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Dietary restriction delays the secretion of senescence associated secretory phenotype by reducing DNA damage response in the process of renal aging

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ABSTRACT

Dietary restriction (DR) has multiple and essential effects in protecting against DNA damage in model organisms. Persistent DNA damage plays a central role in the process of aging. Senescence-associated secretory phenotype (SASP), as a product of cellular aging, can accelerate the process of cellular senescence as a feedback. In this study, we directly observed whether a DR of 30% for 6 months in aged rats could retard SASP by delaying the progression of DNA damage and also found the specific mechanism. The results revealed that a 30% DR could significantly improve renal pathology and some metabolic characteristics. The biomarkers and products of DNA damage were decreased in the process of renal aging on a 30% DR. A series of SASP, notably cytokine, chemokine, and growth factor, were obviously reduced by DR during renal aging. The phosphorylation levels of NF-κB and IκBα in aged kidneys of DR group were markedly reduced. These findings suggest that a 30% DR for 6 months can delay renal aging and reduce the accumulation of SASP by retarding the progression of DNA damage and decreasing the transcription activity of NF-κB, thus providing a target to delay renal aging.

1. Introduction

Dietary restriction (DR) refers to a reduction in food intake without suffering malnutrition. It has multiple and essential effects in protecting against DNA damage in model organisms. It can promote many beneficial health effects, such as a decrease in DNA damage/mutations; resistance to bacterial infection; reduced tumor incidence; extended insulin sensitivity; and protection against age-dependent cardiomyopathy, fatty liver disease, and renal lesions (Fontana et al., 2010; Hallam et al., 2015; Vermeij et al., 2016). At the same time, DR can extend the average and/or maximum life span of various organisms including yeast, flies, worms, fish, rodents, and rhesus monkeys (Fontana et al., 2010). Certainly, DR encompasses various forms, including the reduction of 20% to 50% of total calories, proteins, lipids, and even essential amino acids, either in the short-term or life-long (Xu et al., 2015). It can be continued, kept intermittent, or followed every other day (Xu et al., 2015).

Aging is a complex and progressive process, including loss of function, progressive damage, and increased vulnerability to diseases, leading ultimately to the end of life. As proved in previous studies, the mechanism of aging is closely associated with persistent DNA damage, which can halt the mechanism of tissue repair and then mobilize a series of age-related changes (Campisi, 2013). In the kidneys, DNA damage can lead to changes in both function and structure, including glomerular sclerosis, interstitial fibrosis, tubular atrophy, declined glomerular filtration rate, reduced erythropoietin production, and damaged urinary sodium excretion (Gekle, 2017; Wang et al., 2016). Cellular senescence may be an integral medium that mediates chronic kidney disease and some other age-related diseases, including unrecognized myocardial infarctions and stroke with declining glomerular filtration rate and increasing albuminuria (Masson et al., 2015; Rizk et al., 2012). Therefore, it is essential to take measures to retard the pathology of cellular senescence.

Recently, many research studies have highlighted the significance of DR in retarding renal senescence through multiple mechanisms. In our previous research, we proved that DR reduces the levels of autophagy, inflammation, oxidative stress, and apoptosis in the pathology of renal aging (Ning et al., 2013a; Ning et al., 2013b; Song et al., 2016; Wang et al., 2016). In the process of DNA replication, genomic stress, epigenomic modification, and some other types of stress leads to persistent DNA damage response, followed by the recruitment of γH2AX and 53BP1 to DNA damage sites (Wilson et al., 2016). The damage repair

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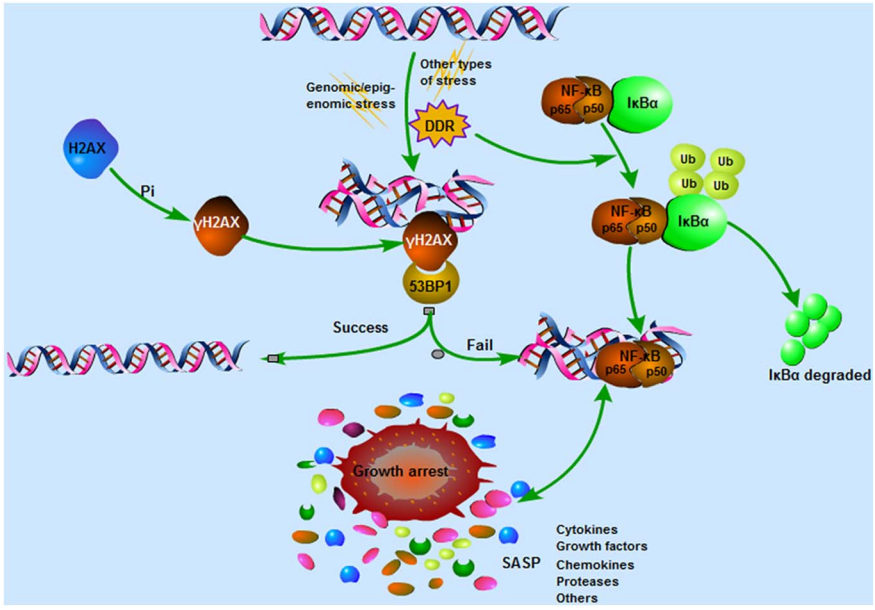

