TNF-α induced pro-inflammation stimulates apolipoprotein-A4 via activation of a TNFR2 and NF-κB signaling pathway in kidney tubular cell

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Directed by Professor Kyung Sup Kim

The Master's Thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Young In Cho

June 2016

This certifies that the Master's Thesis
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June 2016

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ABSTRACT

TNF-α induced pro-inflammation stimulates apolipoprotein-A4 via activation of a TNFR2 and NF-κB signaling pathway in kidney tubular cells

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Acute kidney injury (AKI) is the initial stage of kidney disease and can lead to chronic kidney disease (CKD). Human apolipoprotein A4 (apo-A4) levels are significantly elevated in early-stage CKD. Moreover, elevated apo-A4 levels in the kidney lead to abnormal apo-A4 protein deposits in body tissues and organs, which can cause amyloidosis. Therefore, apo-A4 regulation can affect cell viability following kidney injury.

To determine which types of AKI affect apo-A4 levels, we induced AKI in human kidney cells using a variety of conditions. Apo-A4 expression level was only affected by Tumor necrosis factor alpha (TNF-α) treatment. Our data showed that pro-inflammatory AKI caused by TNF-α induced TNFR2 activation via NF-κB and enhanced apo-A4 expression in human kidney cells and tissues. Moreover, apo-a4 overexpression affected the recovery of kidney cells.

In conclusion, apo-A4 expression was upregulated by TNF-α. In addition, we showed that inflammatory kidney damage occurs through a TNFR2-NFκB complex and that apo-A4 regulation can affect cell viability.

Key words : apolipoprotein-A4, acute kidney injury, TNF-α, TNFR2, NF-κB, kidney function, pro-inflammation

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I. INTRODUCTION

Human apolipoprotein-A4 (apo-A4) is a 46 kDa glycoprotein that is synthesized and secreted by intestinal enterocytes.[1] Lipid absorption increases apo-A4 transcription in enterocytes.[2] Apo-A4 protein circulates freely in blood and lymph. It is a chylomicron structural protein and high-density lipoprotein.[3] Apo-A4 is a satiety factor that suppresses food intake.[4-6] *In vitro* studies have demonstrated that apo-A4 is involved in the reverse cholesterol transport pathway, which removes cholesterol from peripheral cells and directs it to the liver and steroidogenic organs for metabolization.[7-10] In addition, apo-A4 activates lecithin–cholesterol acyltransferase[11] and regulates the activation of lipoprotein lipase.[7] Apo-A4 is a potent endogenous antioxidant that protects against copper-induced oxidation of low-density lipoprotein.[12,13] It was recently shown that apo-A4 has an endogenous anti-inflammatory effect.[14,15]

In the kidney, apo-A4 is expressed in proximal and distal tubular cells, capillaries, and blood vessels, but not in glomeruli. Apo-A4 can be filtered by the glomeruli in its lipoprotein-unbound state and is taken up by proximal tubular cells, where it is degraded.[9] Apo-A4 is related to several kidney diseases, and its expression in kidney tubular cells suggests that apo-A4 is metabolized in the kidney.[16] Creatinine clearance correlated negatively with apo-A4 expression in kidney diseases. Patients with a tubular type of proteinuria had significantly higher amounts of apo-A4 in their urine than those with a pure glomerular type of proteinuria and controls.[17] Apo-A4 levels increased significantly with decreasing GFR and were elevated in early stages of chronic kidney disease (CKD). Patients with kidney disease have significantly higher levels of apo-A4 even when GFR is within the normal range.[18] Therefore, apo-A4 levels represent a novel predictor for kidney disease progression. Elevated apo-A4 levels in response to impaired kidney function may enhance the removal of cholesterol from mesangial cells, but this has not been confirmed.[19]

Significantly higher apo-A4 levels have been observed in the blood plasma and urine of CKD patients, and apo-A4 accumulation increased significantly during the initial phase of the disease.[20,21] Continuous apo-A4 accumulation following kidney injury can cause amyloidosis, which is characterized by amyloid deposits in body tissues and organs.[22] Therefore, apo-A4 accumulation may have a negative impact on in kidney cell recovery.

These findings suggest that apo-A4 levels elevate as kidney injury progresses and that apo-A4 influences the recovery of injured kidney cells. To investigate this further, we induced AKI in kidney cells under a variety of conditions. We also investigated the effect of apo-A4 expression on the recovery of kidney tubular cells. We demonstrated the association of apo-A4 and regulatory function of apo-A4 following AKI and elucidated its effect on cell signaling pathways related to kidney injury *in vitro* and *in vivo*.

II. MATERIALS AND METHODS

1. Cell culture

HK-2 cells were cultured in keratinocyte-serum-free medium(K-SFM, Gibco, Grand Island, NY, US) included 0.05mg/ml bovine pituitary extract (BPE), 5ng/ml human recombinant epidermal growth factor (EGF), 10% fetal bovine serum (FBS), 1% penicillin/streptomycin under an atmosphere of 5% CO2, 95% O_2 at 37°C, and were subcultured every 4 days using 0.02% ethylenediaminetetraacetic acid (EDTA) and 0.05% trypsin. The medium was replaced with fresh medium every 2 days.

