respectively). By contrast, KLF4 overexpression did not enhance the activity of [−100/+73] and [−55/+73] apoE promoter fragments (Fig. 3, columns −100apoE and −55apoE). These results indicated that KLF4 binding sites located in the [−500/−100] region of apoE promoter are functional and play an important role in the up-regulation of apoE gene expression.

Taken together, the results of DNAP, ChIP and transient transfection experiments demonstrated the presence of functionally active KLF4 binding sites in the [−500/−100] region of apoE promoter which have an up-regulatory effect on apoE gene expression.

3.4. The up-regulatory effect of KLF4 on apoE promoter activity is enhanced by CREB

To modulate gene expression, KLF4 may interact with other transcription factors. CREB regulates a variety of immune cell processes including differentiation [12]. To examine the possibility that KLF4 and CREB act coordinately to induce apoE promoter activity, HepG2 cells were transiently transfected with plasmids containing apoE promoter ([−500/+73]apoE-luc) alone (control) or in the presence of expression vectors for KLF4 and/or CREB. The results of these experiments showed that overexpression of CREB or KLF4 alone induced a modest increase (2-fold and ~3.6-fold, respectively, $p < 0.001$) of apoE promoter activity (Fig. 4B, CREB and KLF4 columns), whereas concomitant overexpression of KLF4 and CREB enhanced synergistically the activity of apoE promoter (Fig. 4B, KLF4 + CREB column) at a level significantly higher (~17-fold, $p < 0.001$) than that obtained with CREB or KLF4 overexpression alone. Note that this apoE promoter induction by KLF4 is lower than that obtained in Fig. 3 since the amount of plasmid used in KLF4 and CREB co-transfection experiments was one-half as compared with that used in KLF4 transfection (Fig. 4). These results revealed that the increase in apoE promoter activity induced by KLF4 is further enhanced by CREB.

To test the ability of CREB to directly bind to apoE promoter, we performed DNAP assay, using whole cell extract from PMA-differentiated macrophages (expressing KLF4) and THP-1 monocytes (which do not express KLF4). The results showed that in macrophages, CREB bound to the full-length apoE promoter sequence (Fig. 4B, lane [−500/+4]) and to all apoE promoter deletion mutants tested (Fig. 4B, lanes [−500/−330], [−350/−274], [−300/−170] and [−200/+4]). By contrast, only a very faint binding

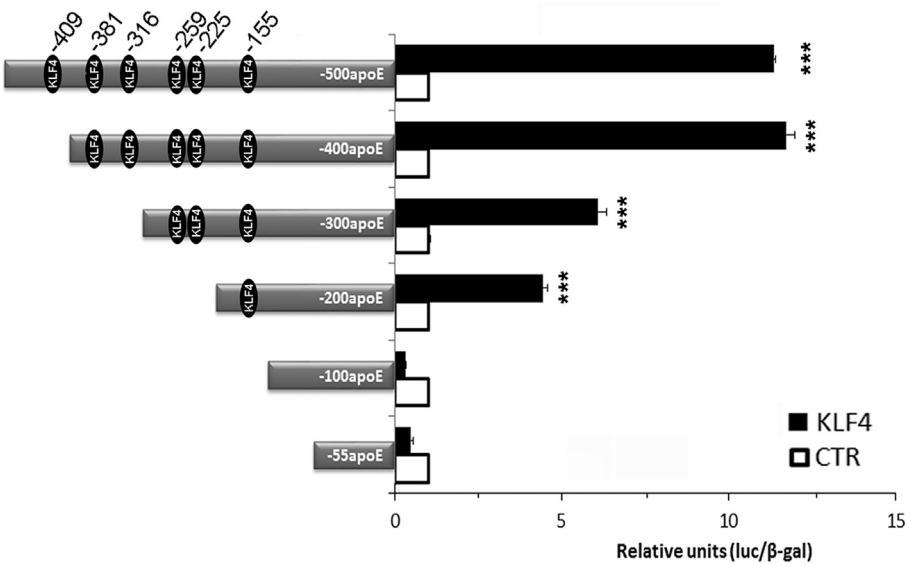

Fig. 3. Deletion of KLF4 binding sites abrogates the up-regulatory effect of KLF4 on apoE promoter activity. HepG2 cells were transiently transfected with plasmids containing apoE promoter ([−500/+4]apoE-luc) or successive deletion mutants in the presence or in the absence of KLF4 expression vector. The presence of KLF4 binding sites localized in positions schematically illustrated, allows KLF4 binding and activation of apoE promoter (−500apoE column) or its fragments (columns −400apoE, −300apoE and −200apoE). The label *** was attributed for $p < 0.001$. Further deletions abrogate the ability of KLF4 to enhance apoE promoter (columns −100apoE and −55apoE), demonstrating that the resulting apoE promoter fragments lack KLF4 binding sites.

on apoE promoter [−500/+4] and traces on fragment [−300/−170] were detected when monocytes cell extract was used (Fig. 4C, lanes [−500/+4] and [−300/−170]). Note that in monocytes no binding was detected for the fragment [−350/−274], for which the strongest binding was obtained when macrophage extract was used (Fig. 4B, lanes [−350/−274]). These data indicated a robust CREB binding on apoE promoter only when KLF4 was present.

