Tubulointerstitial changes in the diabetic kidney correlate closely with renal fibrosis, and transforming growth factor-beta-1 (TGF-β1) is thought to play a key role in this process. In contrast, hepatocyte growth factor (HGF) has shown therapeutic effects on injured renal tubules in animal models. This study was undertaken to test the hypothesis that the preventive effects of HGF may result from interventions in TGF-β1-mediated signaling and collagen III secretion. We examined the expression of HGF/HGF receptor (c-Met) and TGF-β1 in renal fibroblasts at multiple time points. The effects of recombinant human HGF on TGF-β1 expression were studied by RT-PCR and Western blotting, and the levels of collagen III were measured by ELISA. In the high-glucose condition, the expression of HGF and c-Met in renal fibroblasts was detected as early as 6 hours following cell culture while the level of TGF-β1 peaked at 96 hours. The addition of recombinant human HGF to the culture media dose-dependently inhibited TGF-β1 mRNA expression and reduced collagen III secretion by 34%. These results indicate that, during hyperglycemia, HGF inhibits TGF-β1 signaling and type III collagen activation in interstitial fibroblasts. Furthermore, we should recognize that changes in the balance between HGF and TGF-β1 might be decisive in the pathogenesis of chronic renal fibrosis. Therefore, administration of HGF to restore this balance may offer a novel therapeutic intervention in managing renal fibrogenesis in diabetic nephropathy.

Key Words: c-Met, diabetic nephropathy, hepatocyte growth factor, interstitial fibrosis, transforming growth factor-β1

 Plasma protein leakage is accelerated across the glomerular basement membrane into Bowman’s space. Downstream of the tubular compartment, proteinuria induces proinflammatory and profibrogenic injury in tubular cells, which can facilitate the development of interstitial fibrosis and tubular atrophy. In addition to glomerulosclerosis, the molecular basis of tubulointerstitial fibrosis has yet to be clarified because the severity of chronic tubulointerstitial injury is most strongly correlated with impaired renal function and most accurately predicts renal dysfunction in patients with ESRD. The most pronounced feature of chronic kidney disease that progresses to ESRD is...
the relentless accumulation of extracellular matrix (ECM), which leads to widespread glomerular sclerosis and interstitial fibrosis [3]. Several lines of evidence [4] have revealed critical roles of transforming growth factor-beta-1 (TGF-β1) during the progression of DN: (1) TGF-β1 expression is upregulated by glucose and promotes ECM accumulation in mesangial cells; (2) TGF-β1 expression levels are markedly increased in mesangial areas in animals and in patients after the onset of DN [5]; and (3) neutralization of TGF-β1 actions with a specific antibody suppresses kidney hypertrophy and sclerosis in vivo [6]. Thus, TGF-β1 is now considered to be a key factor that can aggravate DN.

Hepatocyte growth factor (HGF) was originally identified as a potent mitogen for mature hepatocytes [7], and was later recognized to have the same effects on renal tubular cells [8]. In the kidney, HGF accelerates renal tubular repair after the onset of acute renal failure, with rapid recovery of tubular morphology and function [9]. Administration of exogenous HGF prevented renal dysfunction and chronic mesangial injuries in a murine model of chronic renal disease, and had therapeutic effects on ESRD [10]. While considerable attention has been given to the effects of HGF in mesangial cells, it remains unclear whether HGF has similar effects on chronic interstitial injuries. Therefore, the aim of this study was to investigate the roles of HGF in fibroblast lesions exposed to high glucose concentrations. Our data suggest that HGF is an effective antifibrogenic factor that can decrease TGF-β1 production and collagen biosynthesis.

**Preparation and characterization of human fibroblast cultures**

Primary cultures of kidney fibroblasts were generated using established methods with some modifications [11,12]. Briefly, the kidney medulla was dissected from the cortex, diced, and digested in a collagenase solution (0.1%) (Shanghai Institute of Pharmaceutical Industry, China) for 30 minutes at 37°C in a water-bath rocker. The enzyme reaction was terminated by adding horse serum. The tissues were centrifuged for 5 minutes at 1,000 revolutions/min. After washing twice in phosphate-buffered saline, the fibroblasts were grown in serum-free DMEM. Cells were incubated at 37°C in a humidified atmosphere of 95% air/5% CO2. After 24 hours, the medium was replaced with DMEM supplemented with 10% fetal bovine serum. Every 2 days, the medium was replaced with fresh medium. When cells reached confluence, they were passaged by trypsinization, resuspended, and replated. The passage number was monitored.