2. Western blot

Total cellular protein extracts was prepared on ice using a PRO-PREP protein extract solution (Intron, Seoul, Korea). Total proteins (20μg) were loaded onto a sodium dodecyl sulfate-polyacrylamide gel (10% polyacrylamide) and transferred to polyvinylidene floride membrane for 1 hr. Membranes were blocked with 5% BSA in TBS-T and incubated 4°C overnight with HIF-1α, apo-A4, TNF receptor 2, IKBa, α-tubulin primary antibody (Abcam, Cambridge, MA, US) diluted 1:1000 in TBS-T. After incubation step, wash the membrane with TBS-T and secondary antibodies were applied (1:10000, horseradish peroxidase-conjugated anti-mouse, anti-rabbit) at room temperature for 1 hrs. Labeled bands were detected by West Pico chemiluminescent kit (Thermo scientific, Rockford, IL, US).

3. Establishment of stable apo-A4 knockdown cells

For depletion of apo-A4, lentiviral vector (pLL-apo-A4-CMV-GFP-puro) (ABM, Richmond, CA) was purchased. Apo-A4 knockdown vectors were transfected with Lipofectamine Plus reagent(Invitrogen, Carlsbad, CA) and viral packing vectors(pMD2.G, pMDLG/PRRE, pRSV-Rev) in HEK293T cell.

Virus packed culture medium was extracted and purified after 48 hrs. Packaging virus and 8mg/ml polybrene were treated in HK-2 cell. Infected cells were selected in 1 g/ml puromycin treated media(Sigma, St. Louis, US). Stable knockdown cells were confirmed by western blotting.

4. Establishment of stable apo-A4 overexpression cells

For over-expression of apo-A4, lentiviral vector (pLL-apo-A4-CMV-GFP-puro) (ABM, Richmond, CA) was purchased.

Apo-A4 overexpression vectors were transfected with Lipofectamine Plus reagent (Invitrogen, Carlsbad, CA) and viral packing vectors (pMD2.G, pMDLG/PRRE, pRSV-Rev) in HEK293T cell. Virus packed culture medium was extracted and purified after 48 hrs. Packaging virus and 8mg/ml polybrene were treated in HK-2 cell. Infected cells were selected in 1 g/ml puromycin treated media (Sigma, St. Louis, US). Optimization of gain of expression was determined with western blotting.

5. MTT assay

HK-2 cell (4×10^6cells/ml) was incubated in 24 well serum free medium with 5% complete medium. 100μl MTT(2mg/ml, sigma) was treated each well and incubation in 37°C for 2 hrs. The supernatant was aspireated, and the precipitates were dissolved in 100ug DMSO. Cellular proliferation was determined using a Beckman Coulter microplate reader at 560nm.

6. Immunofluorescence assay

Cells were washed in phosphate-buffered saline and fixation in 4% paraformaldehyde. Nuclei was permeated in 0.5% Triton X-100/ phosphate-buffered saline for 5 min. Cells were blocked in 5% BSA/phosphate-buffered saline for 20min and incubated with mouse apo-A4 antibody(Abcam, Cambridge, MA, US) in 37°C for 1 hr. Then treated Alexa Fluor 488 anti-mouse

secondary antibody(Invitrogen) 4°C for 1 hr(1:200). Images were taken by confocal microscope(LSM Meta 700, Carl Zeiss, Oberkochen, Germany) and analyze with LSM Image Browser software.

7. Immunohistochemistry

Kidney issue sections were incubated in formalin, rehydrated via graded ethanol series to water, and treated overnight with 0.1 mol/L citrate buffer antigen retrieval solution (pH 6.0) (Dako, Carpinteria, CA, US) at 85°C to retrieve antigen exposure. Endogenous peroxidase was blocked with 3% H2O2 for 5 min. Nonspecific binding was blocked by incubition in 1% BSA in 0.01 mol/L Tris-HCl buffer (pH 8.6) with 0.01%Triton X-100 (BSA-T) at room temperature for 1 hr. Affinity-purified polyclonal rabbit antihuman apo-A4 antibody (0.8 mg/mL) diluted 1:400 in BSA-T were applied for 1 hr at room temperature. Excessive, unbound primary antibody was removed by washing with Tris-HCl buffer. Specific immunoreaction was detected using a secondary horseradish peroxidase–conjugated goat antirabbit antibody (Dako, Carpinteria, CA, US) Incubation with the secondary antibody was performed as recommended by the manufacturer. After washing in Tris-HCl buffer, 3-3-diaminbenzidine tetrahydrochloride (DAB+) (Dako, Carpinteria, CA, US) was used for visualization. Sections were counterstained with hematoxylin, dehydrated, and mounted in DePeX (Serva, Heidelberg, Germany) and examined with a microscope.

8. ELISA

Rat plasma apo-A4 concentrations were determined using a double-antibody enzyme-linked immunosorbent assay (ELISA), which employs an affinity-purified poly-clonal anti-rat apo-A4 antibody for coating and the same antibody coupled to horseradish peroxidase for detection. The amounts of apo-A4 in the rat plasma were estimated using apo-A4 rat ELISA kit(mybiosource, Vancover,

Canada) according to the manufacturer's instructions.

