Oxidized high-density lipoprotein impairs the function of human renal proximal tubule epithelial cells through CD36

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Abstract. Unlike native high-density lipoprotein (HDL), oxidized HDL exerts adverse effects in a number of diseases, including chronic kidney disease (CKD); however, the mechanisms involved in this process remain unclear. In the present study, we investigated the effects of oxidized HDL on renal tubular cells, which play an important role in the progression of CKD. Human renal proximal tubule epithelial cells (HK-2) were cultured and stimulated with various concentrations of oxidized HDL in the absence or presence of CD36 siRNA. The results revealed that oxidized HDL enhanced the production of reactive oxygen species (ROS) and upregulated the expression of pro-inflammatory factors in the HK-2 cells in a dose-dependent manner. Incubation with oxidized HDL also increased the apoptosis of the HK-2 cells and reduced their migration ability in a dose-dependent manner. Src family kinase, mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) were activated following stimulation with oxidized HDL. All these effects mediated by oxidized HDL on HK-2 cells were markedly attenuated by transfection with with CD36 siRNA pior to stimulation with oxidized HDL. These findings suggest that oxidized HDL enhances the pro-inflammatory properties and impairs the function of HK-2 cells, mainly through the scavenger receptor, CD36, as well as through the Src, MAPK and NF-κB pathways.

Introduction

It is widely recognized that tubular lesions participate in the progression of chronic kidney disease (CKD), and there is compelling evidence that the severity of tubular damage has a more significant correlation with the reduction in creatinine clearance as compared with glomerular damage scores (1,2). Renal tubulointerstitial inflammation plays a central role in the loss of renal function in CKD by promoting the expression of pro-inflammatory cytokines, the generation of reactive oxygen species (ROS) and cell apoptosis, ultimately leading to renal fibrosis (3,4). Consistently, the extent of inflammation positively correlates with kidney function and may be used to predict long-term prognosis in some clinical settings (5).

The levels of high-density lipoprotein (HDL) inversely correlate with cardiovascular events by mediating reverse cholesterol transport and exerting potent antioxidant, anti-inflammatory and antithrombotic effects (6,7). However, simply increasing the amount of circulating HDL does not reduce the risk of developing coronary heart disease (CHD), or CHD-related deaths and total deaths (8). Previous studies have demonstrated that HDL is susceptible to damaging structural modifications, including oxidation in atherosclerosis and diabetes in the presence of systemic inflammation (9,10). The oxidative modification of HDL not only deprives HDL of important protective functions, but even transforms it into a pro-oxidant and pro-inflammatory agent (9,11). In the serum of patients with CKD, HDL has also been reported to be in a state of enhanced susceptibility to oxidative modification, particularly in diabetic nephropathy (12,13). However, the role of oxidized HDL in mediating renal tubular damage remains unclear.

Knowing that tubular lesions play an important role in the progression of CKD, in the present study, we aimed to clarify the role of oxidized HDL in inducing inflammatory responses in renal tubular cells, as well as the molecular mechanisms involved.

Materials and methods

HDL oxidation and cell culture. Native HDL (Chemicon International, Inc., Billerica, MA, USA) was incubated with $CuSO_4$ (20 μmol/l final concentration) in 1 mol/l PBS pH 7.4 at a lipoprotein concentration of 1 mg protein/ml for 24 h at 37˚C. Oxidation was terminated by the addition of ethylenediaminetetraacetic acid (EDTA) (200 μmol/l final concentration) and confirmed by measurements of thiobarbituric acid reac-

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tive substances (TBARS) and oxidized HDL was sterilized by passing it through a 0.22-μm filter, as previously described (14).

Human renal proximal tubule epithelial cells (HK-2) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in keratinocyte serum-free medium (K-SFM) (Gibco, Grand Island, NY, USA) supplemented with 5 ng/ml epidermal growth factor (EGF) and 40 mg/ml of bovine extract (Gibco), as well as 100 U/ml of penicillin and 100 Ug/ml of streptomycin. The cells were placed in an atmosphere of 5% CO_2-95% air at 37˚C, and passaged by trypsinization (0.25% trypsin, 0.02% EDTA) following the formation of a confluent monolayer and placed in a serum-free medium 24 h prior to stimulation. Oxidized HDL or native HDL was added to the medium at the indicated concentrations for 24 h, and used for various analyses.

Transfection of CD36 short interference RNA (siRNA). The HK-2 cells were transfected with 100 nM of CD36 siRNA (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and the transfection procedure was performed using Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, 1 day prior to transfection, the cells were plated in growth medium without antibiotics so that they would be 50% confluent at the time of transfection. Subsequently, CD36 siRNA or scrambled control plasmid oligomer-Lipofectamine 2000 complexes were prepared, added to each well containing cells and medium and mixed gently by rocking the plate back and forth. The cells were incubated for 24 h and then treated with various doses of oxidized HDL or native HDL.

Measurement of intracellular ROS. Measurement of intracellular ROS was based on the ROS-mediated conversion of non-fluorescent 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) into dichlorodihydrofluorescein (DCFH), as previously described (15,16). The fluorescence intensity reflects enhanced oxidative stress. Following treatment as described above, the HK-2 cells were incubated with DCFH-DA in medium at 37˚C for 45 min and then washed with PBS. Following incubation, the DCFH fluorescence of the cells from each well was imaged under a fluorescence microscope and analyzed using AxioVision 4.5 software (Carl Zeiss, Jena, Germany).

Apoptosis assay. Annexin V-fluorescein isothiocyanate/propidium iodide (FITC/PI) staining was used to detect the apoptosis induced by treatment with oxidized HDL in HK-2 cells. The HK-2 cells were harvested by centrifugation at 300 x g for 5 min, followed by 2 washes with cold PBS. The cells were stained using an Annexin V-FITC/PI staining kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions. The early stages of apoptosis were assayed using a flow cytometer (BD Biosciences, San Jose, CA, USA).

