대한내과학회지: 제 92 권 제 1 호 2017 https://doi.org/10.3904/kjm.2017.92.1.53

신장에서 노화에 따른 Sirtuin1-NFE2-related Factor 2 Signaling의 변화

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Age-related Changes in the Sirtuin1-NFE2-related Factor 2 Signaling System in the Kidney

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Background/Aims: Renal aging-related changes are characterized by oxidative stress. SIRT1 regulates cellular conditions by activating Nrf2. The present study investigated the processes of renal changes by antioxidant enzymes and the relationship between SIRT1 and Nrf2.

Methods: We used male 2-, 12-, and 24-month-old C57BL/6 mice. We measured renal function, histological changes, oxidative stress, and expression of SIRT1–Nrf2 signaling in the kidneys.

Results: 24-month-old mice exhibited increased albuminuria and serum creatinine. Creatinine clearance was decreased in 24-month-old mice compared with 12-month-old mice. There were increases in mesangial volume and tubulointerstitial fibrosis in 24-month-old mice. Moreover, oxidative stress marker, 3-Nitrotyrosine, expression and apoptosis were increased in 24-month-old mice. The 24 h urinary 8-isoprostane and 8-hydroxy-deoxyguanosine excretion increased with aging. The levels of expression of SIRT1 and nuclear Nrf2 were decreased in 24-month-old mice. The antioxidant enzymes HO-1 and NQO-1 were down-regulated in 24-month-old mice. Another antioxidant enzyme, SOD2, was decreased in 24-month-old mice.

Conclusions: Our results demonstrated that SIRT1 was down-regulated with aging, and this may be related to changes in the expression of target molecules including Nrf2. As a result, oxidative stress was induced. The pharmacological targeting of these signaling molecules may reduce the pathological changes associated with aging in the kidney. (Korean J Med 2017;92:53-61)

Keywords: Aging; Kidney; NF-E2-Related Factor 2; Oxidative stress; Sirtuin 1

Received: 2016. 7. 8
Revised: 2016. 9. 2
Accepted: 2016. 9. 7

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INTRODUCTION

Aging involves accumulation of changes in most species over time [1]; it refers to a multidimensional process of physiologic functions and metabolic processes [2]. Exposure to various cellular conditions or stresses increases damage to homeostatic mechanisms, leading to increased susceptibility to environmental stresses, with increasing rates of morbidity and mortality by diseases [2,3].

Many theories regarding primarily disease-independent renal aging, categorized as evolutionary, molecular, cellular, and systemic changes, have been proposed previously as a result of recent studies [3]. Renal aging-related changes are characterized by oxidative stress, inflammation, glomerular basement membrane thickening and wrinkling, associated with the loss of capillary loops, as well as mesangial matrix expansion, tubulointerstitial fibrosis, renal cell death, and renal dysfunction [4-7].

The aging process is associated with the inactivation of the 'Sirtuin1 (SIRT1)' protein and the activation of oxidative stress [8]. The SIRT1-activated transcription factor 'NF-E2-related factor 2 (Nrf2)' regulates inflammation, senescence, and reactive oxygen species (ROS) [9]. Nrf2 regulates the intracellular redox balance and the cellular anti-oxidant and antiinflammatory machineries. These play important roles in the defense against oxidative stress [10-12]. Nrf2 binds to 'kelch-like ECH-associated protein 1 (Keap1)' in the cytoplasm [8]. Under normal conditions, Keap1 degrades Nrf2 via proteasome. However, upon exposure of cells to oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, to bind to anti-oxidant-responsive elements in genes encoding anti-oxidant enzymes, such as nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase (NQO1), heme oxygenase-1 (HO-1), superoxide dismutase (SOD) 1, and SOD2 [8,9,13]. This activation of Nrf2-dependent anti-oxidants promotes anti-oxidative stress and anti-inflammatory functions [13]. Diabetes and metabolic syndrome are considered to contribute to the same types of organ damage as those caused by the aging process, especially in the kidneys.

In the present study, we investigated the processes of renal changes by assessing the relationship between SIRT1 and Nrf2 during the aging process in mice. We focused on the Nrf2-mediated regulation of the anti-oxidant enzymes HO-1, NQO-1, SOD1, and SOD2.

MATERIALS AND METHODS

Animal model

Old C57BL/6 mice were purchased from the Korea Research Institute of Bioscience and Biotechnology (Chungcheongbuk-do, Korea). The Animal Care Committee of The Catholic University approved the experimental protocol, and the experiments were performed in accordance with our institution's animal care guidelines.

Mice were housed in an environment with controlled temperature and light (12/12-h light/dark cycle). Mice were divided into three groups: a 2-month-old group (2 M group, n = 6), a 12month-old group (12 M group, n = 6), and a 24-month-old group (24 M group, n = 6).