Fig. 1. The roles of DNA damage during cellular senescence in aged kidneys. The genomic/epigenomic stress and some other types of stress lead to the persistent DNA damage response (DDR). γH2AX and 53BP1 were recruited to DNA damage sites to active the damage repair mechanism. If the repair is successful, it will be restored to the ability of cell proliferation. Otherwise, the physical separation and damaged DNA termini will allow NF-κB diffusing into the cell nucleus to activate transcription, causing the consequent secretion of senescence associated secretory phenotype (SASP) and inducing the permanent growth arrest, finally delaying cellular senescence.

mechanism was instantly activated, and the ability of cells to proliferate was restored. However, if the physical separation and damaged DNA termini cannot be effectively repaired, NF-κB will diffuse into the cell nucleus to activate transcription, causing the consequent secretion of SASP (Campisi, 2013; Mine-Hattab and Rothstein, 2012). As a result, the cells with damaged DNA permanently cease dividing, that is, cellular senescence (Li et al., 2008). Alternatively, the damaged cells are eventually eliminated by apoptosis and autophagy (Lieber, 2010). The schematic diagram of this process is shown in Fig. 1. Evidence of DR on DNA damage during renal aging is lacking; therefore, this article reports the effect of 30% DR for 6 months on persistent DNA lesions.

In this study, we systematically and comprehensively demonstrated that DNA damage, and the corresponding changes of the expression profile existing in aged kidneys, can be effectively attenuated by a 30% DR for 6 months. We also identified the different pathways involving in renal aging, namely p53/p21-, p16-, and p27-dependent mechanisms. Our findings have essential implications for targeting persistent DNA damage to restrict the cell cycle arrest and a variety of expression profiles and thus retard renal aging.

2. Methods

2.1. Rat models and tissue samples

The young (3 months old) male Fischer 344 rats ($n = 16$) were kept in a separate cage under a specific pathogen-free condition with a 12:12 light/dark cycle. The room temperature was 22 ± 1 °C and the relative humidity was 50% ± 10%. All the rats had free access to water. This research was approved by the Military Medical Postgraduate College and the Chinese PLA General Hospital. It was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. When the rats were 24 months old, we recorded the average food consumption by the ad libitum (AL) group for a week, and then calculated 70% of the food intake to determine the food consumption of the DR group in the next week. The old rats (24 months, $n = 16$) in the AL group were divided into the AL group ($n = 8$) and the DR group ($n = 8$). After a 30% DR for 6 months, all the rats in these two groups were sacrificed together. Before the animals were sacrificed, their urine was collected for 24 h in individual metabolic cages. Next, we used the sarcosine oxidase and Coomassie Brilliant Blue methods to detect the urinary protein/creatinine ratio in the urine samples. The blood samples were extracted and further analyzed. The colorimetric method was used to detect triglyceride and blood urea nitrogen. Enzymatic methods were used to detect serum creatinine and total cholesterol, and the hexokinase method was used to determine blood sugar. The kidney tissues for staining and western blot were stored at − 80 °C.

2.2. Senescence-associated β-galactosidase staining

The levels of senescence-associated β-galactosidase (SA-β-gal) were detected with the β-galactosidase staining kit (Beyotime, Jiangsu, China). The cryostat sections of kidney tissue were fixed in 0.2% formaldehyde and 2% glutaraldehyde for 15 min at room temperature. Tissue sections were washed with PBS for at least 15 min and then were incubated in freshly prepared SA-β-gal staining solution (staining solution A 10 μl, staining solution B 10 μl, staining solution C 930 μl, and X-gal solution 50 μl) overnight at 37.0 °C without CO_2. After washing with PBS solution, the kidney tissue sections were further counterstained with eosin solution. Two investigators examined the sections and performed image analyses using a microscope; the sample identities were masked from the investigators. Quantitative analysis of SA-β-gal positive was conducted with Image-Prosoftware from 15 random fields of view per rat at × 200 magnification.

2.3. Renal histology and histological grading

Kidney tissues were fixed in a 10% formalin solution at 4 °C for at least 12 h. After dehydration, kidney tissues were embedded, sectioned, and stained with Periodic acid–Schiff in turn. The images were captured at × 200 magnification with Nikon Element software (Nikon Instrument, Nikon Inc., Melville, NY, USA) and were also analyzed by two professors according to the standards of glomerular lesion and tubulointerstitial damage defined in supplementary Table S1 online (Wang et al., 2016). Each specimen was determined from 15 random fields of view per rat using the National Institutes of Health (NIH, Bethesda, MD, USA) Semi-Quantitative Score.

2.4. Western blot analysis

The kidney tissues were lysed with a radio immunoprecipitation assay buffer. The extracted protein was measured with the Pierce BCA Protein Assay kit (Thermo Fisher Scientific, Rockford, IL, USA). The extracted proteins were denatured with a 5% sodium dodecyl sulfate-