Migration assays. The migratory function of the HK-2 cells was measured using 24-well-modified Boyden chambers (Corning Life Sciences, Oneonta, NY, USA). Following transfection and treatment as described above, the cells ($1x10^4$) were detached, resuspended and seeded onto the filters (8-μm pore size) in the top compartment of the chamber. Following incubation at 37˚C for 24 h, the membrane was washed with PBS and fixed with 4% paraformaldehyde for 15 min at room temperature. Non-migrating cells were gently removed from the upper side of the Transwell, and the membrane of the Transwell filter was then stained using hexamethyl pararosaniline solution for a further 15 min, and the upper surface of the filters was carefully wiped with a cotton-tipped applicator. For quantification, the migrated cells were counted in 6 random microscopic fields (x40) in a blinded manner.

Real-time reverse transcription PCR. Total RNA was extracted using the RNase mini kit (Invitrogen Life Technologies) and was reverse-transcribed. The primer sequences were as follows: tumor necrosis factor-α (TNF-α) forward, 5'-TGCTTGTTCCTCAGCCTCTT; and reverse, 5'-GGTTTGCTACAACATGGGCT; monocyte chemoattractant protein-1 (MCP-1) forward, 5'-CCCCAGTCACCTGCTGTTAT; and reverse, 5'-AGATCTCCTTGGCCACAATG; regulated upon activation normal T cell expressed and secreted (RANTES) forward, 5'-GAAGGAAGTCAGCATGCCTC; and reverse, 5'-AGCCGATTTTTCATGTTTGC; CD36 forward, 5'-GAGAGCCTGTGCCTCATTTC; and reverse, 5'-GACTGGCTCCAGAGTCTTGC; 18S forward, 5'-CGCACGGCCGGTACAGTGAA; and reverse, 5'-GGGAGAGGAGCGAGCGACCA. Real-time PCR was performed using SYBR-Green PCR Master mix (Toyobo Co., Ltd., Osaka, Japan) and Rotor-Gene-3000. A real-time PCR system (Corbett Research Pty, Ltd., Sydney, Australia) was used according to the manufacturer's instructions. In brief, the PCR amplification reaction mixture (25 μl) contained 1 μl cDNA, 0.4 mM sense and antisense primer and 12.5 μl SYBR-Green I. After the initial denaturation at 95˚C for 10 min, the reaction was cycled 35 times. Each cycle consisted of denaturation at 95˚C for 30 sec, primer annealing at 60˚C for 30 sec and primer extension at 72˚C for 30 sec. The results are presented as the relative expression of TNF-α, MCP-1, RANTES and CD36 normalized to the expression of 18S.

Enzyme-linked immunosorbent assay (ELISA). The protein levels of TNF-α, MCP-1 and RANTES under the different experimental conditions were determined in the cell supernatants using commercial ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions, and each protein level was normalized with the cell numbers, as previously described (17).

Western blot analysis. The HK-2 cells were washed twice with cold PBS and lysed in protein extract buffer (1 ml protein extract buffer with 10 μl mixture of protease inhibitors and 10 μl 100-mM PMSF) at 4˚C for 30 min. The lysates were centrifuged at 14,000 x g and 4˚C for 10 min. The supernatant was collected and the concentration of total soluble protein was quantified using the BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). The extracts were employed for immunoblot analysis. Cellular proteins were electrophoresed through a 10% SDS-PAGE gel before transferring to PVDF membranes. After blocking for 1 h at room temperature in blocking buffer (1% gelatin in PBS with 0.05% Tween-20), the membranes were incubated

Figure

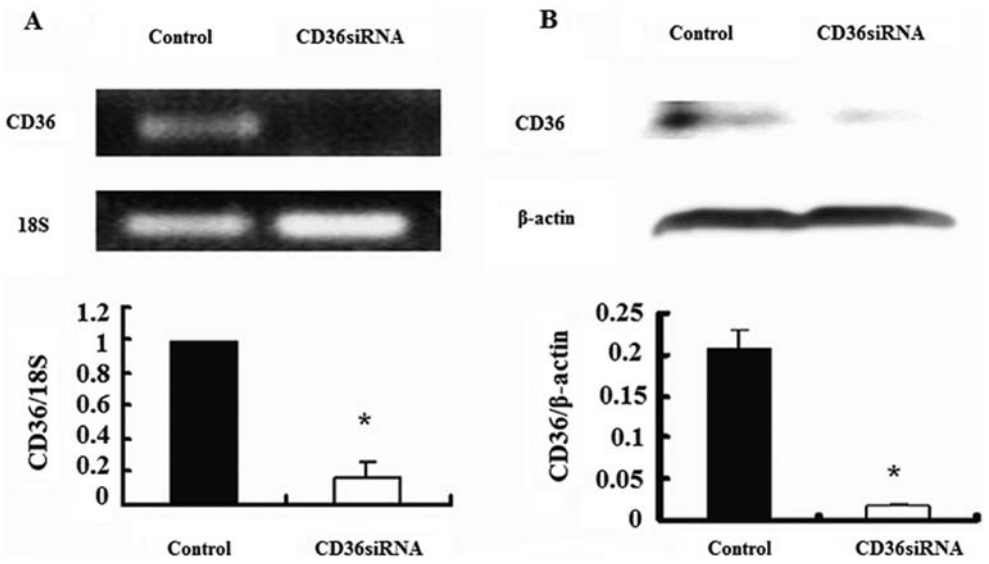

Figure.1. Effects of CD36 short interference RNA (siRNA) on scavenger receptor CD36 expression in HK-2 cells. CD36 (A) mRNA and (B) protein expression following transfection with CD36 siRNA. Data are the means ± standard deviation (SD), *P<0.05 vs. control. n≥3, each experiment was performed in triplicate.

for 16 h with monoclonal mouse anti-human β-actin antibody (1:1,000; Santa Cruz Biotechnology); monoclonal mouse anti-human CD36 antibody (1:1,000; Abcam, Cambridge, UK), monoclonal mouse anti-human poly(ADP-ribose) polymerase (PARP) antibody, polyclonal rabbit anti-human Src antibody, polyclonal rabbit anti-human phosphorylated (phospho)-Src (tyr416) antibody, polyclonal rabbit anti-human phospho-Src (tyr527) antibody, monoclonal mouse anti-human extracellular regulated kinase (ERK)1/2 antibody, monoclonal mouse anti-human phospho-ERK1/2 antibody, monoclonal mouse anti-human p38 antibody, monoclonal mouse anti-human phospho-p38 antibody, monoclonal mouse anti-human c-Jun N-terminal kinase (JNK) antibody, monoclonal mouse anti-human phospho-JNK antibody, monoclonal mouse anti-human p65 antibody and monoclonal mouse anti-human phospho-p65 antibody (1:1,000; Cell Signaling Technology, Danvers, MA, USA) in PBS Tween-20. The membranes were washed and incubated for 1 h at room temperature with a secondary antibody for 1 h at room temperature and then visualized by enhanced chemiluminescence detection reagents. Relative intensities of the protein bands were analyzed using ImageJ software.