4. Discussion

Macrophage-derived apoE has a protective effect on the initiation and progression of atherosclerotic lesions, by promoting cholesterol efflux from these sites [23]. The absence of apoE secretion from the macrophages is pro-atherogenic, and determines the development of atherosclerosis in apoE-deficient mice [24]. Thus, to increase apoE gene expression in macrophages, an elaborate study of the transcription factors involved in cell-specific up-regulation would constitute a notable strategy to prevent or treat atherosclerosis. In this study, we questioned whether KLF4 could be involved in the regulation of apoE gene expression since this transcription factor regulates several cellular processes [6,25,26] and was reported to have a protective role in atherosclerosis [27]; moreover, KLF4 deficiency is considered a major risk factor for this pathology [10].

Our experiments showed that KLF4 and apoE have similar expression patterns in monocytes and macrophages, namely they are absent or below the limit of detection in monocytes, but they are strongly expressed in macrophages (Fig. 1A). These results corroborate and are in line with previous reports showing an association of KLF4 expression with monocyte differentiation [6,9]. We also have data showing that KLF4 induction is related to monocyte differentiation, but not to PMA treatment, since the latter is unable to induce KLF4 in other cell lines such as HepG2 cells, HEK-293, smooth muscle cells or endothelial cells (data not shown).

It is worthwhile mentioning that KLF4 is induced in response to pro-inflammatory stimuli, like in lipopolysaccharides (LPS) - treated monocytes [9,28]. Similarly, apoE gene expression is absent in THP-1 monocytes, but is induced in activated macrophages (LPS- stimulated monocytes), as we have previously demonstrated [20].

Considering this differential expression pattern in monocytes versus macrophages for both apoE and KLF4, we then searched for the possible action of KLF4 on apoE induction in macrophages.

In silico analysis using TRANSFAC database predicted six binding sites for KLF4 transcription factor on apoE promoter. Therefore, we searched for the capability of KLF4 to bind and modulate apoE promoter activity. Herein we bring evidence that KLF4 interacts with apoE promoter (Fig. 1C) and positively regulates its activity in a dose-dependent manner (Fig. 1B). Moreover, DNA pull-down assays confirmed that KLF4 binds to the proximal apoE promoter sequence and to all apoE promoter fragments tested (Fig. 2B). All these fragments contain one or two putative KLF4 binding sites determined by TRANSFAC analysis, as schematically illustrated in Fig. 2B.

To determine the activity of these binding sites we employed a series of deletion mutants of apoE promoter in transient transfection experiments. The data showed that successive deletion of KLF4 binding sites led to a decreased ability of KLF4 to enhance apoE promoter activity, up to the last two fragments tested: [−100/+73] apoE and [−55/+73] apoE, which were not transactivated by KLF4 since they lack the corresponding binding sites (Fig. 3).

It was reported that KLF4 interacts with other transcription factors and modulates the expression of numerous genes. Previous studies have reported that KLF4 cooperates with CREB to modulate the promoter activity of ghrelin [29] or bradykinin B2 receptor [30] genes.

CREB is a transcription factor ubiquitously distributed in various tissues. We have data showing that THP-1 monocytes or derived macrophages, HEK-293, HepG2 cells and other cells express CREB (unpublished results). Moreover, in an animal model, loss of CREB function was associated with cardiovascular risk factors like obesity, hypertension and hyperlipidemia [31]. Furthermore, CREB could promote anti-inflammatory responses through the inhibition