9. *In vivo*

Procedures were conducted using sterile (autoclaved) surgical instruments and consumables. An 8-week-old male SD rat was used for model of renal ischemic reperfusion injury. Animals were prepared and experiment was started with laparotomy, ureter division and right nephrectomy. Next, we conducted left Kidney Ischemia using artery clamp and performed reperfusion for 48 hrs. Rats were sacrificed and kidney and blood sample were collected for western blot, IHC, ELISA. [23]

III. RESULTS

1. Localization and expression of apo-A4 in kidney cell lines and human kidney tissues

To investigate the expression of apo-A4 in the kidney, we used two kidney cancer cell lines (caki-1 and caki-2) and two normal kidney cell lines (HK-2 and HEK-293). We measured apo-A4 expression level in these cells by western blotting (Figure 1A) and normalized this to α-tubulin expression. We observed apo-A4 protein expression in all four kidney cell lines. Next, we investigated apo-A4 expression in normal kidney and CKD tissues by immunohistochemistry (IHC) using a mouse anti-apo-A4 antibody. IHC revealed apo-A4 expression in normal and CKD tissue (Figure 1B). We observed apo-A4 expression in the glomerulus and tubule. Apo-A4 expression was stronger in CKD tissue than in normal kidney tissue.

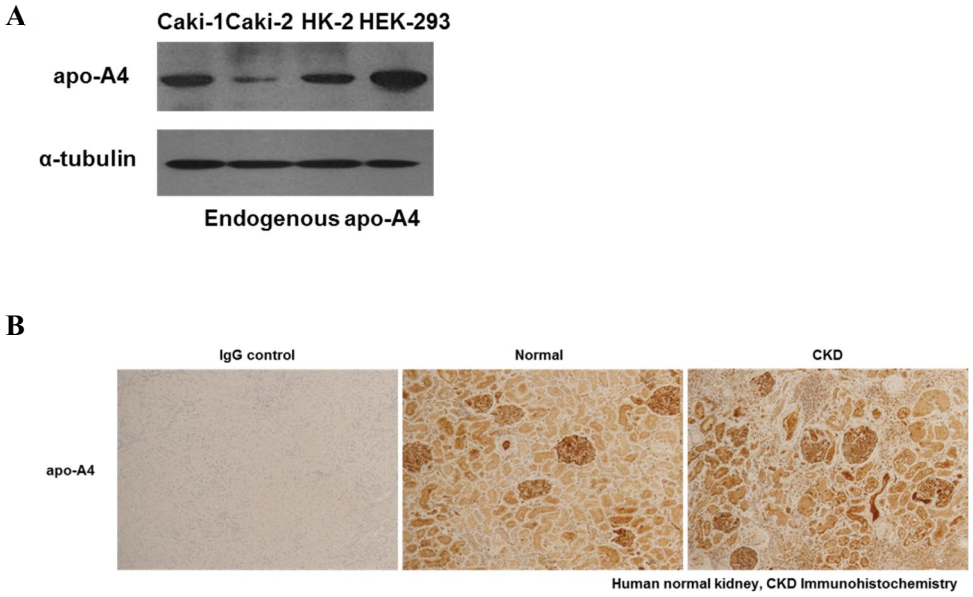


Figure 1. Apo-A4 expression in kidney cell lines and human kidney tissues.

(A) Endogenous apo-A4 protein expression was measured in Caki-1, Caki-2, HK-2, and HEK-293 cells by western blotting. α-tubulin was used as a loading control. (B) Immunohistochemistry of apo-A4 on formalin-fixed, paraffin-embedded human kidney tissue. Apo-A4 expression was detected in the tubule and glomerulus of normal kidney and CKD tissue.

2. Apo-A4 expression in kidney cell lines following AKI

To determine which type of AKI affects apo-A4 expression in kidney cells, we exposed human kidney cells to various AKI-inducing conditions. We exposed Caki-1, HK-2, and HEK293 cells to hypoxic condition for 72 hr (Figure 2A). In addition, we induced chemical hypoxia using $CoCl_2$ (0, 100, and 200 uM) for 8, 16, and 24 hr in HK-2 cells (Figure 2B), but this had no effect on apo-A4 expression. Next, we treated HK-2 cells with cisplatin, A23187, TNF-α, and IL-β. Cisplatin is a nephrotoxic cancer drug, and A23187 is a calcium ionophore, which induces a calcium influx that causes apoptosis. TNF-α and IL-1β are well-known pro-inflammatory cytokines that cause inflammatory injury. We measured apo-A4 protein expression levels by western blotting (Figure 2C). We found that only TNF-α affected apo-A4 expression. This indicated that TNF-α induced inflammatory AKI by regulating apo-A4 expression.