Statistical analysis. The results are expressed as the means ± standard deviation (SD) from at least 3 experiments. Significance was determined by analysis of variance (ANOVA) and a t-test using StatView 4.0. Differences with P-values <0.05 were considered statistically significant.

Results

CD36 siRNA suppresses the expression of CD36 in HK-2 cells. In order to elucidate the role of CD36 in the impairment of cellular function induced by oxidized HDL, we transfected the cells with CD36 siRNA. As shown in Fig. 1, 100 nM CD36 siRNA effectively suppressed CD36 mRNA (approximately 80% inhibition) and protein expression (approximately 90% inhibition) in the HK-2 cells. Hence, CD36 siRNA was applied in the following experiments.

Oxidized HDL increases ROS production in HK-2 cells. The HK-2 cells were co-incubated with various concentrations of oxidized HDL (0, 10, 20 and 50 μg/ml), HDL (50 μg/ml) or oxidized HDL (50 μg/ml) plus CD36 siRNA (100 nM). Oxidized HDL has been shown to induce oxidative stress in human umbilical vein endothelial cells by promoting the generation of intracellular ROS (18). As shown in Fig. 2, oxidized HDL increased the generation of ROS in the HK-2 cells in a concentration-dependent manner and this effect was markedly attenuated by CD36 siRNA, while native HDL had no effect on ROS production.

Oxidized HDL increases the mRNA and protein expression of pro-inflammatory factors in HK-2 cells. Real-time reverse transcription PCR revealed that the mRNA expression of TNF-α, MCP-1 and RANTES markedly increased in a concentration-dependent manner following exposure to oxidized HDL for 24 h, and this effect was markedly inhibited by treatment with CD36 siRNA, while native HDL had no effect on the expression levels of these inflammatory factors, apart from TNF-α (Fig. 3A-C). ELISA revealed that the TNF-α, MCP-1 and RANTES protein levels in the conditioned culture medium were altered in a pattern similar to that of the mRNA expression (Fig. 3D-F).

Oxidized HDL promotes HK-2 cell apoptosis. Early apoptosis of the HK-2 cells was detected using an Annexin V-FITC kit. To exclude dead cells, only the Annexin V-positive and PI-negative cells were counted. As shown in Fig. 4A-E, oxidized HDL markedly promoted the early apoptosis of the HK-2 cells compared with the control group. This effect was markedly inhibited by treatment with CD36 siRNA; native HDL also attenuated the early apoptosis of the cells compared with the control group.