Renal function

Mice were placed in individual mouse metabolic cages (Tecniplast, Gazzada, Italy) with access to water and food for 24 hours (h), to collect urine. Albuminuria (Albuwell M, Exocell; Philadelphia, PA, USA) and urine creatinine (Creatinine Companion, Exocell) were measured using ELISA kits. Serum creatinine concentrations were measured using i-STAT system cartridges (CHEM8+, Abbott Point of Care, IL, USA). Creatinine clearance was calculated using a standard formula: urine creatinine (mg/dL) × urine volume (mL/24 h) / serum creatinine (mg/dL) × 1,440 (min/24 h).

Histology

Kidney samples were fixed in 10% formalin. The tissues were embedded in low-temperature melting paraffin wax, and 4 μm sections were processed and stained with hematoxylin and eosin, periodic acid-Schiff (PAS), and Masson's trichrome staining. The glomerular volume and mesangial area were determined by examining PAS-stained sections. The relative mesangial area was expressed as the mesangial/glomerular surface area. Finding of tubulointerstitial fibrosis was defined as matrix-rich ex-

pansion of the interstitium of the tubular basement membrane. To assess oxidative stress, we performed immunohistochemistry (3-nitrotyrosine, Santa Cruz Biotechnology Inc., Dallas, TX, USA). Sections were treated with an antigen-unmasking solution consisting of 10 mM sodium citrate, pH 6.0, and were then washed with phosphate-buffered saline. Sections were incubated with 3% H_2O_2 in methanol to block endogenous peroxidase activity. Non-specific binding was blocked with 10% normal horse serum. After incubation with the primary antibody (3-nitrotyrosine 1:200) at 4°C overnight, antibodies were visualized with a peroxidase-conjugated secondary antibody using the Vector Impress kit (Vector Laboratories, Burlingame, CA, USA). Apoptosis was quantified using an *in situ* detection terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay kit (Chemicon-Millipore, Billerica, MA, USA). All of the sections were examined in a blinded manner using a color-image analyzer (TDI Scope Eye, ver. 3.5 for Windows; Olympus, Tokyo, Japan) and results were quantified using ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA).

Western blot analysis

Total proteins from renal cortical tissues were prepared using a Pro-Prep Protein Extraction Solution (Intron Biotechnology, Gyeonggi, Korea) according to the manufacturer's protocol. Nuclear proteins were extracted using the NE-PER nuclear and cytoplasmic extraction kit (Thermo Fisher Scientific, Rockford, IL, USA). Western blot analysis was performed using the following antibodies: SIRT1 (1:2,000; Cell Signaling Technology Inc., Danvers, MA, USA), Nrf2 (1:1,000; Santa Cruz Biotechnology Inc., Dallas, TX, USA), Keap1 (1:1,000; Santa Cruz Biotechnology Inc.), Lamin B1 (1:1,000; Cell Signaling Technology Inc.), HO-1 (1:1,000: Cell Signaling Technology Inc.), NQO-1 (1:500; Santa Cruz Biotechnology Inc.), SOD1 (1:10,000; Enzo Life Sciences, New York, NY, USA), SOD2 (1:20,000; Abcam, Cambridge, UK), cytochrome c oxidase I (1:400; Santa Cruz Biotechnology) and IV (1:2,500; Santa Cruz Biotechnology), and β-actin (1:10,000; Sigma, St. Louis, MO, USA) antibodies.

Assessment of renal oxidative stress

To evaluate oxidative stress, we measured 24-h urinary 8-epi-prostaglandin F2α (isoprostane; OXIS Health Products, Portland, OR, USA) and 24 h urinary 8-hydroxy-deoxyguanosine (8-OH-dG; OXIS Health Products) concentrations.

Statistical analyses

Data were expressed as means ± standard error. The statistical significance of differences between the groups was examined using ANOVA with the Bonferroni correction (SPSS ver. 19.0; IBM Inc., Armonk, NY, USA). A p-value < 0.05 was considered to indicate statistical significance.

RESULTS

Renal function

Our results showed that albuminuria in the 24 M group was higher than in the 2 M and 12 M groups (13.4 ± 0.5 vs. 31.8 ± 1.01 vs. 67.1 ± 3.9, respectively; Fig. 1A). Moreover, the 24 M group had a higher serum creatinine level than the other groups (0.2 ± 0.007 vs. 0.28 ± 0.01 vs. 0.72 ± 0.04, respectively; Fig. 1B), and creatinine clearance was decreased in the 24 M group versus the 12 M group (0.12 ± 0.003 vs. 0.35 ± 0.03 vs. 0.18 ± 0.01, respectively; Fig. 1C).