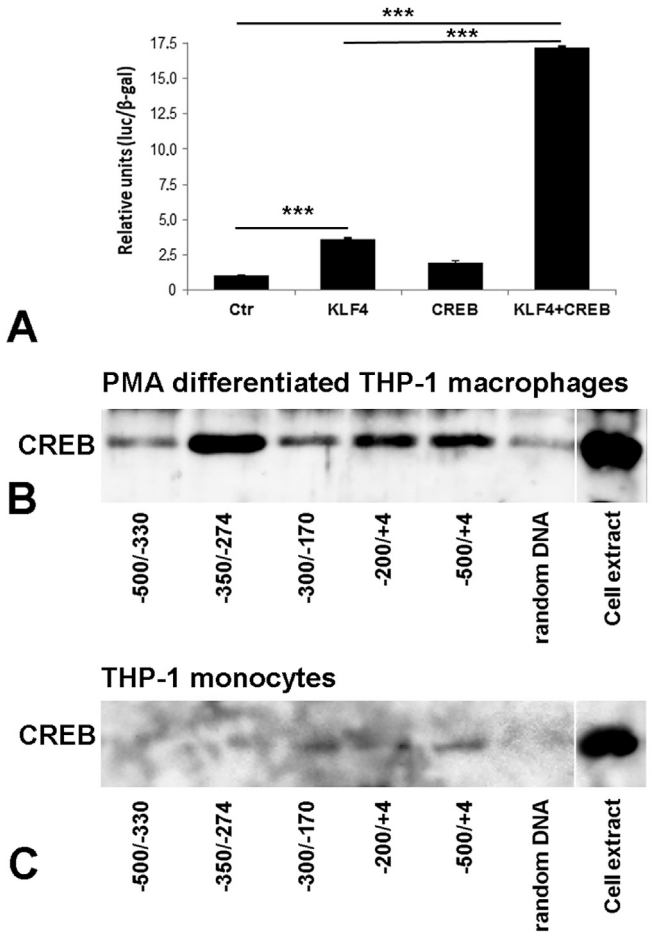

Fig. 4. Synchronized and enhanced action of KLF4 and CREB transcription factors on apoE promoter activity. *Panel A.* HepG2 cells were transiently co-transfected using plasmids containing apoE promoter in the presence of expression vectors for KLF4 and/or CREB. Note that simultaneous overexpression of KLF4 and CREB enhanced significantly the activity of apoE promoter (~17-fold, CREB + KLF4 column) as compared to the cells that overexpress KLF4 (~3.6-fold, KLF4 column) or CREB (~1.9-fold, CREB column) alone. *Panel B and C.* DNA pull-down assays were performed, using biotinylated DNA for apoE promoter ([−500/+4]) or different apoE promoter fragments ([−500/−330], [−350/−274], [−300/−170], [−200/+4]) and cell extracts obtained from THP-1 derived macrophages (B) or from THP-1 monocytes (C). Note that in macrophages, CREB binds to apoE promoter (lane [−500/+4]) and to all apoE promoter fragments used (Panel B, lanes [−500/−330], [−350/−274], [−300/−170] and [−200/+4]). By contrast, in monocytes (that do not express KLF4) a very weak binding of CREB on [−500/+4] apoE promoter and traces on [−300/−170] fragment are detected (lanes [−500/+4], [−300/−170]). As negative control, random DNA was used (lanes random DNA). Note that CREB is strongly expressed in both cell types (lanes cell extract).

of NF-κB [12], a transcription factor we previously found to down-regulate apoE expression [19,20].

In the experiments reported herein, we showed that over-expression of CREB in HepG2 cells did not affect, or, at most, induced a very modest up-regulatory effect on apoE promoter (Fig. 4A). Moreover, CREB did not bind to apoE promoter in monocytes (Fig. 4C). Interestingly, both these cell types, HepG2 cells (data not shown) and THP-1 monocytes (Fig. 1) do not express KLF4. Hence, we questioned the role of CREB transcription factor in KLF4- mediated apoE gene induction. Our transient co-transfection experiments revealed that KLF4 and CREB overexpression had a synergistic effect on apoE promoter activity (Fig. 4A). To depict CREB binding on apoE promoter in the presence or absence of KLF4, we used macrophages and respectively, monocytes, both heavily expressing CREB (Fig 4B and C, lanes Cell extract). DNAP assay showed that CREB bound strongly to apoE promoter only in macrophages, the cells that express KLF4 (Fig. 4B), whereas in monocytes, the binding was very weak or even absent (Fig. 4C).

In conclusion, the novel findings of the current study are: *(i)* there is a strong synchronized induction of KLF4 and apoE expression during differentiation of monocyte-derived macrophages; *(ii)* KLF4 up-regulates apoE gene in a dose-dependent manner; *(iii)* biologically active KLF4 binding sites are present on apoE promoter and *(iv)* the interaction of KLF4 with CREB results in an enhanced up-regulatory effect of KLF4 on apoE promoter.

Taken together these data provide novel knowledge on apoE gene modulation mechanism, and recommend KLF4 as a promising target for atherosclerosis therapy.

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