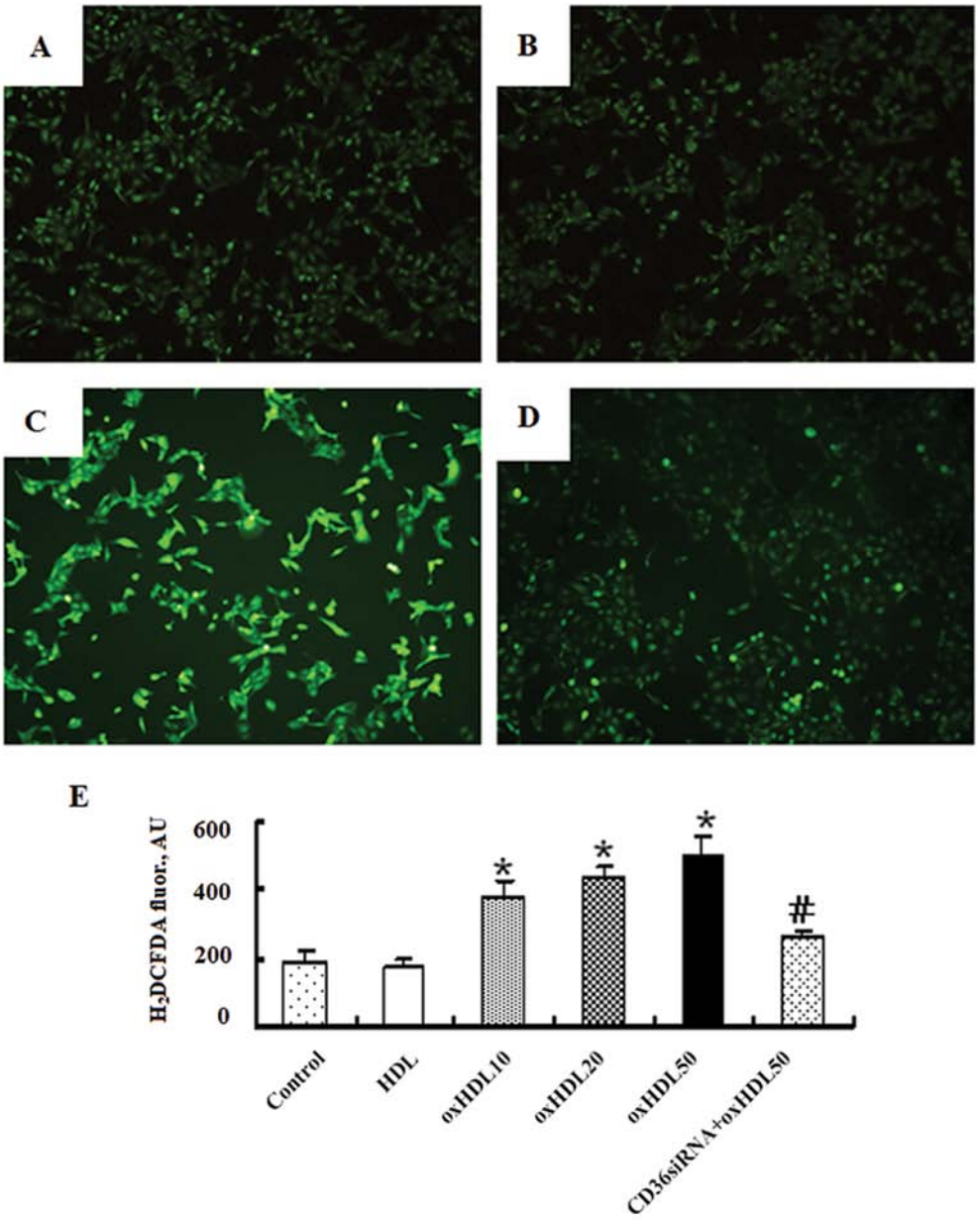

Figure 2. Effects of oxidized high-density lipoprotein (HDL) on reactive oxygen species (ROS) production in HK-2 cells. Fluorometrical images of ROS in HK-2 cells after 24 h of incubation with (A) 0 μg/ml oxidized HDL (control), (B) 50 μg/ml HDL, (C) 50 μg/ml oxidized HDL or (D) 50 μg/ml oxidized HDL following transfection with CD36 short interference RNA (siRNA) (E) Generation of ROS in HK-2 cells following treatment with HDL or various concentrations of oxidized HDL. Data are the means ± standard deviation (SD), *P<0.05 vs. control, $^{\#}$P<0.05 vs. 50 μg/ml oxidized HDL. n≥3, each experiment was performed in triplicate.

In order to further confirm that the cell death induced by oxidized HDL was primarily caused by apoptosis, the cleavage of the caspase substrate, PARP, which is considered a biochemical hallmark of apoptosis, was investigated by western blot anaysis. The results revealed that the expression of cleaved PARP increased as the concentration of oxidized HDL increased from 10 to 50 μg/ml, and this increment was reduced following the transfection of the cells with CD36 siRNA (Fig. 4F and G).

Oxidized HDL inhibits HK-2 cell migration through CD36. The migration of the HK-2 cells was inhibited by oxidized HDL in a dose-dependent manner, and this effect was markedly attenuated by transfection with CD36 siRNA. There was no significant difference between the control group and the native HDL-treated group (Fig. 5).

Oxidized HDL activates Src family proteins in HK-2 cells. The Src family of protein tyrosine kinases is important in the regulation of the growth and differentiation of eukaryotic cells (19). Src activity can be regulated by tyrosine phosphorylation at 2 sites, with opposing effects. The phosphorylation of tyr416 in the activation loop of the kinase domain upregulates enzyme activity, while the phosphorylation of tyr527 in the carboxy-terminal tail triggers enzyme inactivation (20). Our results revealed that the phosphorylation of the Src-family