Histology

Histological examinations showed that the mesangial area was increased in the 24 M group compared with the 2 M and 12 M groups (10.7 ± 2.1% vs. 16.8 ± 2.2% vs. 45.7 ± 2.73%, respectively; Fig. 1D). There was also a significant increase in tubulointerstitial fibrosis in the 24 M group versus the other groups (1.02 ± 0.17% vs. 1.13 ± 0.14% vs. 17.4 ± 2.1%, respectively; Fig. 1E).

Immunochemistry for 3−nitrotyrosine and TUNEL assay

Oxidative stress in the 24 M group, as indicated by the presence of 3-nitrotyrosine-positive signals, was compared with that in the 2 M and 12 M groups. Oxidative stress was increased in the 24 M group (0.04 ± 0.02% vs. 0.26 ± 0.1% vs. 1.05 ±

0.49% respectively; Fig. 2A). We observed a difference in the number of TUNEL-positive cells in the kidneys of animals in the 2 M, 12 M, and 24 M groups; the number of TUNEL-positive cells in the glomeruli (0.27 ± 0.07% vs. 0.53 ± 0.1% vs. 2.7 ± 0.3%, respectively; Fig. 2B) and cortical tubular areas (0.27 ± 0.07% vs. 0.53 ± 0.1% vs. 2.8 ± 0.33%, respectively; Fig. 2C) were increased significantly in the 24 M group versus the other groups. These results demonstrated that aging was associated with increased mesangial expansion and tubulointerstitial fibrosis, which could be related to inflammation and apoptosis.

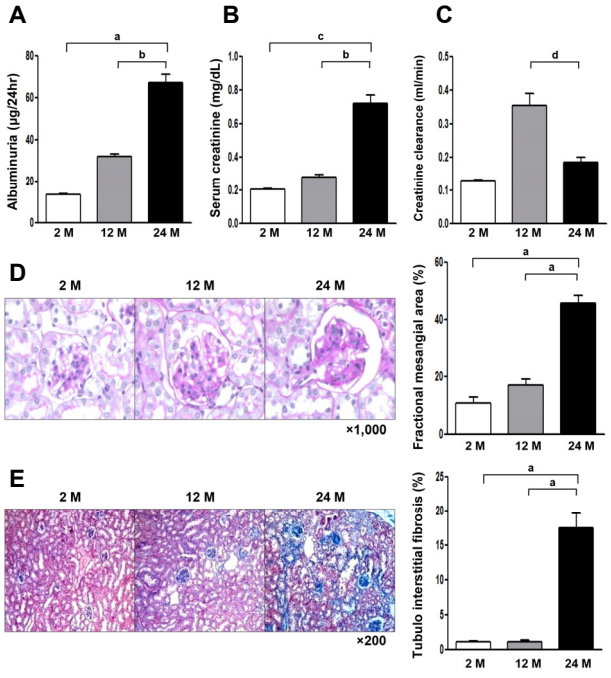

Figure 1. Changes in renal function and histology in aged mice. The 24 M group showed higher 24 h albuminuria than the other groups (A). Serum creatinine was increased in the 24 M group versus the 2 M and 12 M groups (B). Creatinine clearance was increased in the 12 M group compared with the other groups, and was decreased in the 24 M group compared with the 12M group (C). Representative images of PAS (D, ×1000) and trichrome (E, ×200) staining of renal tissues, as indicated. There was an increase in the fractional mesangial area in the 24 M group compared with other groups and a significant increase in the tubulointerstitial fibrosis area in the 24 M group compared with the other groups. Values are reported as means ± standard error. $^{a}p <$ 0.0001 vs. 2 M. $^{b}p < 0.01$ vs. 12 M. $^{c}p < 0.001$ vs. 2 M. $^{d}p < 0.05$ vs. 12 M. M, months.

24h urinary levels of 8-Epi-PGF2α and 8-Hydroxy-deoxyguanosine

The 24 h urinary concentration of 8-isoprostane, a product of lipid peroxidation and albuminuria, increased with aging (7.8 ± 0.29% vs. 17.3 ± 0.59% vs. 22 ± 0.91%, respectively; Fig. 3A). Moreover, we determined the levels of 8-hydroxy-deoxyguanosine, to assess oxidative damage to DNA in the three groups. 8-Hydroxy-deoxyguanosine levels were increased markedly in the 24 M group compared with the 2 M group (36.9 ± 2.91% vs. 63.1 ± 6.58% vs. 86.5 ± 9.27%, respectively). They were also increased versus the 12 M group, although this was not statistically significant (Fig. 3B). These findings indicate that oxidative damage increased in renal cells of aged mice.

