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The Mechanism by Which AGE Activates EGFR-mediated Diabetic Kidney Disease Fibrosis by Regulating the Balance of Tyrosine Phosphatase SHP1/SHP2

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Abstract: *Objective*: To investigate the mechanism by which advanced glycation end products (AGEs) promote diabetic kidney disease fibrosis by regulating the tyrosine phosphatase SHP1/SHP2 balance and activating the epidermal growth factor receptor (EGFR) pathway. *Methods*: Animal experiments and in vitro cell experiments were conducted using Western blot analysis and tissue cell staining to detect the expression of relevant proteins and cellular morphological changes. *Results*: AGEs disrupt the SHP1/SHP2 balance, activate the EGFR and TGFβ pathways, and promote fibrosis in diabetic nephropathy. *Conclusion*: AGEs regulate the balance of tyrosine phosphatases SHP1/SHP2, activate the EGFR-mediated signaling pathway, promote the release of inflammatory factors, and ultimately lead to fibrosis in diabetic nephropathy through a novel mechanism.

Keywords: Advanced glycation end products; Tyrosine phosphatase; Epidermal growth factor receptor; Diabetic nephropathy; Fibrosis

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1. Introduction

Diabetic kidney disease is one of the common microvascular complications of diabetes, with renal fibrosis as its primary pathological feature, severely threatening patients' health and quality of life [1]. The pathogenesis of this condition is complex, and current research generally suggests that its occurrence is primarily associated with multiple factors such as impaired glucose metabolism, haemodynamic changes, endothelial dysfunction,

inflammatory responses, cytokines, oxidative stress, genetic factors, the kinin system, and autophagy [2]. Common clinical treatments for diabetic kidney disease include controlling blood glucose and blood pressure, managing blood lipids, and inhibiting the renin-angiotensin system. However, all metabolic control measures and other interventions fail to effectively prevent progressive renal function decline in some patients. Therefore, it is essential to conduct in-depth research into the pathogenesis of diabetic kidney disease to identify new treatment strategies [3]. With further research, it is now widely recognized in clinical practice that one of the key factors contributing to the development of diabetic kidney disease is the accumulation of advanced glycation end products (AGEs) in the body, the balance of tyrosine phosphatases SHP1 and SHP2 in cellular signal transduction, and the activation of epidermal growth factor receptors (EGFR) in relation to renal fibrosis [4]. However, whether AGEs activate EGFR through regulating the SHP1/SHP2 balance to mediate diabetic kidney disease fibrosis remains unclear. This study will investigate this mechanism through animal experiments and in vitro cell experiments, aiming to provide a theoretical basis for the prevention and treatment of diabetic kidney disease.

2. Materials and methods

2.1. Experimental materials

(1) Experimental animals: Healthy male C57BL/6 mice, 8 weeks old, purchased from a laboratory animal centre.

(2) Cells: Human renal tubular epithelial cells (HK-2), purchased from a cell bank.

(3) Main reagents: Streptozotocin (STZ), AGE, TGFβ1, SHP1 antibody, SHP2 antibody, p-SHP1 antibody, p-SHP2 antibody, EGFR antibody, p-EGFR antibody, α-smooth muscle actin (α-SMA) antibody, type I collagen (Col I) antibody, etc.

(4) Main instruments: Microscope, Western blot-related equipment, centrifuge, etc.

2.2. Methods

2.2.1. Animal experiment section

(1) Animal grouping and modelling: Ten healthy mice were randomly selected as the control group. The experimental group consisted of model mice, which were modelled by intraperitoneal injection of streptozotocin (STZ) (60 mg/kg). After successful modelling, the experimental group was divided into a negative experimental group (model mice, no special treatment, 10 mice) and a positive experimental group (model mice, treated with AGE intervention, 10 mice).

(2) Sample collection: After the experiment, the mice were euthanized, and kidney tissue was collected. Part of the tissue was used for histological staining, and the remaining portion was used for Western blot analysis.

2.2.2. In vitro experiment section

(1) Cell grouping

(a) Cell groups without TGFβ pathway stimulation: Normal control group (HK-2 cells, normal culture), AGE-treated group (HK-2 cells, treated with AGE).

