Original Article
Changes in inflammatory factors in SV40MES13 mesangial cells after silencing ApoM gene


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Abstract: Objective: Inflammation is an important process in the occurrence and development of nephropathy, and ApoM is closely related to inflammation. This article aims to investigate the inflammatory changes of SV40 MES13 cells after ApoM gene silencing by western blot and to explore the relationship between ApoM and inflammation. Methods: Control group glomerular mesangial cells (SV40 MES13), and the same cells after adding a small interfering RNA silencing ApoM gene for 24 h were observed under a microscope and photographed. After extracting the protein western blot was used to explore the associated inflammation of IL-6, P-Jak2, Erk, TNF-α, P-JNK, IKKβ, P-p38, IκBα, P-IKKα/β, NF-κB and P-NF-κB expression. Results: Western blot showed that ApoM gene was successfully silenced in SV40 MES13 cells after adding small interfering RNA. The decrease of inflammatory factors IL-6 and P-Jak2 in Jak/Stat pathway was statistically significant. Inflammatory cytokines TNF-α and P-JNK in the NF-κB pathway decreased statistically significantly, while the inflammatory factor IKKβ increased statistically significantly. Conclusion: Inflammation was suppressed in SV40 cells with ApoM gene silencing.

Keywords: Apolipoprotein, inflammation, kidney disease

Introduction

The incidence of chronic kidney disease (CKD) has been increasing year by year and is an important risk factor that endangers public health [1]. Inflammation is an important pathologic change in pathogenesis of kidney disease. The main clinical manifestations of nephritis are fatigue, abnormal renal function, hematuria, and proteinuria. Inflammation plays an important role in renal insufficiency and kidney fibrosis. The human ApoM gene is located on chromosome 6 p21. 3, the histocompatibility complex III region, immediately adjacent to the gene encoding tumor necrosis factor (TNF). This suggests that it may be closely related to the inflammatory response [2]. Recent studies have shown that platelet activating factor, tumor necrosis factor alpha, interleukin-1 alpha, transforming growth factor-alpha/beta, epidermal growth factor, hepatocyte nuclear factor-1 alpha, leptin, and insulin can regulate ApoM gene expression [3].

Apolipoprotein M (ApoM) is a protein isolated from triglyceride rich lipoproteins (TGRLP) by Xu and Dahlback in 1999 and has a unique N-terminal amino acid sequence [4]. The protein has a molecular weight of 26 kD and consists of 188 amino acids [5]. It is mainly found in plasma high-density lipoprotein (HDL), and a small part of it is present in TGRLP and low-density lipoprotein (LDL). ApoM expression is highly specific, mainly distributed in the liver and kidneys, especially liver cells and renal tubular epithelial cells, and is also low in embryos, stomach, skeletal muscle cells, small intestine, heart, brain, spleen, and testis [6]. SV40 cells are mouse mesangial cells. In this study, We will observe the effect of ApoM on the inflammatory signaling pathway of SV40 cells and explore its potential relevance in renal inflammatory diseases.
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Materials and methods

Materials

Mesangial cells (SV40 MES 13) were purchased from Shanghai Cellular Bank of Chinese Academy of Sciences. The basal medium was purchased from Biological Industries (BI). Fetal bovine serum was purchased from Shanghai Shuangye Biotechnology Co., Ltd. SDS-PAGE protein loading buffer was purchased from Guangzhou Biosharp. Protein Ladder (10-170 kU) was purchased from Piere, polyvinylidene fluoride (PVDF) membrane was purchased from Bio-RAD, and chemiluminescence kit was purchased from Thermo Fisher. ApoM antibody, actin mouse monoclonal antibody was purchased from Sigma; P-Jak-2 (Thy1007/1008) antibody, Erk antibody, IL-6 antibody, P-JNK (Thr183/Tyr185) antibody, NF-κB antibody, P-NF-κB antibody was purchased from CST (Cell Signaling), TNFα antibody was purchased from ABGENT, IKKβ antibody, P-p38 antibody, and IκBα antibody was purchased from Proteintech. Digestion of pancreatic cells, radioimmunoprecipitation assay (RIPA), Benzincnyl fluoride (PMSF) bicinchonininc acid (BCA) protein concentration assay kit, Horseradish peroxidase (HRP) labeling of the goat anti-rabbit IgG (H+L) and goat anti-mouse IgG (H+L) antibodies were purchased from Shanghai Biyunian Biotechnology Co., Ltd. All other reagents were of domestic analytical grade.