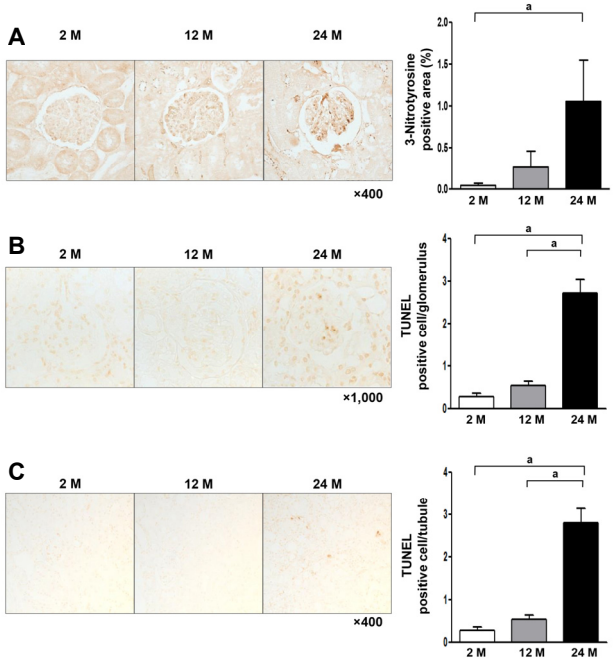

Figure 2. Immunohistochemistry for 3-Nitrotyrosine and TUNEL assay of apoptotic cells. Results of immunohistochemical staining for 3-nitrotyrosine and quantitative analyses in the three groups (A, ×400). There was a significant increase in the 3-nitrotyrosine-positive area in the 24 M group compared with the other groups. Representative images of TUNEL-positive mesangial cells in the glomeruli (B, ×1000) and cortical tubular (C, ×400) areas of animals from all groups. Values are reported as means ± standard error. $^{a}p < 0.0001$ vs. 2 M, 12 M. M, months; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

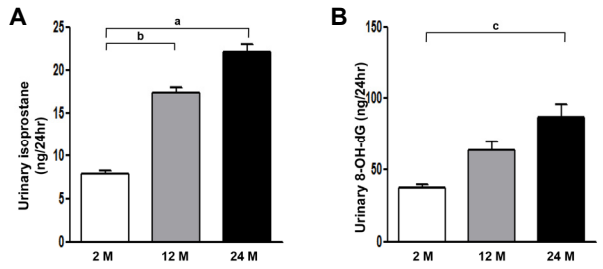

Figure 3. Increase in the intrarenal 24h urinary 8-epi-prostaglandin F2α (isoprostane) and 24h urinary 8-hydroxy-deoxyguanosine with aging. Quantitative analyses of 24-h urinary isoprostane showed that it increased in the 24 M group with aging (A). Moreover, the 24 h concentration of urinary 8-hydroxy-deoxyguanosine increased in the 24 M group versus the 2 M and 12 M groups (B). Values are reported as means ± standard error. $^{a}p < 0.0001$ vs. 2 M. $^{b}p < 0.05$ vs. 2 M. $^{c}p < 0.05$ vs. 2 M. M, months.

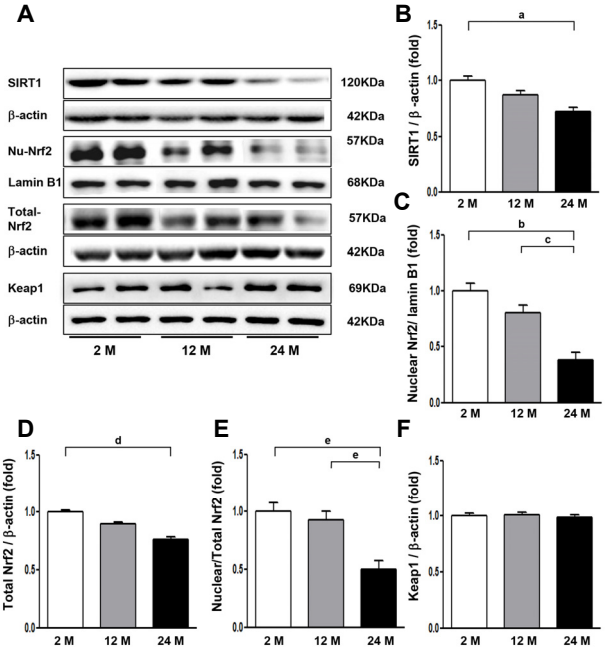

Figure 4. Levels of SIRT1 and Nrf2 signaling. Protein lysates (30 μg) from whole kidneys were separated by SDS-PAGE and analyzed by Western blotting. Representative blots from the kidney are shown (A). SIRT1 levels (B) and Nrf2 expression in nuclear (C) and total protein (D) were decreased in the 24M group compared with the other groups. Consequently, the nucleus:total protein ratio of Nrf2 (E) was decreased in the 24M group versus the 2M and 12M groups. Keap1 protein expression among the three groups. Quantitative analyses of the results are shown. $^{a}p < 0.05$ vs. 2 M. $^{b}p < 0.0001$ vs. 2 M. $^{c}p < 0.001$ vs. 12 M. $^{d}p < 0.01$ vs. 2 M. $^{e}p < 0.01$ vs. 2 M, 12 M. SIRT1, silent information regulator T1; Nrf2, NFE2-related factor 2; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; M, months.