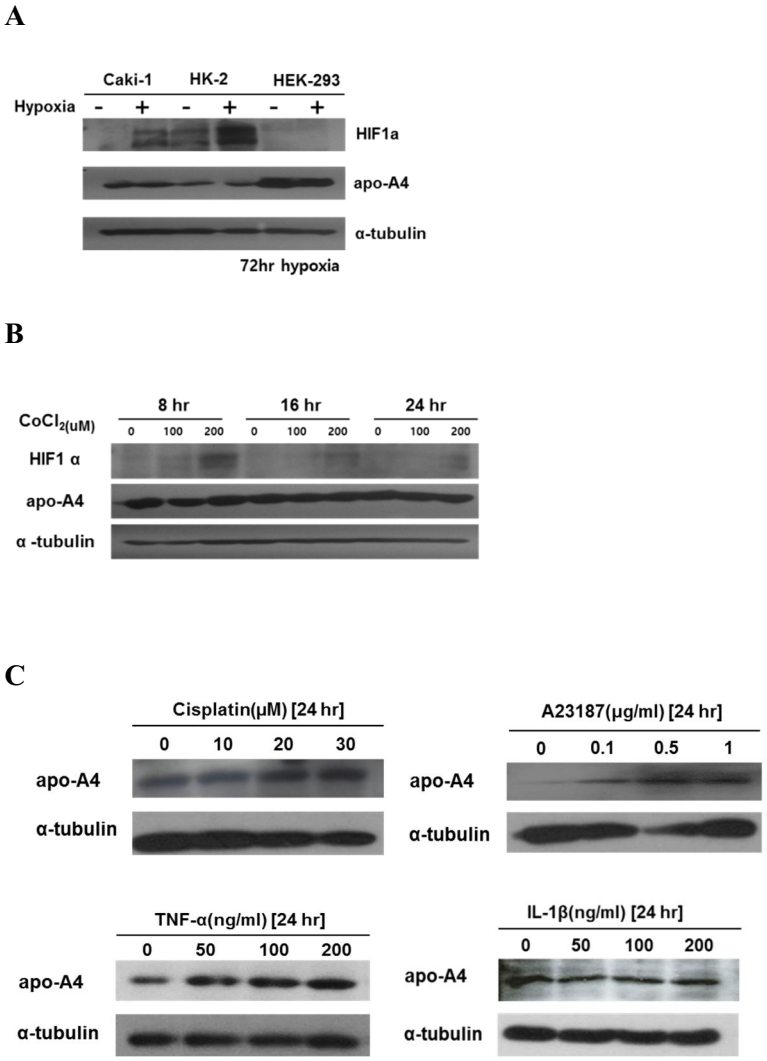


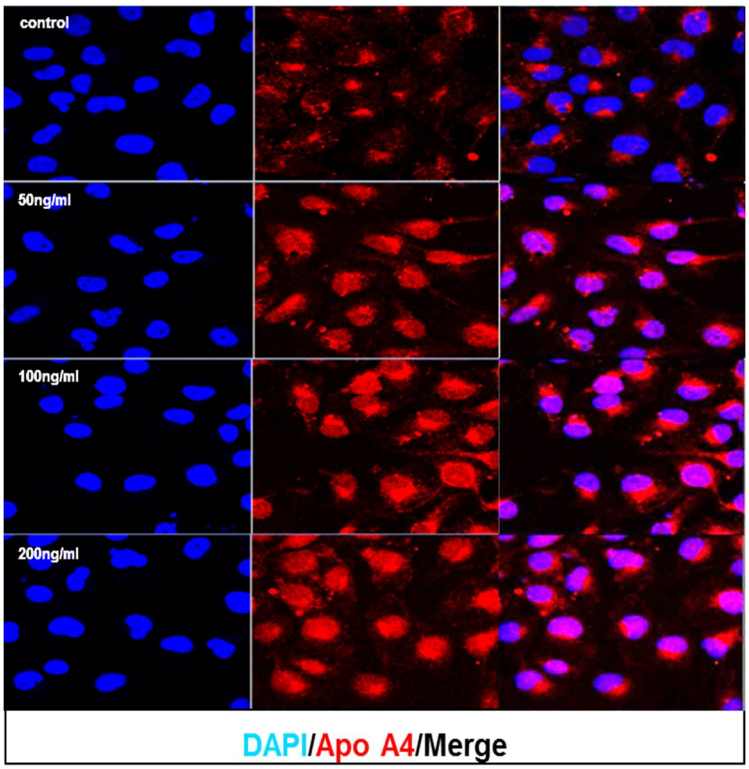
Figure 2. Effect of AKI on apo-A4 expression in kidney cell lines.

(A) HIF-1α and apo-A4 expressions in caki-1, HK-2, and HEK-293 cells exposed to hypoxia for 72 hr (B) HIF-1α and apo-A4 expression in HK-2 cells treated with different concentrations of $CoCl_2$ (0, 100, and 200 uM) for 8, 16, and 24 hr. (C) Apo-A4 expression in HK-2 cells treated with cisplatin (0, 10, 20, and 30 μM), A23187 (0, 0.1, 0.5, and 1 g/ml), TNF-α (0, 50, 100, and 200 ng/ml), and IL-1β (0, 50, 100, and 200 ng/ml) for 24 hr.