Methods

Design of Small Interfering RNA: The ApoM gene sequence was first obtained from the mouse gene bank and submitted to the Ribobio company (Guangzhou, China) for design (siRNA sequence: GCCTTC TCTTTAACTCCT).

Silencing of the ApoM gene with small interfering RNA: SV40 MES 13 cells were taken from the -80°C refrigerator for cell resuscitation. Cell density was observed after several days of medium exchange. Cells were passaged while waiting for appropriate cell density. Cells were passaged from a dish to two dishes. The appropriate number of cells was present in one dish of cells to add small interfering RNA, and another dish as a control. After 24 hours, the cells were observed under a microscope and photographed. After the photographing was completed, the cellular proteins were extracted from the two dishes.

To identify whether ApoM gene is silent: We extracted the control group and the SV40 MES 13 cells to which was added small interfering RNA, and detected the expression of ApoM gene by using western blot to identify whether the ApoM protein was silent.

Western Blot Detection of Related Inflammatory Factors. Western Blot Detection by Related Antibodies: (1) SV40 MES 13 Cell Protein Treatment. The SV40 MES 13 cells of the control group and the SV40 MES 13 cells added with the small interfering RNA group were respectively taken, and the RIPA and PMSF were added in strict accordance with the instructions of the RIPA kit operation method. The cells were fully lysed by an ultrasonic cell crusher to extract the proteins, and the protein concentration was determined using BCA. After the kit was used to measure the protein concentration, the SDS-PAGE protein loading buffer and protein samples were mixed at a ratio of 1:4. After being bathed in a metal bath at 100°C for 10 minutes, the protein was cooled to room temperature and used as a backup. The remaining protein samples were stored at -80°C until use. (2) Configuring SDS-PAGE gel with glue material ultrapure water, acrylamide, 1.5 M Tris (pH 8.8), 0.5 M Tris (pH 6.8), 10% SDS, 10% AP and TEMED. We prepared 15% or 12% or 10% or 8% of the separation gel and 5% of the concentration gel according to the gluing scheme, and condensed the gel on top. (3) Detection of related antibodies by SDS-PAGE was done using 80 V electrophoresis concentration gel, and 110 V electrophoresis separation gel. (4) After electrophoresis, there was a 100 V 70MIN transfer transfer of the protein on SDS-PAGE gel to PVDF membrane, it was sealed with 5% skimmed milk powder, the relevant antibody stock solution was diluted in proper proportion according to instructions, and incubated at 4°C overnight. The HRP-labeled goat anti-rabbit IgG or goat anti-mouse IgG diluted 1:5000 was used as the secondary antibody to wash the membrane after 90 min incubation. The luminescence solution and PVDF membrane were uniformly added. The western blot results were analyzed in a multifunction imager.

Statistical analysis

All statistics were expressed as mean ± standard deviation. The t-test was used to compare
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Results

Morphology of microscopic cells before and after the addition of small interfering RNA. In the control group, cells before siRNA were added, and the siRNA group of cells at 24 hours after adding siRNA are shown (Figure 1A and 1B).

After the addition of siRNA, we identify whether the ApoM protein is silent (Figure 2). The first three wells were control cellular proteins and the last three wells were cellular proteins added to the siRNA (Figure 2A). Observing the bands, it can be seen that the ApoM protein in the latter three wells was significantly reduced (Figure 2B), indicating that the siRNAs were successful in silencing the ApoM gene. The data are mean ± standard deviation of 3 independent measurements, n = 3, *P < 0.05. Thus, it was found that the NF-κB pathway of SV40 MES13 cells was inhibited and inflammation was weakened when apoM was subjected to the small interfering RNA, the Jak/Stat pathway of SV40 MES13 cells was suppressed and inflammation was weakened.