(b) Cell groups with TGFβ pathway stimulation: TGFβ1 stimulation group (HK-2 cells, treated with TGFβ1), TGFβ1 + AGE treatment group (HK-2 cells, treated with TGFβ1 and AGE concurrently).

(2) Cell treatment

Cells were treated according to their respective groups, cultured for a specified period, and then harvested.

2.2.3. Tissue cell staining and morphological observation

Perform HE staining and Masson staining on kidney tissue and cells. Observe morphological changes in cells from each group under a microscope, and collect and analyze images.

2.2.4. Western blot analysis

Extract total protein from tissues and cells of each group, perform Western blot analysis, and determine the expression levels of SHP2, p-SHP2, p-SHP1, EGFR, p-EGFR, α-SMA, and Col I. Use GAPDH as an internal control and analyze protein relative expression levels using grey scale values.

2.3. Statistical analysis

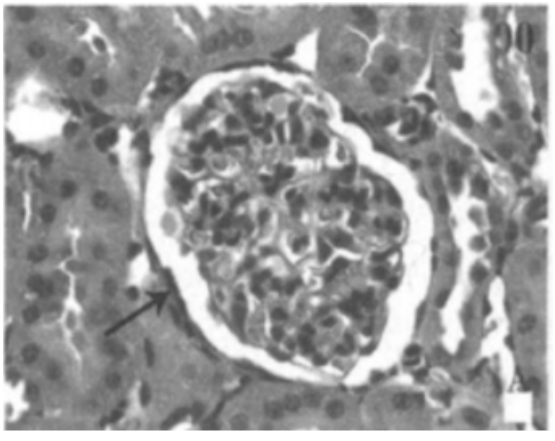
Data analysis was performed using SPSS 22.0 statistical software. Quantitative data were expressed as mean ± standard deviation ($\bar{x} \pm s$). For comparisons among multiple groups, one-way analysis of variance (ANOVA) was used, and LSD-t tests were used for pairwise comparisons between groups. A P value < 0.05 was considered statistically significant.

3. Results

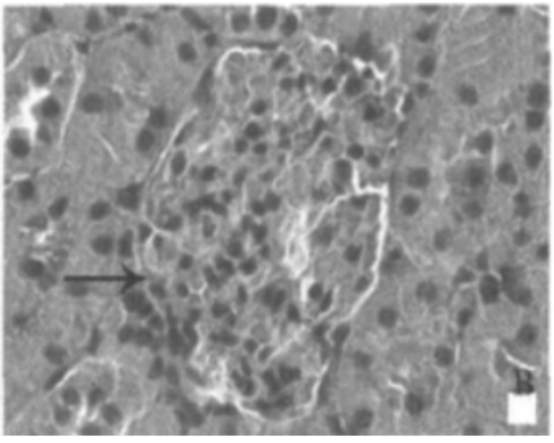
3.1. Animal experiment results

3.1.1. Morphological changes in renal tissue cells of mice in different groups

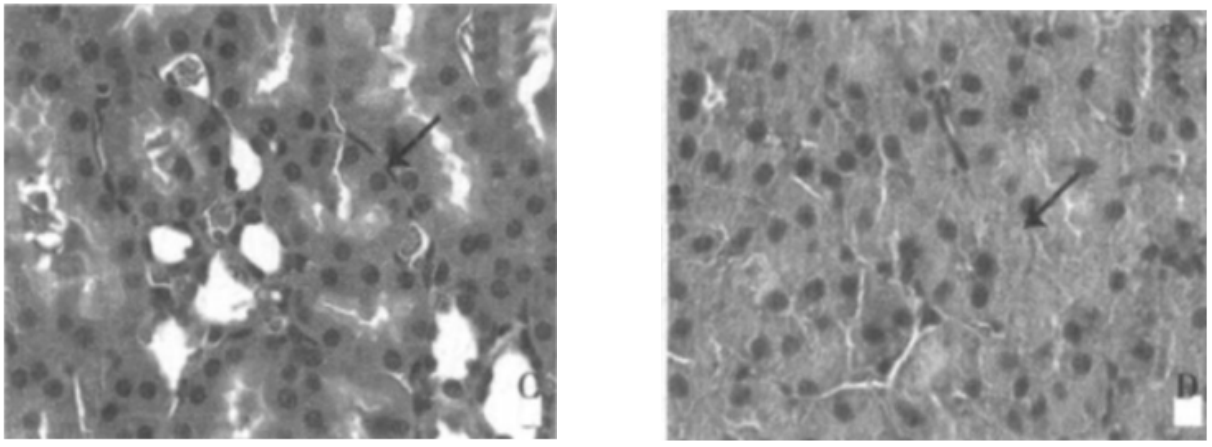
Histological staining revealed that the renal glomeruli, proximal tubules, and distal tubules of the control group mice showed no obvious abnormalities, with intact structures. In the positive experimental group, renal tissue exhibited varying degrees of pathological changes, such as severe swelling of the glomeruli, narrowing of the glomerular capsules, and disappearance of the interglomerular spaces; renal tubular epithelial cells were severely swollen and degenerated, while the distal tubules showed no obvious abnormalities, as shown in **Figure 1**.