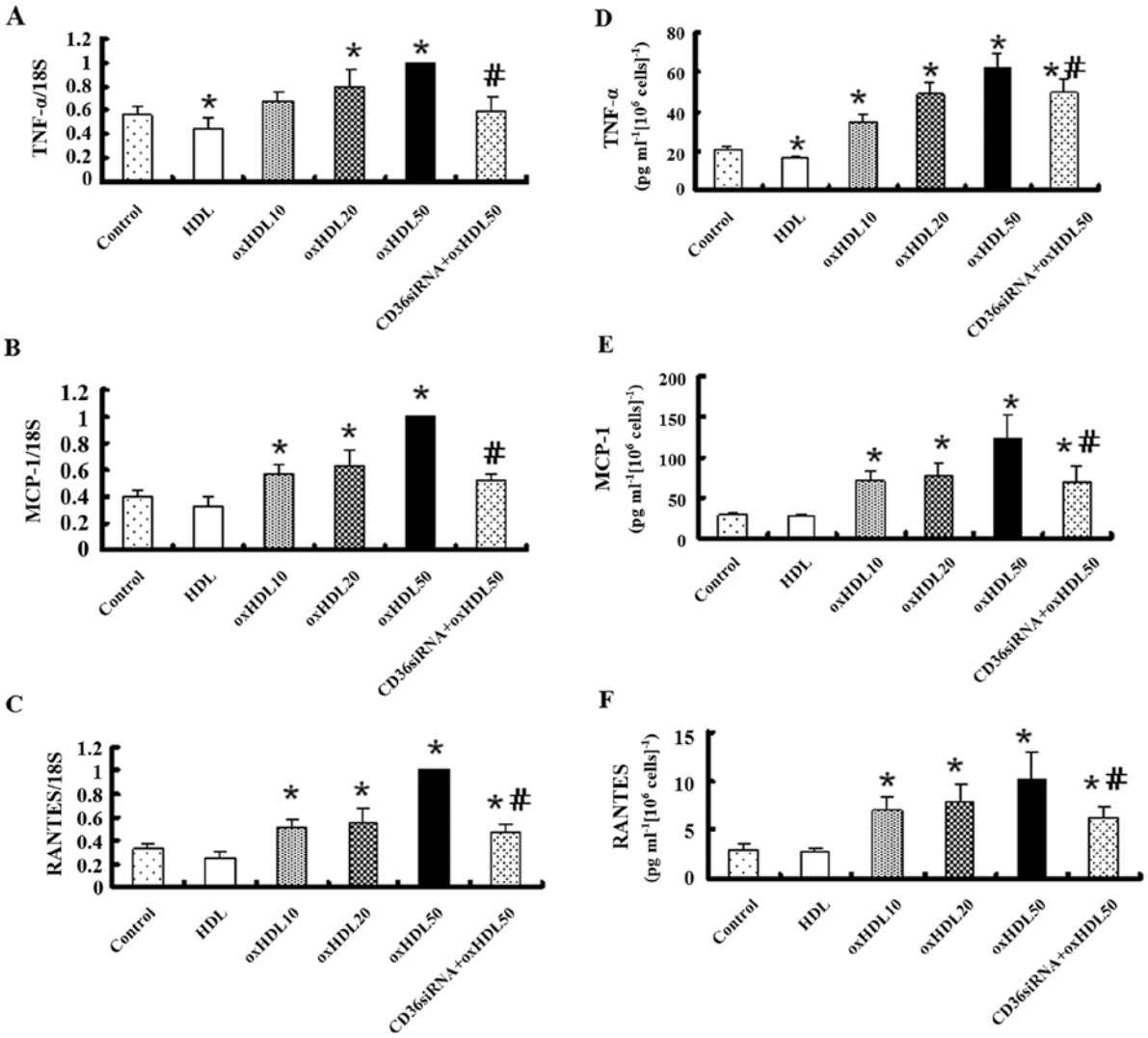

Figure 3. Effects of oxidized high-density lipoprotein (HDL) on mRNA and protein levels of pro-inflammatory factors. (A and D) Tumor necrosis factor-α (TNF-α), (B and E) monocyte chemoattractant protein-1 (MCP-1) and (C and F) regulated upon activation normal T cell expressed and secreted (RANTES) mRNA and protein levels in proximal tubular cell line (HK-2) cells following 24 h of incubation with 50 μg/ml HDL, oxidized HDL at the concentrations of 0, 10, 20, 50 or 50 μg/ml oxidized HDL following transfection with CD36 short interference RNA (siRNA). Control, untreated group. Data are the means ± standard deviation (SD), *P<0.05 vs. control, #P<0.05 vs. 50 μg/ml oxidized HDL. n≥3, each experiment was performed in triplicate.

kinase tyr527 was downregulated by oxidized HDL in a dose-dependent manner, and this effect was eliminated by treatment with CD36 siRNA, while the incubation of the HK-2 cells with oxidized HDL did not affect the expression of phospho-Src (tyr416) (Fig. 6).

Oxidized HDL regulates mitogen-activated protein kinase (MAPK) family proteins in HK-2 cells. MAPK mainly consists of 3 different pathways (p38/MAPK, ERK/MAPK and JNK/MAPK) linking growth, differentiation, proliferation and apoptotic signals with transcription in the nucleus (21). In the present study, the expression of MAPK family proteins was detected by western blot analysis in order to determine whether it was affected by oxidized HDL. The incubation of HK-2 cells with oxidized HDL increased the phosphorylation of p38, JNK and ERK in a dose-dependent manner. Pre-treatment with CD36 siRNA partially attenuated the upregulation of phosphorylated p38, JNK and ERK by oxidized HDL (Fig. 7).

Oxidized HDL activates nuclear factor-κB (NF-κB) proteins in HK-2 cells. The activation of the transcription factor, NF-κB, is considered to be a vital signaling factor for apoptosis, ROS generation, and in particular, inflammatory responses in HK-2 cells (22). The phosphorylation of the p65 unit of NF-κB has been reported to initiate its activation and plays an important role in regulating the specificity of NF-κB-dependent gene expression (23). In this study, to investigate whether NF-κB activation is involved in the effects of oxidized HDL, the HK-2 cells were pre-incubated with various concentrations of oxidized HDL or HDL, as already mentioned in the previous sections. The results of western blot analysis indicated that oxidized HDL induced the phosphorylation of p65 and this effect was attenuated by CD36 siRNA (Fig. 8).

Discussion

In the present study, we investigated the effects of oxidized HDL on HK-2 cells, as well as the mechanisms involved.