These cells exhibited fibroblast-specific characteristics on immunohistochemical and morphologic observations (Figure 1). Briefly, the fibroblasts were positive for vimentin and fibronectin expression, but negative for the expression of cytokeratin.

**In vitro experimental design**

The cells were seeded in six-well tissue culture plates. After reaching confluence, the medium was replaced with fresh serum-free DMEM for 24 hours because all of the cells were in quiescent states. In the high-glucose groups, D-glucose was added to the medium at a

---

**Materials and Methods**

**Materials**

Dulbecco’s modified Eagle medium (DMEM) was purchased from Gibco BRL (Gaithersburg, MD, USA). Secondary antibodies were from Fuzhou Maixin Biotechnology Development Co. Ltd. (Fuzhou, Fujian, China). Glucose and mannitol were purchased from Sigma-Aldrich Corp. (St Louis, MO, USA). The collagen III enzyme-linked immunosorbent assay (ELISA) kit was purchased from Shanghai SenYong Science and Technology Co. (Shanghai, China). The HGF ELISA kit and goat anti-human c-Met antibody were purchased from R&D Systems Inc. (Minneapolis, MN, USA).

---

**Figure 1.** Primary human renal fibroblasts.
concentration of 25 mmol/L. The concentration of glucose in the control group was 5.5 mmol/L. At the same time, we used mannitol as an osmolarity control (5.5 mmol/L glucose + 20 mmol/L mannitol). The osmolarity was the same as that in the high-glucose group. The medium was changed every 2 days in each group. Cells were then collected at 6, 12, 24, 48 and 96 hours from each experimental group to measure collagen III biosynthesis, and HGF, c-Met and TGF-β1 mRNA and protein levels. Each experimental condition was tested in six replicate wells and the mean was taken to represent an individual experiment.

To examine the effects of HGF on fibroblasts, human recombinant (rh) HGF (R&D Systems Inc.) was added to the high-glucose medium at concentrations of 25, 50, 100 or 200 ng/mL. To investigate the role of transcription and protein synthesis of TGF-β1 induced by HGF, cells were pretreated with or without HGF for 48 hours. To evaluate the ECM of fibroblasts, type III collagen secretion was measured in the supernatants of cultured cells. Each experimental condition was tested in six replicate wells and the mean was taken to represent an individual experiment.

### Effects of glucose concentrations on expression of HGF, TGF-β1 and c-Met mRNA

After the cells had reached confluence under serum stimulation, RNA was extracted from cells using RNAzol. The levels of HGF and its receptor c-Met mRNA were measured by reverse transcription-polymerase chain reaction (RT-PCR). Glyceraldehyde 3-phosphate dehydrogenase was used as the normal control. The primers are shown in Table 1 [13].

RNA was quantified by measurement of the ultraviolet absorbance at 260 nm and its purity was assessed by measuring the optical density ratio at 260 nm and 280 nm. First-strand cDNA was synthesized using 2 μg of total RNA isolated from cells by reverse transcription with random primers at 37°C for 1.5 hours, in accordance with the manufacturer’s instructions. The cDNA was then amplified with specific primers designed for human HGF, c-Met and TGF-β1. The HGF gene was amplified for 35 cycles of 1 minute at 94°C, 1 minute at 48°C and 1 minute at 72°C. c-Met was amplified for 35 cycles of 1 minute at 94°C, 1 minute at 48°C and 1 minute at 72°C. The TGF-β1 gene was amplified for 35 cycles of 1 minute at 94°C, 40 seconds at 54°C and 40 seconds at 72°C. The amplification products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide. Quantitation was carried out by scanning and analyzing the intensity of the band signals using the FR200 instrument (Shanghai FuRi Institute of Biotechnology, Shanghai China), installed by Smart View analysis software (OPTON Co., Germany).