Control group glomeruli



Glomeruli in the positive group



Control group proximal tubules

Positive group proximal tubules

Figure 1. Histopathological images of kidney tissues from the two groups of rats stained with HE (× 400)

3.1.2. Expression levels of relevant proteins in kidney tissues of mice in each group

Western blot analysis revealed that, compared with the control group, the expression levels of SHP2, p-SHP2, p-EGFR, α-SMA, and Col I were elevated in the kidneys of mice in the negative experimental group, while the expression level of p-SHP1 was decreased. However, some differences were not statistically significant ($P > 0.05$). The trends in the expression levels of the aforementioned proteins in the positive experimental group were consistent with those in the negative experimental group, and the differences were statistically significant ($P < 0.05$). Compared with the negative experimental group, the expression levels of SHP2, p-SHP2, p-EGFR, α-SMA, and Col I were significantly increased, while the expression level of p-SHP1 was significantly decreased in the positive experimental group ($P < 0.05$). Specific data are shown in **Table 1**.

Table 1. Relative expression levels of relevant proteins in kidney tissues of mice in each group ($\bar{x} \pm s$, n=10)

Group	*SHP2*	*p-SHP2*	*p-SHP1*
Control	1.02± 0.11	1.01 0.± 0.09	1.03 0.± 0.10
Negative control group	1.21± 0.13	1.25± 0.15	0.85± 0.12
Positive control group	1.89± 0.21	1.95± 0.23	0.42± 0.08
t	3.5282/8.7065	4.3386/8.0614	3.6440/9.4284
p	0.0024/0.0000	0.0004/0.0000	0.0019/0.0000

Note: t-value, p-value: compared with the control group/compared with the negative control group

3.2. In vitro experimental results

3.2.1. Expression levels of related proteins in cell groups without stimulation of the TGFβ pathway

In the absence of TGFβ pathway stimulation, compared with the normal control group, the expression levels of SHP2, p-SHP2, p-EGFR, α-SMA, and ColI were significantly increased, while the expression level of p-SHP1

was significantly decreased in the AGE-treated group ($P < 0.05$). Specific data are shown in **Table 2**.

Table 2. Relative expression levels of proteins in cell groups without stimulation of the TGFβ pathway ($\bar{x} \pm s$, n=3)

Group	*SHP2*	*p-SHP2*	*p-SHP1*
Normal control group	1.00 ± 0.07	1.01 (±) 0.06	1.03 (±) 0.09
AGE-treated group	1.65 ± 0.18	1.72 ± 0.21	0.58 ± 0.12
t	5.8293	5.6307	5.1962
p	0.0043	0.0049	0.0065

3.2.2. Expression levels of proteins associated with TGFβ pathway stimulation in cell groups

Under TGFβ pathway stimulation, compared with the TGFβ1 stimulation group, the expression levels of SHP2, p-SHP2, p-EGFR, α-SMA, and ColI were significantly increased, while the expression level of p-SHP1 was significantly decreased in the TGFβ1+AGE treatment group ($P < 0.05$). Specific data are shown in **Table 3**.