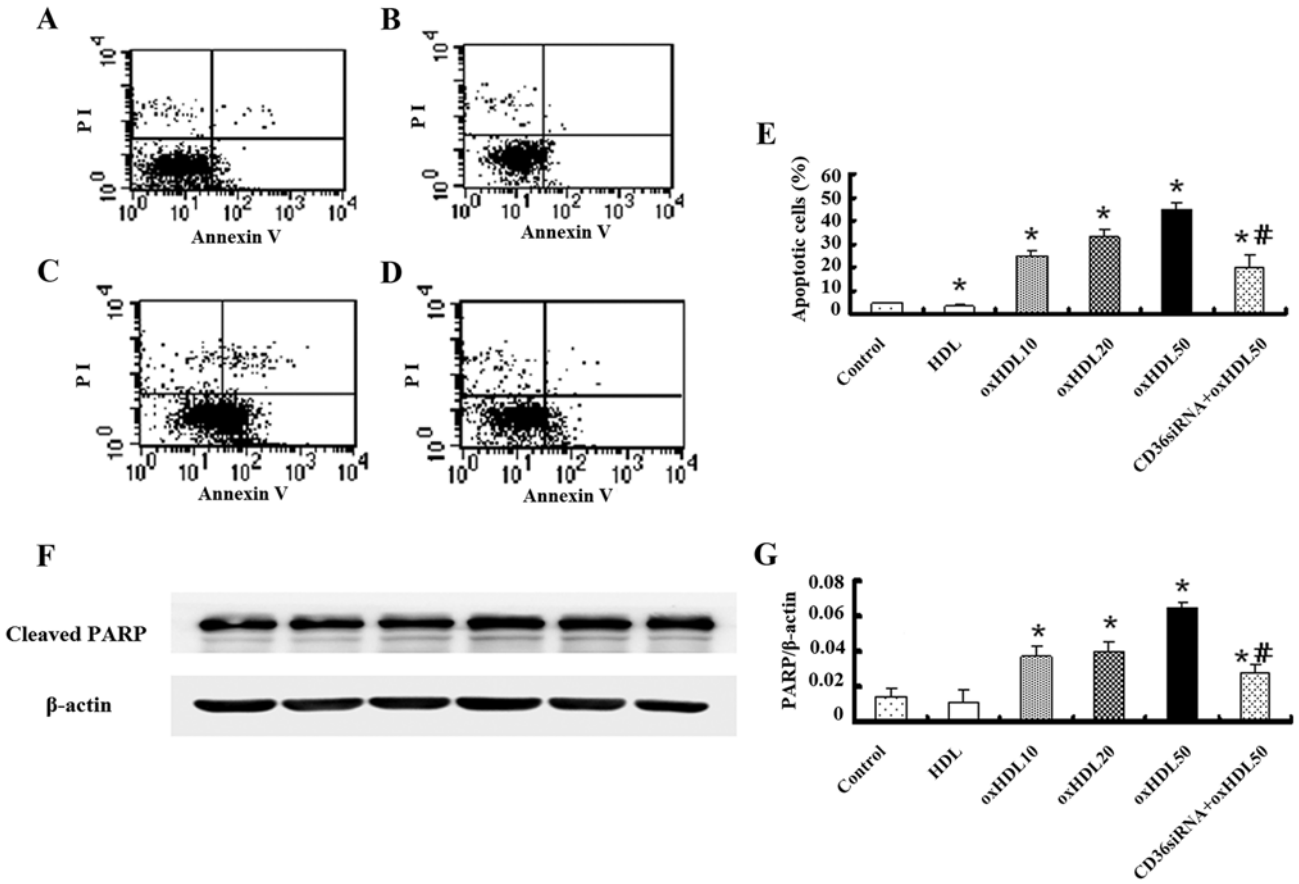

Figure 4. Effects of oxidized high-density lipoprotein (HDL) on apoptosis of HK-2 cells. Early apoptosis of HK-2 cells after 24 h of incubation with (A) 0 μg/ml oxidized HDL (control), (B) 50 μg/ml HDL, (C) 50 μg/ml oxidized HDL or (D) 50 μg/ml oxidized HDL following transfection with CD36 short interference RNA (siRNA); (E) Percentage of early apoptotic cells. (F) Cleaved poly(ADP-ribose) polymerase (PARP) protein expression as shown by western blot analysis and (G) normalized OD values. Data are the means ± standard deviation (SD), *P<0.05 vs. control, $^{\#}$P<0.05 vs. 50 μg/ml oxidized HDL. n≥3, each experiment was performed in triplicate.

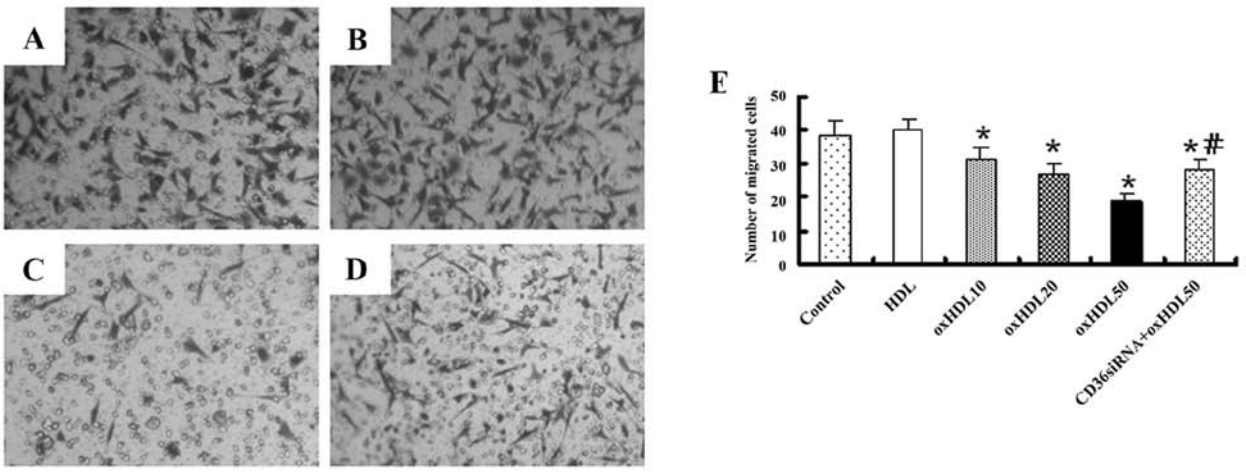

Figure 5. Effects of oxidized high-density lipoprotein (HDL) on the migration ability of HK-2 cells analyzed by modified Boyden chambers. Representative images of migration of HK-2 cells stained with hexamethylpararosaniline after a 24 h of incubation with (A) 0 μg/ml oxidized HDL (control), (B) 50 μg/ml HDL, (C) 50 μg/ml oxidized HDL or (D) 50 μg/ml oxidized HDL following transfection with CD36 short interference RNA (siRNA). (E) Number of migrated cells. Data are the means ± standard deviation (SD), *P<0.05 vs. control, $^{\#}$P<0.05 vs. 50 μg/ml oxidized HDL. n≥3, each experiment was performed in triplicate.

The principal finding of this study was that oxidized HDL increased intracellular the generation of ROS, promoted inflammation and apoptosis, and inhibited the migration ability of HK-2 cells. Oxidized HDL exerted negative effects on HK-2 cells which were mediated through the scavenger receptor, CD36, inducing the activation of the Src, MAPK and