3. Inflammatory AKI induced by TNF-α increased apo-A4 expression

TNF-α treatment increased apo-A4 expression in HK-2 cells. To confirm this finding, we performed fluorescence immunocytochemistry. The expression of apo-A4 increased 24 hr after TNF-α treatment compared with untreated HK-2 cells (Figure 3A). To test whether this was true *in vivo*, we induced ischemic reperfusion injury (IRI) in rat kidneys. W removed kidney tissue 48 hr after reperfusion and isolated protein lysates to measure apo-A4 expression levels by western blotting. Apo-A4 protein expression was elevated in IRI tissue compared with control tissue (Figure 3B). In addition, we isolated serum samples from rats and measured apo-A4 levels measured by ELISA (Figure 3C). Our findings indicated that apo-A4 expression is increased by inflammatory AKI *in vitro* and *in vivo.*

A



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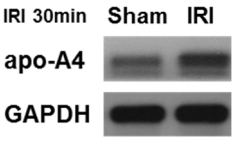

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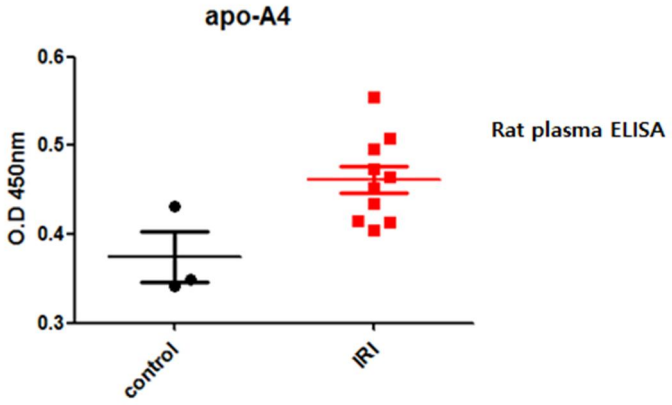


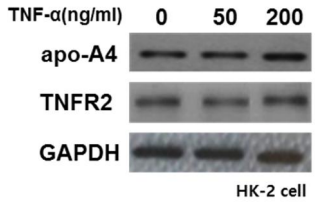
Figure 3. Inflammatory AKI induced by TNF-α increased apo-A4 expression.

(A) Immunofluorescence showing localization of apo-A4 (red) in HK-2 cells after 24 hr treatment with TNF-α. (B) Expression of apo-A4 protein detected by western blotting of kidney tissue from an IRI rat model. (C) Apo-A4 expression in rat plasma measured by ELISA.

4. Inflammatory AKI by TNF-α increases apo-A4 via TNF receptor 2

Some studies have reported that TNF receptor 2 (TNFR2) expression is increased in tubular epithelial cells during IRI.[24] Therefore, we measured apo-A4 and TNFR2 expression after the induction of AKI. Western blotting showed that apo-A4 and TNFR2 expression increased following TNF-α treatment (Figure 4A). Next, we detected apo-A4 and TNFR2 expression by IHC in rat kidney tissue. IHC revealed positive apo-A4 and TNFR2 staining in IRI rat kidneys compared with sham rats (Figure 4B). Our data revealed a positive correlation between apo-A4 and TNFR2 expression after TNF-α treatment.

A



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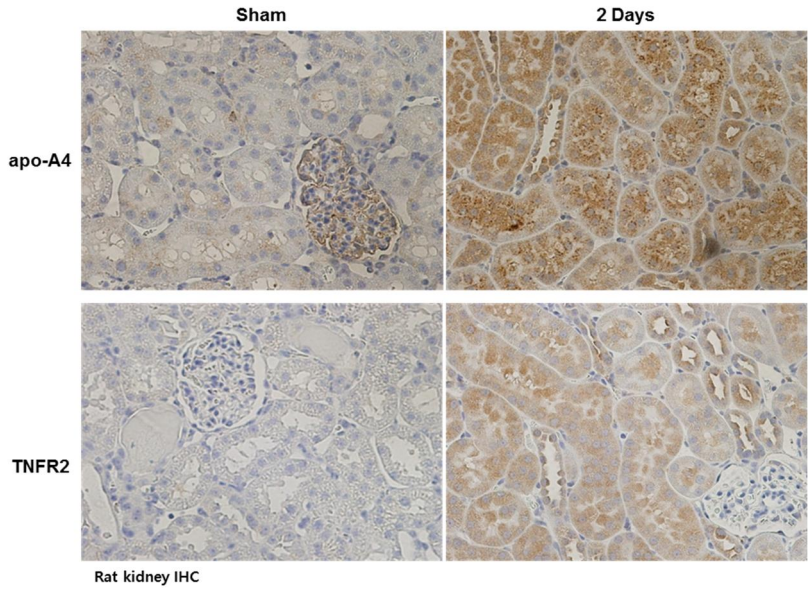


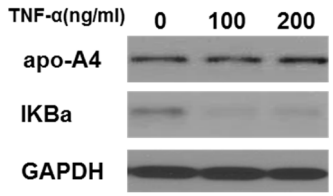
Figure 4. Inflammatory AKI by TNF-α increased apo-A4 expression via TNFR2.

(A) HK-2 cells were treated with different concentrations of TNF-α (0, 50, and 200 ng/ml) for 24 hr. Apo-A4 protein expression was detected by western blotting. (B) Apo-A4 and TNFR2 immunohistochemistry on formalin-fixed, paraffin-embedded ischemic reperfusion injured rat kidney tissues.