Expression of SIRT and Nrf2 signaling

SIRT1 activation can be influenced by several cellular conditions, such as oxidative stress. Western blot analysis showed that SIRT1 protein levels were decreased markedly in the 24 M group compared with the 2 M group (Fig. 4A and 4B). The activation of Nrf2 expression can be influenced by the expression of anti-oxidant proteins in the nucleus. The relative content of the Nrf2 protein was measured in the nucleus and in total proteins by Western blot analyses. Nrf2 protein levels in the nucleus were decreased markedly in the 24 M group compared with the 2M and 12M groups (Fig. 4A and 4C). Total Nrf2 protein expression was decreased in the 24 M group compared with the 2 M group (Fig. 4A and 4D). Thus, the Nrf2 nuclear: total protein ratio was decreased in the 24 M group, compared with the 2 M and 12 M groups, by the aging process (Fig. 4A and 4E). There was no difference in total Keap1 protein levels among the three groups (Fig. 4A and 4F).

Levels of anti–oxidant proteins

We used Western blot analysis to examine changes in the levels of the anti-oxidant proteins HO-1, NQO-1, SOD1, and SOD2. The level of HO-1 was decreased in the 24 M group compared with the 2 M and 12 M groups (Fig. 5A and 5B). Moreover, NQO-1 expression was decreased in the 24 M group versus the 2 M and 12M groups (Fig. 5A and 5C). The level of SOD1 was decreased in the 24 M group, although this was not statistically significant (Fig. 5A and 5D). SOD2 protein levels were decreased in the 24 M group compared with the 2M and 12M groups (Fig. 5A and 5E).

Levels of cytochrome C oxidase I and cytochrome C oxidase IV

Cytochrome C oxidase, also known as complex IV, is an enzyme in the electron transport chain of the mitochondria. Levels of cytochrome c oxidase I were decreased in the 24 M group compared with the other groups. The cytochrome c oxidase IV protein level showed no significant difference (Fig. 6A). Thus, the cytochrome c oxidase I / cytochrome c oxidase IV ratio decreased significantly in the 24 M group versus the 2 M and 12 M groups (Fig. 6B).

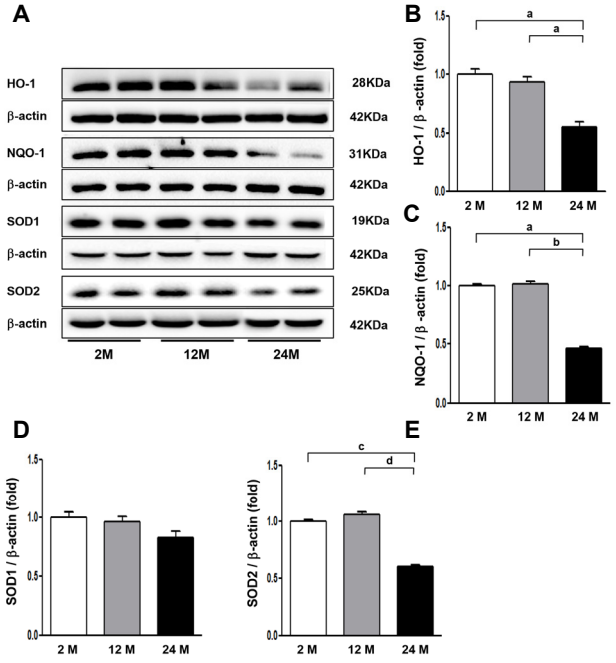

Figure 5. Levels of Anti-oxidant proteins. Protein lysates (30 μg) from whole kidneys were separated by SDS-PAGE and analyzed by Western blotting. Representative Western blot analyses of the levels of HO-1, NQO-1, SOD1, and SOD2 in total protein extracts (A). Our results showed that HO-1 (B) and NQO-1 (C) protein levels were decreased in the 24 M group compared with the other groups. The level of SOD1 was decreased in the 24 M group, although this was not statistically significant (D). The SOD2 protein level was decreased in the 24 M group compared with the other groups (F). Quantitative analyses of the results are shown. $^{a}p < 0.0001$ vs. 2 M, 12 M. $^{b}p < 0.01$ vs. 24 M. $^{c}p < 0.05$ vs. 2 M. $^{d}p < 0.01$ vs. 12 M. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; HO-1, heme oxygenase-1; NQO-1, NADPH quinone oxidoreductase; SOD, superoxide dismutase; M, months.