### Type III collagen secretion

The level of type III collagen was determined using an ELISA following the manufacturer’s protocol. At the end of the procedure, the reaction was stopped and the optical density of each well was determined at 492 nm using an automated microplate reader. The results of the assay were compared as the mean of duplicate samples against a 10-point standard curve.

### Protein HGF secretion

HGF protein levels were determined by an ELISA (R&D Systems Inc.). The kit utilizes a sandwich method consisting of three antigen–antibody reaction steps. Briefly, a monoclonal antibody specific to human HGF was coated onto 96-well microplates. Fifty-microliters of standard human HGF or culture supernatant samples were added to the wells and were incubated for 2 hours at room temperature. After washing three times with phosphate-buffered saline, 200 μL of an enzyme-linked polyclonal antibody specific to human HGF was added and the plates were incubated for another

---

**Table 1.** Primers for hepatocyte growth factor (HGF), c-Met and transforming growth factor-beta-1 (TGF-β1)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF</td>
<td>Sense</td>
<td>5'-GAT GTC CAC GGA AGA GGA GA-3'</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-GAG TCA CCT TCC CTC GAT GA-3'</td>
</tr>
<tr>
<td>c-Met [13]</td>
<td>Sense</td>
<td>5'-TCT TGG GAC ATC AGA GGG TC-3'</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-TGA CTT CAG GAC TGG AAA TG-3'</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Sense</td>
<td>5'-TCT GAA TTC ATG GCC TGG ACA CCA ACT A-3'</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5'-TCT GTC GAC ACC TCA GCT GCA CTT GCA GGA-3'</td>
</tr>
</tbody>
</table>
2 hours. They were then washed three times before the enzyme substrate was added. The plates were allowed to stand for 30 minutes, and the reaction was stopped by the addition of 50 μL of 1 N H2SO4. The absorbance was read at 450 nm with a BioTek EL340 automatic microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

Protein c-Met and TGF-β1 secretion
The samples were solubilized with lysis buffer at 4°C for 10 minutes, and the lysates were collected after centrifugation at 13,000 g at 4°C for 10 minutes. The protein concentration was then determined using Bradford’s assay. Samples were heated at 100°C for 3 minutes before loading and separated on a 7% SDS-PAGE gel. The proteins were electrotransferred to a PVDF (polyvinylidene fluoride) membrane. Non-specific binding to the membrane was blocked with 2% milk and the membrane was incubated overnight at 4°C with specific anti-human TGF-β1 antibodies or c-Met antibodies, followed by incubation for 1 hour at room temperature with the secondary IgG conjugate. The signals were visualized using the ImageJ system (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis
All data are expressed as mean ± standard deviation. Groups were compared using unpaired t tests. Statistical significance was considered at p < 0.05.

RESULTS

Effects of high glucose on secretion of collagen III
The secretion of collagen III was increased in response to the high-glucose medium (25 mmol/L glucose) at all three time points (24, 48 and 96 hours), and peaked at 96 hours with a 3.2-fold increase compared with that observed in the control groups (5.5 mmol/L glucose), as shown in Table 2.

Although a similar trend was observed in the mannitol group, the difference across the three groups remained statistically significant. These data suggest that at least part of the observed increase in collagen III levels is due to the cellular response to higher osmolality induced by higher concentrations of mannitol or glucose.

Effects of high glucose concentration on gene expression of TGF-β1, HGF and c-Met
RT-PCR and a Smart View algorithm were used to quantify the mRNA levels of TGF-β1, HGF and c-Met at 6, 12, 24, 48 and 96 hours after exposure to the control, high glucose or mannitol conditions, as shown in Figure 2. In the TGF-β1 study, the expression of TGF-β1 was increased in a time-dependent manner across the five time periods. No difference among the three groups in TGF-β1 mRNA was observed at 6 or 12 hours. However, a >2-fold increase in TGF-β1 mRNA was recorded at the remaining three time points and peaked at 96 hours, which was associated with the greatest difference between the control and high-glucose groups (43.5 ± 3.42 vs. 103.7 ± 6.21, respectively). Figures 2B and 2C demonstrate the biphasic mRNA expression profile of HGF and c-Met. A peak in HGF mRNA expression, which represented almost a 3-fold increase, was observed as early as 12 hours in the high-glucose group. This overexpression then gradually declined and returned to the baseline level at 96 hours. Although slightly delayed, a parallel peak corresponding to a 2.75-fold increase in c-Met gene expression was found 24 hours after high-glucose induction.