5. Increased apo-A4 expression caused by inflammatory AKI related to the NF-κB complex

The NF-κB complex plays an important role in inflammation.[25] We treated HK-2 cells with TNF-α as described earlier and measured IKBa expression by western blotting. We observed an inverse correlation between apo-A4 and IKBa expression (Figure 5A). In addition, we neutralized TNF-α using a TNF-α antibody and then examined NF-κB location by immunocytochemistry. Fluorescent immunocytochemistry revealed that NF-κB expression in the cytoplasm translocated to the nucleus after TNF-α neutralization compared with untreated HK-2 cells (Figure 5B). This finding suggests that NF-κB expression is promoted by TNF-α treatment, which elevates apo-A4 expression levels.

A



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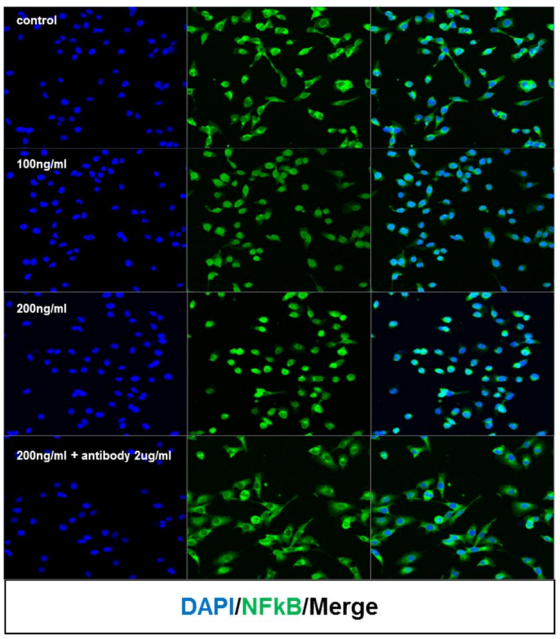

Figure 5. Inflammatory AKI increases apo-A4 expression through the NF-κB complex.

(A) Western blotting revealed an inverse relationship between apo-A4 and IKBa protein expression in HK-2 cells following TNF-α treatment. (B) Immunofluorescence showing apo-A4 and NF-κB expression after 24 hr treatment with TNF-α in HK-2 cells. A TNF-α neutralizing antibody was used to examine TNF-α function. Representative images of apo-A4 (red) and NF-κB (green) immunolocalization are shown.

6. Regulation of apo-A4 expression in kidney tubular cells affects cell recovery following inflammatory AKI

To investigate whether apo-A4 expression in HK-2 cells is related to cell viability, we introduced an apo-A4 expression vector. After transfection, we selected cells in conditioned media and confirmed apo-A4 expression by GFP fluorescence (Figure 6A, right). Apo-A4 protein expression level was measured by western blotting (Figure 6A, left). Next, we suppressed apo-A4 expression using shRNA. We selected transfected HK-2 cells using conditioned media. GFP fluorescence confirmed successful transfection (Figure 6B, right). We detected apo-A4 protein expression by western blotting (Figure 6B, left). We performed MTT assays to confirm cell viability after TNF-α treatment in cells overexpressing apo-A4 and in cells where apo-A4 was knocked down. Cell survival was not affected by TNF-α in apo-A4 knocked down cells (Figure 6C, left). In contrast, there was a positive correlation between apo-A4 expression and cell survival following TNF-α treatment in cells overexpressing apo-A4 (Figure 6C, right). Taken together, these results suggest that apo-A4 promotes cell survival following inflammatory AKI.