Western blot was used to detect relevant inflammatory factors of the NF-κB pathway. Observing the bands, we found that the expression of tumor necrosis factor-α (TNF-α) was decreased while the expression of IKKβ was increased. There was no trend in the expression of IκBα, P-IKKα/β, NF-κB, and P-NF-κB (Figure 4A). Figure 4B is a statistical graph of the result of Figure 4A. The data are mean ± standard deviation of 3 independent measurements, n = 3, *P < 0.05. Thus, it was found that the NF-κB pathway of SV40 MES13 cells was inhibited and inflammation was weakened when apoM gene was subjected to small interfering RNA.

After the addition of small interfering RNA, the Jak/Stat pathway of SV40 MES13 cells was suppressed and inflammation was weakened.

Extraction of proteins from the control group SV40 MES13 cells and SV40 MES13 cells with siRNA. Western blot was used to detect relevant inflammatory factors of the Jak/Stat pathway. Observing the band results, the expression of interleukin-6 (IL-6) and P-Jak2 (Tyr1007/1008) was reduced (Figure 3A). Figure 3B is a statistical graph of the result of Figure 3A. The data are mean ± standard deviation of 3 independent measurements, n = 3, *P < 0.05. Thus, it was found that where apoM was subjected to the small interfering RNA, the Jak/Stat pathway of SV40 MES13 cells was suppressed and inflammation was weakened.

Discussion

Inflammation is the main pathologic change and mechanism in the development of kidney disease. Apolipoprotein M is closely related to inflammation. In this paper, the expression of inflammatory cytokines in the silenced apoM gene in SV40 MES13 cells was studied by western blot. The decrease in inflammatory cytokines IL-6, P-Jak2, TNF-α, and P-JNK was statistically significant, and the increase in inflammatory factor IKKβ was statistically significant. In this experiment, when the ApoM gene was inhibited, the inflammation of the SV40 cells was weakened. Whether this condition will
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Figure 2. The protein level of ApoM expression before and after the addition of small interfering RNA. (A) shows the results of the ApoM protein detected by the western blot. The first three groups are the control group and the latter three groups are cells that have undergone siRNA; (B) is the statistical data of (A) as a graph. Data are mean ± standard deviation of 3 independent measurements, n = 3, *P < 0.05.

Figure 3. Changes in inflammatory factors related to the Jak/Stat pathway. (A) shows the western blot results and P-JAK2 in the inflammatory pathway. The first three groups are the control group and the latter three groups are the cells with small molecule interfering RNAs; (B) is the statistical graph of (A). The data are mean ± standard deviation of 3 independent measurements, n = 3, *P < 0.05.

Figure 4. Changes in NF-κB pathway-related inflammation. (A) shows the western blot results of TNF-α, IKKβ, IκBα, NF-κB, and P-NF-κB in the inflammatory pathway. The first three groups are the control group and the latter three groups are the small molecule interfering RNAs; (B) is the statistical data from (A) as a chart. The data are mean ± standard deviation of 3 independent measurements, n = 3, *P < 0.05.
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The mechanism of weakened inflammation in SV40 MES13 cells after ApoM gene silencing may be related to its location on chromosome 6 p21. 3. The histocompatibility complex III region is closely related to the gene encoding tumor necrosis factor (TNF). S1P is the ligand of ApoM and ApoM is the carrier of S1P. The apolipoprotein M-sphingosine-1-phosphate (apoM-S1P) axis is a new signaling axis proposed by Arkensteijn et al. in 2013 and is involved in the regulation of the 1-phospho A series of biologic functions downstream of the S1 Preceptor (S1PR) signaling pathway, such as lipid metabolism, inflammatory response, and coagulation function [7]. ApoM may carry S1P binding to its receptor and regulate S1P-related signaling pathways, thus playing an important role in inflammation-related diseases [8].

Conclusions

We silenced the ApoM gene in SV40 MES13 cells by means of using siRNA, then we used western blot to evaluate inflammatory factors in those cells. Our data indicate that inflammatory factors in those SV40 MES13 cells in which ApoM is silenced are suppressed contrast with the control cells. The study is the first to explore a significant relationship between Apolipoprotein M and nephritis. The relationship is important for us to understand Apolipoprotein M function. It also adds a new way to think about how to cure nephritis and how to prevent kidney disease. We hope the study ultimately can be helpful for clinicians to treat nephritis and reduce the burden of kidney disease.

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Disclosure of conflict of interest

None.

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