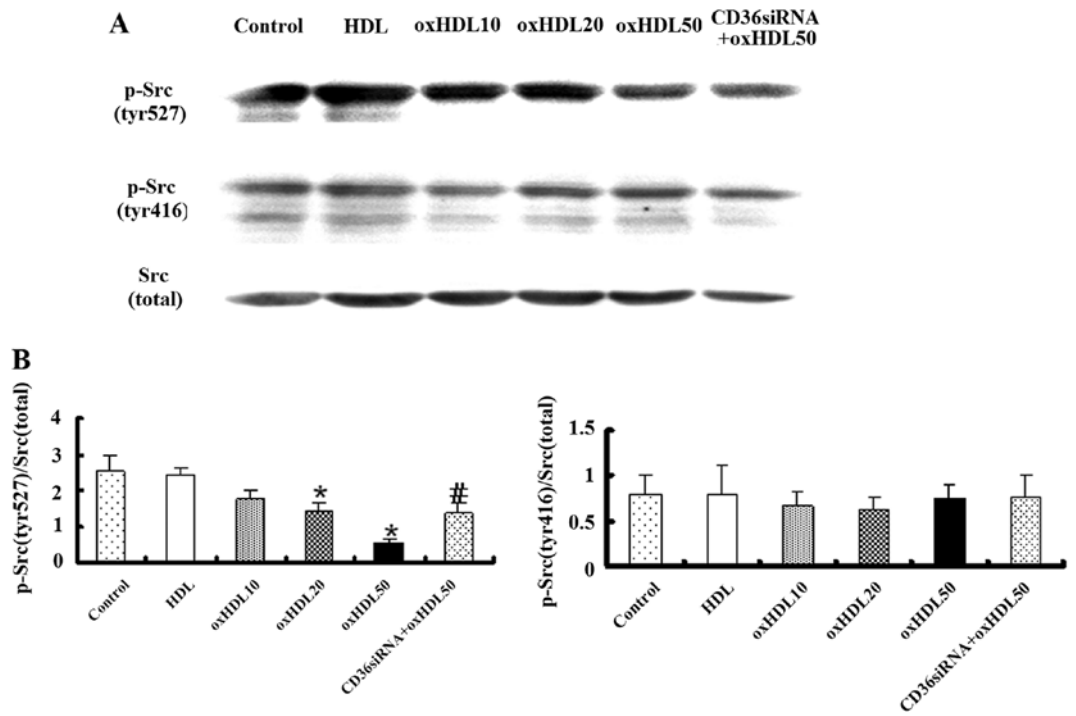

Figure 6. Effects of oxidized high-density lipoprotein (HDL) on Src proteins in HK-2 cells. (A) Representative western blot showing the expression of phosphorylated (p)-Src (tyr527) and p-Src (tyr416) following incubation of the cells with 50 μg/ml HDL, or oxidized HDL at the concentrations of 0, 10, 20, 50 or 50 μg/ml oxidized HDL following transfection with CD36 short interference RNA (siRNA). (B) Normalized OD values for the Src proteins. Data are the means ± standard deviation (SD), *P<0.05 vs. control, $^{\#}$P<0.05 vs. 50 μg/ml oxidized HDL. n≥3, each experiment was performed in triplicate.

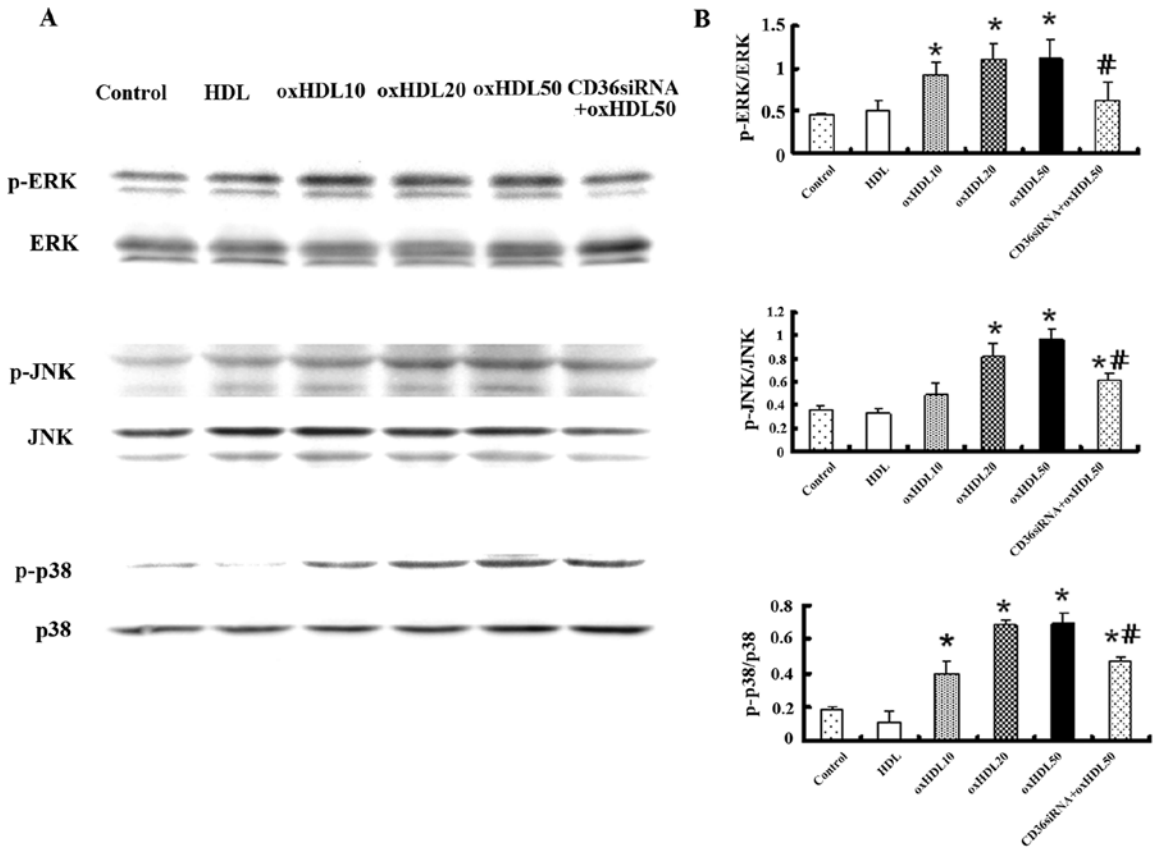

Figure 7. Effects of oxidized high-density lipoprotein (HDL) on mitogen-activated protein kinase (MAPK) proteins in HK-2 cells. (A) Representative western blot showing the activation (phosphorylation) of p38, c-Jun N-terminal kinase (JNK) and extracellular regulated kinase (EPK)1/2 following incubation of the cells with 50 μg/ml HDL, or oxidized HDL at the concentrations of 0, 10, 20, 50 or 50 μg/ml oxidized HDL following transfection with CD36 short interference RNA (siRNA). (B) Normalized OD values for MAPK proteins. Data are the means ± standard deviation (SD), *P<0.05 vs. control, $^{\#}$P<0.05 vs. 50 μg/ml oxidized HDL. n≥3, each experiment was performed in triplicate.