Table 3. Relative expression levels of proteins associated with TGFβ pathway stimulation in cell groups ($\bar{x} \pm s$, n=3)

Group	SHP2	p-SHP2	p-SHP1
TGFβ1-stimulated group	1.52 ± 0.16	1.58 (±, 0.18)	0.72 (±) 0.10
TGFβ1 + AGE treatment group	2.15 ± 0.22	2.23 ± 0.24	0.35 ± 0.07
t	4.0113	3.7528	5.2501
p	0.0160	0.0199	0.0063

3.2.3. Morphological changes in cells of each group

When the TGFβ pathway was not stimulated, the cell morphology of the normal control group was regular and neatly arranged. In the AGE-treated group, cell morphology changed, with varying degrees of deformation and abnormal proliferation. When the TGFβ pathway was stimulated, the cell morphology of the TGFβ1-stimulated group showed some changes, while the cell morphology of the TGFβ1+AGE-treated group changed more significantly, with disordered cell arrangement and more pronounced fibrotic features.

4. Discussion

Fibrosis, as an important manifestation of chronic inflammation, plays a significant role in the pathological classification of DKD. The histological manifestations of glomerular and tubular fibrosis are similar, primarily characterized by the accumulation of extracellular matrix components (such as type I, III, and IV collagen, as well as fibronectin and desmin) and the proliferation of interstitial fibroblasts [5]. Immune cells, as the primary executors of the inflammatory process, also play an important role in the fibrosis process of DKD [6]. In recent years, renal cell differentiation and fibrosis-related signalling pathways have received increasing attention [7]. Numerous studies have shown that AGE levels are highly correlated with the progression of (sugar) metabolic diseases and play an important role in the pathological mechanisms of these diseases; The EGFR signaling pathway plays a crucial role

in physiological processes such as cell growth, proliferation, and differentiation; studies have shown that EGFR-mediated diabetic kidney disease fibrosis [8]; while research into the mechanisms by which AGEs participate in diabetic kidney disease fibrosis may provide new therapeutic targets for the treatment of diabetic kidney disease.

This study investigated the mechanism by which AGEs activate GFR-mediated diabetic kidney disease fibrosis through regulating the SHP1/SHP2 balance via animal experiments and in vitro cell experiments. In animal experiments, it was found that in the positive control group of mice, the expression levels of SHP2 and p-SHP2 in kidney tissue were significantly increased, while the expression level of p-SHP1 was significantly decreased. Additionally, the expression of p-EGFR, α-SMA, and Col I was significantly increased, and the degree of kidney tissue fibrosis was markedly exacerbated. This suggests that AGEs may promote the fibrotic progression of diabetic nephropathy by disrupting the SHP1/SHP2 balance and activating EGFR [9]. In vitro experiments showed that AGE treatment led to similar results regardless of whether the TGFβ pathway was stimulated or not, with increased expression of SHP2, p-SHP2, p-EGFR, α-SMA, and ColI, and decreased expression of p-SHP1, with these changes being more pronounced when the TGFβ pathway was stimulated. The TGFβ pathway is an important signaling pathway promoting renal fibrosis [10]. This result suggests that AGEs may synergistically interact with the TGFβ pathway by regulating the SHP1/SHP2 balance to further activate EGFR, thereby exacerbating renal fibrosis. SHP1, as a phosphodiesterase associated with tumor suppressor genes, typically exerts negative regulation on cellular signaling pathways; whereas SHP2 often acts as a positive regulator in signal transduction [11]. AGE may inhibit SHP1 activity through a certain mechanism while promoting SHP2 activation, disrupting the SHP1/SHP2 balance, thereby lifting the inhibition on EGFR, leading to elevated p-EGFR levels, and subsequently activating downstream fibrosis-related signals, promoting the expression of fibrosis markers such as α-SMA and Col I, ultimately resulting in renal fibrosis [12].

5. Conclusion

In summary, AGE can promote the fibrotic progression of diabetic kidney disease by regulating the balance of tyrosine phosphatases SHP1/SHP2 and activating EGFR, with this effect being more pronounced when the TGFβ pathway is stimulated. This suggests that regulating the SHP1/SHP2 balance may be a potential therapeutic target for treating fibrosis in diabetic kidney disease.

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Disclosure statement

The authors declare no conflict of interest.

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