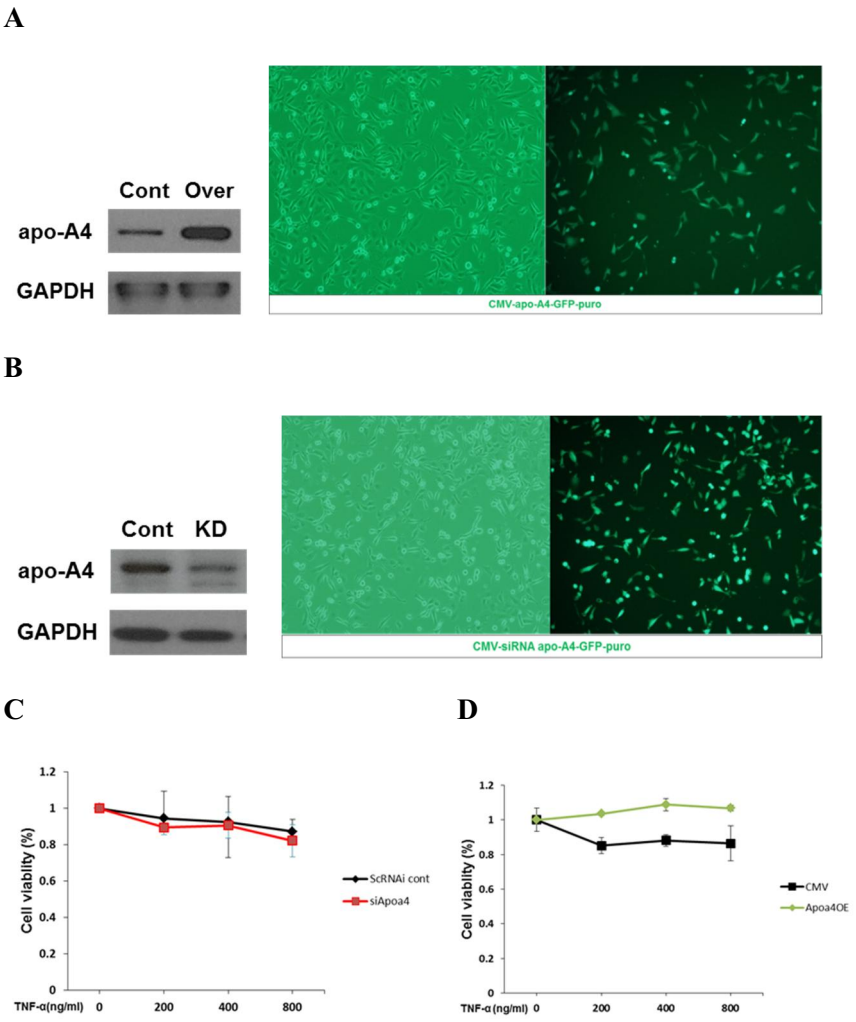

Figure 6. Regulation of apo-A4 expression in kidney tubular cells affects cell recovery following acute AKI.

(A) Apo-A4 expression was downregulated by siRNA in HK-2 cells. (B) Apo-A4 was overexpressed in HK-2 cells by an apo-A4 overexpression plasmid vector. Expression of apo-A4 was confirmed by western blot analysis and GFP fluorescence. (C,D) Cells were plated in 96-well culture plates and treated with different concentrations of TNF-α (0, 50, 100, and 200 ng/ml) for 24 hr. Cell viability was measured by the MTT assay after TNF-α treatment.

A B

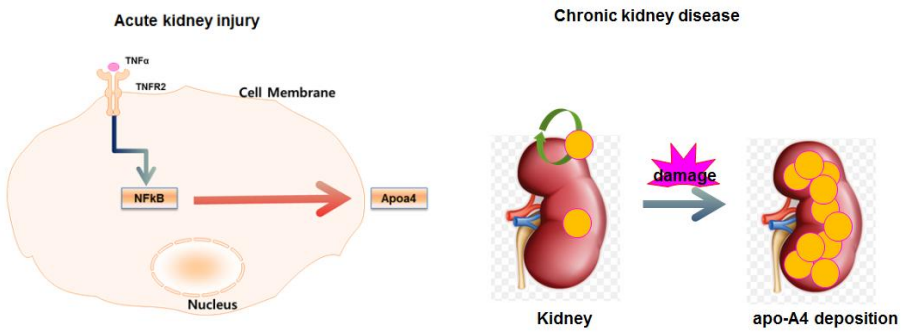

Figure 7. Schematic diagram of the apo-A4 mediated signaling pathway in kidney injury.

(A) The TNF-α-mediated increase in apo-A4 expression during inflammatory AKI. TNF-α activates apo-A4 expression via the TNFR2-NFκB pathway. (B) Continuous kidney damage caused by IRI promotes apo-A4 deposition in kidney cells.

IV. DISCUSSION

AKI occurs in the initial stages of kidney disease and is followed by CKD.[26] Some reports have indicated that apo-A4 expression levels are significantly elevated during AKI.[22] We have previously shown that apo-A4 expression levels are strongly reduced in pig kidney when ischemic preconditioning was performed prior to renal IRI. In this report, we investigated whether the downregulation of apo-A4 expression affected kidney cell recovery from IRI.[27] We hypothesized that CKD would be affected by an increased apo-A4 expression level in the kidney. To address this, we examined the role of apo-A4 and apo-A4-associated inflammation pathways in kidney injury.

First, we observed endogenous apo-A4 expression levels in kidney cells and tissues. It has been reported that apo-A4 is expressed in proximal and distal tubular cells, capillaries, and blood vessels, but not in the glomeruli of normal kidneys.[16] We observed apo-A4 expression in kidney tubular cells and the glomeruli of normal and CKD tissues. In this study, we focused on the differences in apo-A4 accumulation in the glomerulus. We anticipated apo-A4 accumulation in the glomerulus during IRI. Indeed, apo-A4 expression levels were elevated in CKD tissue compared with those in normal kidney tissue. This indicated that apo-A4 was upregulated in response to kidney injury. Protein accumulation has been reported in the blood plasma and urine of CKD patients during initial phases of the disease.[28,29] Therefore, apo-A4 expression can predict the progression of kidney disease.[30]

Next, we induced injury in human kidney cells using different conditions. AKI is caused by decreased renal blood flow (renal ischemia), exposure to harmful substances, inflammatory processes, or urinary tract obstruction, which impedes the flow of urine.[31-34] To mimic renal ischemia, we exposed human kidney cells to hypoxic conditions. Second, we induced nephrotoxicity by treating kidney cells with cisplatin. [35] Next, we induced apoptosis by treating

kidney cells with the calcium ionophore A23187, which increased intracellular Ca^{2+} levels.[36] We did not observe any correlation between apo-A4 expression and AKI induced by the abovementioned conditions.