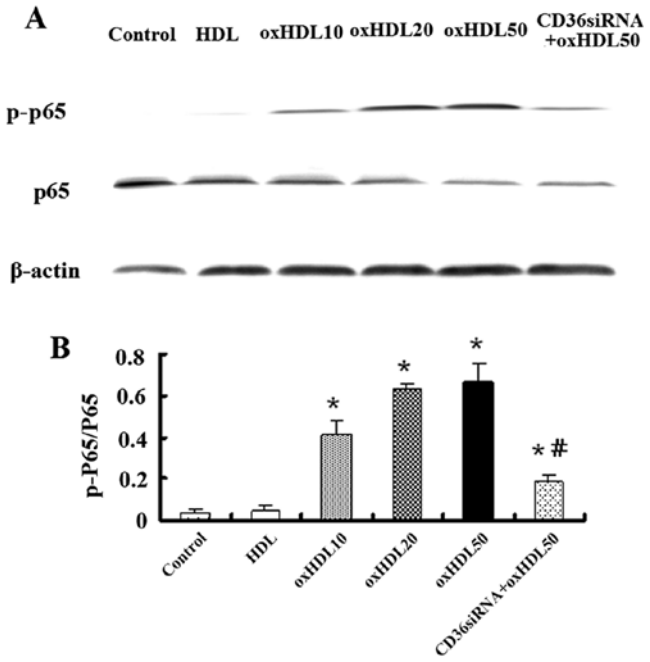

Figure 8. Effects of oxidized high-density lipoprotein (HDL) on p65 activity in HK-2 cells. (A) Representative western blot showing the activation (phosphorylation) of p65 following incubation of the cells with 50 μg/ml HDL, or oxidized HDL at the concentrations of 0, 10, 20, 50 or 50 μg/ml oxidized HDL following transfection with CD36 short interference RNA (siRNA). (B) Normalized OD values of western blot analysis. Data are the means ± standard deviation (SD), *P<0.05 vs. control, $^{\#}$P<0.05 vs. 50 μg/ml oxidized HDL. n≥3, each experiment was performed in triplicate.

NF-κB pathways. These results indicate that oxidized HDL, which negatively affects renal tubular epithelial cell biology through CD36, may play an important role in the pathogenesis of CKD.

Several factors are involved in the pathogenesis of CKD, including oxidative stress, inflammation and dyslipidemia. Lipid-mediated renal injury is an important component of CKD, particularly in diabetic nephropathy (24,25). Under normal conditions, HDL plays a protective role through reverse cholesterol transport and also serves as a potent antioxidant, anti-inflammatory and antithrombotic factor (6,7). However, HDL can lose its protective capacity and even become a pro-inflammatory agent known as oxidized HDL in the setting of systemic inflammation or under oxidative damage (26). Recently, Vaziri *et al* (13) reported that a decrease in the HDL concentration was compounded by the severe reduction in its antioxidant capacity in patients with CKD. However, although renal tubulointerstitial inflammation plays a central role in the progression of CKD, little is known about the role of oxidized HDL in mediating renal tubular cell damage. In this study, we found that oxidized HDL exerted several deleterious effects on HK-2 cells. The exposure of renal tubular cells to oxidized HDL potently increased the generation of ROS and stimulated the production of pro-inflammatory factors, including TNF-α, MCP-1 and RANTES. These factors may contribute to the pathogenesis of cell injury, either by modulating the immune system or by directly promoting renal damage, eventually resulting in cell apoptosis and the inhibition of migration ability (3,27). Other factors, including TGF-β, also play an important role in the pathogenesis of inflammatory response during the course of chronic kidney damage. Our preliminary experiments found that TGF-β remained statistically unaltered (data not shown). We also measured the release of lactate dehydrogenase (LDH) and no significant cell lysis was detected up to a concentration of 100 μg/ml of oxidized HDL (data not shown), indicating that oxidized HDL did not increase the necrosis of renal tubular cells.

Scavenger receptors comprise a family of 9 classes of structurally similar receptors that share oxidized lipoproteins as their primary ligands (28). CD36 has been identified as a class B transmembrane scavenger receptor, which is known to be expressed by multiple cell types (29). Previous studies have shown that CD36 is predominantly expressed in tubular epithelial cells with specific modulation of its expression patterns during chronic renal injury (30-32). Apart from binding to a variety of ligands, including oxidized low-density lipoprotein, it has also been suggested to be a major receptor for oxidized HDL (29-33). Our finding that the negative effects exerted by oxidized HDL were blocked by transfection with CD36 siRNA prior to treatment with oxidized HDL supports the hypothesis that the oxidized HDL-induced effects on HK-2 cells are largely mediated through the scavenger receptor, CD36.

Src family kinases are essential components of cell growth and proliferation signaling at inflammatory sites (19,34). Previous studies have shown that Src is activated in renal tubular injury and may be an important regulator of tubular cell proliferation (19,35). The MAPK and NF-κB pathways are both important signaling routes that are activated in response to a variety of environmental stresses and inflammatory signals, and promote apoptosis and growth inhibition (21,22). Downstream targets of Src, MAPK and the transcription factor, NF-κB, regulate inflammation, and the binding of oxidized HDL to CD36 may also activate these intracellular signaling pathways that lead to pro-inflammatory reactions (32,34). In accordance with these data, our findings indicated that the incubation of HK-2 cells with oxidized HDL inhibited the phosphorylation of Src-family kinase tyr527 in a dose-dependent manner, mainly through CD36, without affecting the expression of phospho-Src (tyr416). Since the phosphorylation of tyr527 renders the enzyme less active, oxidized HDL upregulated Src enzyme activity. Oxidized HDL also increased the phosphorylation of p38, JNK and ERK in a dose-dependent manner. NF-κB activity was altered with the level of Src and MAPK. These results further confirm the involvement of the Src, MAPK and NF-κB pathways in the negative effects induced by oxidized HDL on HK-2 cells.

In conclusion, our data are in support of the hypothesis that oxidized HDL enhances oxidative stress and alters the phenotype of proximal tubule epithelial cells to a more dysfunctional pro-inflammatory state, ultimately succumbing to apoptosis and the inhibition of migration. These effects are largely mediated through the scavenger receptor, CD36, as well as through the Src, MAPK and NF-κB signaling pathways. The ability of oxidized HDL to negatively affect proximal tubule cell biology may represent a novel pathological mechanism for the development and progression of CKD.

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