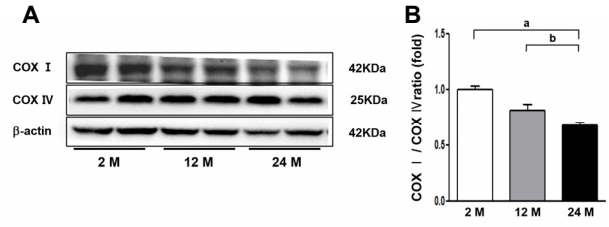

Figure 6. Expression of cytochrome C oxidase I and cytochrome C oxidase IV. Protein lysates (30 μg) from whole kidney were separated by SDS-PAGE and analyzed by Western blotting. Representative Western blot analysis of cytochrome C oxidase I and cytochrome C oxidase IV levels (A). The cytochrome C oxidase I / cytochrome C oxidase IV ratio was significantly decreased in the 24 M group compared with the 2 M and 12 M groups (B). Quantitative analyses of the results are shown. $^{a}p < 0.001$ vs. 2 M. $^{b}p < 0.01$ vs. 12 M. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; M, months.

DISCUSSION

Aging is a multifactorial process, characterized by progressive decline in physiological function. In this study, we investigated aging-related physiologic and pathologic changes in the kidney. Creatinine clearance decreased and albuminuria increased with aging. There were also increases in mesangial volume, tubulointerstitial fibrosis, oxidative stress (3-nitrotyrosine-positive area), and apoptosis. The rate of pathological changes with aging may vary depending on the levels of angiotensin II, nitric oxide (NO), transforming growth factor-β, advanced glycosylation end product, oxidative stress, and lipid levels [14]. In this study, the aging process in the kidney occurred via oxidative stress, mediated via the SIRT1/Nrf2 signaling system.

Aging is associated with progressive functional deterioration of, and structural changes in, the kidney. The glomerular filtration rate declines by about 1 mL/min per year; moreover, the percentage of glomerulosclerosis and tubulointerstitial fibrosis increases and hyalinization of afferent arterioles may develop with aging. Changes in the activity or responsiveness of hormonal systems also occur with aging, leading to alterations in homeostatic mechanisms in the elderly [15-17]. In this study, we demonstrated the presence of decreased renal function and increased proteinuria and tubulointerstitial fibrosis with aging. Tissue injury in aging can be caused by free radical production and/or anti-oxidant enzyme deficiency, with subsequent lipid peroxidation and oxidative stress. We demonstrated that the inactivation of Nrf2 decreased its translocation into the nucleus, where it binds to anti-oxidant-responsive elements in genes encoding anti-oxidant enzymes, such as NQO1, HO-1, SOD1, and SOD2.

We showed previously, and in this study, that the aging-induced suppression of SIRT1 signaling may increase oxidative stress in cooperation with adenosine monophosphate-activated protein kinase (AMPK) [6]. This signaling may also affect ca-

tabolism, mitochondrial function, angiogenesis, inflammation, and insulin resistance [18]. The hypothetical Sirt1 and AMPK cycles regulate each other and share many common target molecules, such as peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α, PPARs, forkhead box proteins, and NF-κB [18]. It has been suggested that the activation of AMPK and SIRT1 allows the concurrent deacetylation and phosphorylation of their target molecules and decreases susceptibility to aging. Among the seven mammalian sirtuins, sirtuin 1 (Sirt1) and sirtuin 3 (Sirt3) are considered anti-aging molecules that are expressed in the kidney [19]. It has been shown that Sirt1 activation protects the mouse renal medulla from oxidative injury and has anti-apoptotic and anti-fibrotic effects in obstructed mouse kidneys [20].

In this study, we also showed that Sirt1 may influence the activation of Nrf2. A previous study showed that SIRT1 can deacetylate Nrf2, which is a master regulator of the cellular redox state, thereby decreasing Nrf2 transcriptional activity and nucleocytoplasmic localization in a human cancer cell line [21]. The oxidative stress theory of aging postulates that oxidative damage to biological macromolecules is a key driver of aging and that, conversely, mechanisms that delay the accumulation of oxidation products in the cells and tissues of an organism can promote longevity [22]. Consistent with this and the established role of Nrf2 and of its invertebrate homologs as master regulators of anti-oxidant gene expression, the results of several studies support a function for the Nrf2 pathway in the regulation of life-span [9]. A role for Nrf2 in renoprotection has also been suggested by various studies. In a model of ischemia-reperfusion injury, renal function, vascular permeability, and survival of Nrf2-knockout mice were significantly worse than those of wild-type mice [23]. Renal damage and interstitial fibrosis by cyclosporin A treatment were also higher in Nrf2-knockout mice [24]. The streptozotocin-induced diabetic nephropathy model showed that Nrf2-null mice developed severe renal injury, with greater oxidative DNA damage, than wild-type mice [25]. Accordingly, several studies showed favorable effects of Nrf2 inducers. Pharmacological intervention using Nrf2 activators showed protective effects against injuries from oxidative stress and inflammation in various *in vitro* and *in vivo* experimental models [26]. Treatment with resveratrol (Nrf2 activator) also induced Sirt1 overexpression and suppressed AT1 receptor expression in cultured smooth muscle cells; moreover, resveratrol improved angiotensin II-induced hypertension in mice [27]. Thus, it seems likely that the activation of the SIRT1/Nrf2 signaling system ameliorates the changes observed in aging kidneys via downregulating oxidative-stress-mediated cellular senescence [8].