Finally, we investigated the effect of inflammation on apo-A4 expression. Inflammation is an important response to pathogenic infection. However, the excessive production of inflammatory cytokines such as TNF-α and IL-1β may induce cell streaks. We found that TNF-α enhanced apo-A4 expression levels during inflammatory AKI. Triglyceride (TG) concentrations were elevated in patients with TNF-α-induced acute inflammatory disorders and sepsis during the acute episode of the disease.[37,38] Apo-A4 circulates in the blood and lymph as a free structural protein of chylomicron, which includes TG.[3] We assumed that the increase in apo-A4 expression level caused by TNF-α was related to pro-inflammatory AKI in human kidney cells.

Next, we induced IRI in rats to investigate the effect of apo-A4 expression on specific kidney inflammation. We induced IRI using an artery clamp instead of TNF-α treatment because TNF-α may cause inflammation in the whole body rather than local inflammation in the kidney.

TNFR2 plays an important role during inflammation. It was upregulated during intestinal inflammation TNFR2-deficient mice showed less severe intestinal inflammation.[24] In this study, we demonstrated that apo-A4 expression increased during inflammatory injury stimulated by TNF-α and therefore directly induced TNFR2 activation.

TNF-α-mediated activation of the NF-κB complex initiates phosphorylation and proteosomal degradation of IκB inhibitors within the cytoplasm,[39] which promotes nuclear translocation of the p65 subunit of NF-κB, enabling heterodimeric NF-κB to enter the nucleus. In the nucleus, NF-κB binds to enhancers of its target genes.[40] We have shown that inflammatory damage

induced TNFR2 activation via NF-κB in human kidney cell and tissues. This is supported by the finding that the promoter region of TNFR2 contains a number of transcription factors including NF-κB, AP-1, IRF and GAS.[41]

Next, we observed whether apo-A4 affected the viability of kidney cells. To examine this, we overexpressed apo-A4 in HK-2 cells. The overexpression of apo-A4 improved the viability of human kidney cells after inflammatory injury induced by TNF-α. Furthermore, apo-A4 knockdown in human kidney cells did not affect cell viability following TNF-α-induced inflammation. The mechanisms underlying these observations remain to be elucidated.

Our findings have shown that inflammatory AKI induced by TNF-α increases apo-A4 expression via the TNFR2-NFκB pathway (Figure 7A). In addition, apo-A4 may promote kidney cell recovery following AKI. In contrast to our findings that apo-A4 overexpression in human kidney cells promotes recovery, previous data have indicated a negative effect of apo-A4 in early stages of CKD.[18] Elevated apo-A4 levels in the kidney can cause amyloidosis, which is a consequence of apo-A4 deposits in kidney tissue.[22]

We suggest that continuous kidney damage induced by inflammatory AKI disrupts apo-A4 metabolism, which promotes apo-A4 deposition in kidney cells. This may lead to CKD or amyloidosis. [22] However, mechanisms involving apo-A4, including how apo-A4 is metabolized, in injured kidney tubular cells remain unclear (Figure 7B). In addition, the factor that causes apo-A4 deposition during CKD needs to be identified.

Further investigations are required to identify molecular mechanisms that link NF-κB-induced inflammation to apo-A4. It is also important to determine whether the protective role of apo-A4 is specific to NF-κB. Our results should encourage further examinations of the therapeutic effects of apo-A4 in amyloidosis or CKD.

V. CONCLUSIONS

We demonstrated that apo-A4 expression is increased by TNF-α and that inflammatory kidney damage and inflammation occur through a TNFR2-NFκB complex.

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ABSTRACT(IN KOREAN)

TNF-α로 유도된 염증반응에서 apolipoprotein-A4는 TNFR2와 NF-κB 경로를 통해 촉진된다

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급성 신 손상은 신장 질환의 초기 단계에 일어나는 현상으로 만성 신부전으로까지 이어질 수 있다. 이 때 apolipoprotein-A4(apo-A4) 발현은 만성 신부전의 초기 단계에서 매우 증가 되어 있는 것으로 알려져 있다. 또한 신장에서 증가되어 있는 apo-A4 발현양은 조직 및 장기의 비정상 적인 축적으로 인한 아밀로이드증을 유발할 수 있다고 알려져 있다. 따라서 신장 손상에서 apo-A4의 조절은 신 세포 생존에 영향을 줄 수 있을 것이라 예상하였다.

먼저 급성 신 손상 시에 apo-A4 발현에 영향을 줄 수 있는 인자를 결정하기 위해, 다양한 조건을 사용하여 급성 신 손상을 유발 하였다. 그 결과, Apo-A4 발현은 Tumor necrosis factor alpha(TNF-α)처리에 의해서만 영향을 받게 되었다. 또한 신장 세포 및 조직에서 TNF-α 의해 유발된 염증성 급성 신손상은 TNF receptor 2(TNFR2)의 활성화를 일으켜 NF-κB 경로를 통해 apo-A4를 증가시켰다. 또한, apo-A4의 과

발현은 신장세포의 회복에 영향을 미쳤다.

결과적으로 apo-A4의 발현은 TNF-α에 의해 증가 되었으며, 염증성 신장 손상은 TNFR2와 NF-κB 경로를 통해 발생하였고 이러한 apo-A4의 조절은 신장 세포 생존에 영향을 줄 수 있을 것이다.

핵심되는 말 : 아포지질단백질 A4, 급성 신 손상, 종양괴사인자-α, 종양괴사인자수용체2, 자연살해세포 핵심인자, 신장 기능, 염증촉진