The aging process in the kidney is a complex process, caused by various molecular pathways and biochemical events. Of them, angiotensin II plays a key role in the aging process. Angiotensin II promotes inflammation, cell growth, and ROS generation at cellular and mitochondrial levels and promotes the progression of cell senescence [17]. Systemic and intrarenal activation of the renin-angiotensin system (RAS) induces increasing tissue and mitochondrial oxidative stress and, furthermore, end-stage organ injury associated with aging [28]. Changes in RAS predispose the elderly to various fluid and electrolyte imbalances, as well as to acute kidney injury and chronic kidney disease. Among the multiple pathways that are involved in renal aging, RAS plays a key role in this process [17]. In the liver of enalapril- and losartan-treated rats, the expression of the *Nrf2* gene remained similar to that detected in the young-age group, and was lower than that observed in the old-age group. This suggests that the upregulation of Nrf2 detected in old rats may correspond to an unsuccessful effort to compensate for both the failure to attain adequate Nrf1 and PGC-1α transcription levels and for the negative effects of angiotensin II on mitochondrial function [29]. We also demonstrated that Nrf2 expression was decreased with aging in the kidney, and that RAS may be related to the preservation of renal mitochondria via the Nrf2 signaling system.

In conclusion, our results demonstrated that the aging process is associated with deterioration of renal function and increased mesangial expansion and tubulointerstitial fibrosis, which may be accompanied by inflammation, apoptosis, and oxidative stress. SIRT1 is downregulated with aging and may regulate targets of the Nrf2 signaling system. Recently, studies have established the role of NRF2 signaling in renal protection against oxidative damage, and in the modulation of the inflammatory

response. The pharmacological targeting of Nrf2 signaling molecules that share antioxidant and anti-inflammatory efficacy may reduce the pathological changes of aging observed in the kidney.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (2011-0023126).

요 약

목적: 노화에 따른 변화는 산화스트레스가 주요 역할을 하며 SIRT1과 Nrf2가 관여하여 조절하는 것으로 알려져 있다. 이에 본 연구에서는 항산화 효소와 SIRT1-Nrf2의 관계에 의한 신장의 변화를 관찰하였다.

방법: 본 실험에서는 2, 12, 24개월의 C57BL/6 수컷 마우스를 이용하여 신장 기능, 조직학적 변화, 산화스트레스 및 SIRT1-Nrf2 신호전달 변화를 관찰하였다.

결과: 24개월군에서 알부민뇨가 증가되었으며, 크레아티닌 청소율은 12개월군에 비해 24개월군에서 감소되었다. 조직학적 변화에서 사구체 혈관 간세포질과 세뇨관 간질 섬유화가 24개월군에서 증가되었다. 또한, 24개월군에서 산화스트레스 지표인 3-Nitrotyrosine 면역조직화학 발현과 세포사멸이 증가되었다. 24시간 소변내 8-isoprostane과 8-hydroxy-deoxyguanosine 농도는 노화에 따라 증가하였다. 반면에 SIRT1과 핵내 Nrf2 단백질 발현은 24개월군에서 감소되었다. 항산화 효소 heme oxygenase-1과 NADPH quinone oxidoreductase 1의 발현이 24개월군에서 감소되었으며 또 다른 항산화 효소로 알려진 superoxide dismutase 2도 감소되었다.

결론: SIRT1의 발현이 노화에 따라 감소됨을 증명하였으며 이 결과는 Nrf2를 포함한 SIRT1의 하위분자들의 발현에 관여하여 산화스트레스를 유도하였다. 이러한 신호 전달체계의 약물학적 조절은 신장 노화와 관련된 변화를 감소시킬 수 있을 것이다.

중심 단어: 노화; 신장; Nrf2; 산화스트레스; Sirtuin 1

REFERENCES

1. Bowen RL, Atwood CS. Living and dying for sex. A theory of aging based on the modulation of cell cycle signaling by reproductive hormones. Gerontology 2004;50:265-290.
2. Li YN, Guo Y, Xi MM, et al. Saponins from Aralia taibaiensis attenuate D-galactose-induced aging in rats by activating FOXO3a and Nrf2 pathways. Oxid Med Cell Longev 2014;2014:320513.
3. O'Hare AM, Bertenthal D, Covinsky KE, et al. Mortality risk stratification in chronic kidney disease: one size for all ages? J Am Soc Nephrol 2006;17:846-853.
4. Lee HB, Yu MR, Yang Y, Jiang Z, Ha H. Reactive oxygen species-regulated signaling pathways in diabetic nephropathy. J Am Soc Nephrol 2003;14(8 Suppl 3):S241-S245.
5. Alpers CE, Hudkins KL. Mouse models of diabetic nephropathy. Curr Opin Nephrol Hypertens 2011;20:278-284.
6. Lim JH, Kim EN, Kim MY, et al. Age-associated molecular changes in the kidney in aged mice. Oxid Med Cell Longev 2012;2012:171383.
7. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci U S A 2004;101:15998-16003.
8. Yao H, Rahman I. Perspectives on translational and therapeutic aspects of SIRT1 in inflammaging and senescence. Biochem Pharmacol 2012;84:1332-1339.
9. Sykiotis GP, Habeos IG, Samuelson AV, Bohmann D. The role of the antioxidant and longevity-promoting Nrf2 pathway in metabolic regulation. Curr Opin Clin Nutr Metab Care 2011;14:41-48.
10. Xing X, Zhang C, Shao M, et al. Low-dose radiation activates Akt and Nrf2 in the kidney of diabetic mice: a potential mechanism to prevent diabetic nephropathy. Oxid Med Cell Longev 2012;2012:291087.
11. Li W, Khor TO, Xu C, et al. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. Biochem Pharmacol 2008;76:1485-1489.
12. Kim HJ, Vaziri ND. Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure. Am J Physiol Renal Physiol 2010;298:F662-F671.
13. McMahon M, Itoh K, Yamamoto M, Hayes JD. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. J Biol Chem 2003;278:21592-21600.
14. Taal MW, Chertow GM, Marsden PA, Skorecki K, Alan S, Brenner BM. Brenner and Rector's the Kidney. Amsterdam: Elsevier, 2011.
15. Neugarten J, Gallo G, Silbiger S, Kasiske B. Glomeruloscle-

rosis in aging humans is not influenced by gender. Am J Kidney Dis 1999;34:884-888.
16. Hill GS, Heudes D, Bariéty J. Morphometric study of arterioles and glomeruli in the aging kidney suggests focal loss of autoregulation. Kidney Int 2003;63:1027-1036.
17. Yoon HE, Choi BS. The renin-angiotensin system and aging in the kidney. Korean J Intern Med 2014;29:291-295.
18. Ruderman NB, Xu XJ, Nelson L, et al. AMPK and SIRT1: a long-standing partnership? Am J Physiol Endocrinol Metab 2010;298:E751-E760.
19. Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D. Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. Clin Sci (Lond) 2013;124:153-164.
20. He W, Wang Y, Zhang M-Z, et al. Sirt1 activation protects the mouse renal medulla from oxidative injury. J Clin Invest 2010;120:1056-1068.
21. Kawai Y, Garduño L, Theodore M, Yang J, Arinze IJ. Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. J Biol Chem 2011;286:7629-7640.
22. Harman D. Free radical theory of aging: an update: increasing the functional life span. Ann N Y Acad Sci 2006;1067:10-21.
23. Liu M, Grigoryev DN, Crow MT, et al. Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice. Kidney Int 2009;76:277-285.
24. Shin DH, Park HM, Jung KA, et al. The NRF2−heme oxygenase-1 system modulates cyclosporin A-induced epithelial−mesenchymal transition and renal fibrosis. Free Radic Biol Med 2010;48:1051-1063.
25. Jiang T, Huang Z, Lin Y, Zhang Z, Fang D, Zhang DD. The protective role of Nrf2 in streptozotocin-induced diabetic nephropathy. Diabetes 2010;59:850-860.
26. Choi BH, Kang KS, Kwak MK. Effect of redox modulating NRF2 activators on chronic kidney disease. Molecules 2014;19:12727-12759.
27. Miyazaki R, Ichiki T, Hashimoto T, et al. SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2008;28:1263-1269.
28. Kang SJ, You A, Kwak MK. Suppression of Nrf2 signaling by angiotensin II in murine renal epithelial cells. Arch Pharm Res 2011;34:829-836.
29. de Cavanagh EM, Flores I, Ferder M, Inserra F, Ferder L. Renin-angiotensin system inhibitors protect against age-related changes in rat liver mitochondrial DNA content and gene expression. Exp Gerontol 2008;43:919-928.