As discussed above, medium rendered hypotonic by mannitol or glucose can lead to increases in osmolality. This factor might alter the expression pattern and partially contribute to the overexpression of these proteins.

Table 2. High glucose (25 mmol/L) enhances type III collagen secretion and suppresses hepatocyte growth factor (HGF) expression (n = 6)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>5.5 mmol/L glucose</th>
<th>25 mmol/L glucose</th>
<th>Mannitol</th>
<th>25 mmol/L glucose + 50 ng/mL HGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>42.69 ± 8.62</td>
<td>98.00 ± 12.33†</td>
<td>70.57 ± 10.19†</td>
<td>96.69 ± 16.33</td>
</tr>
<tr>
<td>48</td>
<td>47.47 ± 6.69</td>
<td>120.02 ± 8.20†</td>
<td>77.35 ± 12.51†</td>
<td>102.01 ± 5.43†</td>
</tr>
<tr>
<td>96</td>
<td>59.31 ± 4.53</td>
<td>189.31 ± 49.34*</td>
<td>93.44 ± 11.33†</td>
<td>124.36 ± 18.92†</td>
</tr>
</tbody>
</table>

*p < 0.05 and †p < 0.01 vs. 5.5 mmol/L glucose; ‡p < 0.05 and §p < 0.01 vs. high-glucose group (25 mmol/L).
HGF attenuates injury in renal fibroblasts

Figure 2. Expression of transforming growth factor-beta-1 (TGF-β1), hepatocyte growth factor (HGF) and c-Met mRNA. (A) Time-course of TGF-β1 mRNA expression: TGF-β1 mRNA expression reached a peak at 96 hours. (B) Time-course of HGF mRNA expression: HGF mRNA expression reached a peak at 12 hours. (C) Expression of c-Met mRNA: c-Met mRNA expression reached a peak at 24 hours. Control group: the concentration of glucose was 5.5 mmol/L. High-glucose group: the concentration of glucose was 25 mmol/L. Mannitol group: osmolarity was the same as the high-glucose group and comprised 5.5 mmol/L glucose plus 20 mmol/L mannitol. Error bars represent standard deviation. The experiments were repeated six times. *p < 0.01 and †p < 0.05, compared with the control groups at the appropriate times.

Table 3. Relative hepatocyte growth factor expression (n = 6)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>5.5 mmol/L glucose</th>
<th>25 mmol/L glucose</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>97.67 ± 11.40</td>
<td>157.00 ± 30.81*</td>
<td>66.00 ± 7.00*‡</td>
</tr>
<tr>
<td>12</td>
<td>102.73 ± 15.40</td>
<td>177.67 ± 18.15†</td>
<td>40.08 ± 8.88†‡</td>
</tr>
<tr>
<td>24</td>
<td>91.33 ± 9.93</td>
<td>105.50 ± 12.76</td>
<td>38.97 ± 8.73†‡</td>
</tr>
<tr>
<td>48</td>
<td>94.40 ± 13.56</td>
<td>97.42 ± 11.57</td>
<td>35.00 ± 9.17†‡</td>
</tr>
<tr>
<td>96</td>
<td>89.60 ± 13.38</td>
<td>86.60 ± 8.32</td>
<td>25.67 ± 4.04†‡</td>
</tr>
</tbody>
</table>

*p < 0.05 and †p < 0.01 vs. 5.5 mmol/L glucose; ‡p < 0.01 vs. high-glucose group (25 mmol/L).

three genes in the mannitol (25 mmol/L) group compared with the normal glucose control group (TGF-β1: 56.5 ± 10.43 vs. 43.5 ± 3.42, p < 0.05; HGF: 41.50 ± 6.61 vs. 28.23 ± 6.59, p < 0.05; c-Met: 63.37 ± 6.01 vs. 29.87 ± 5.05, p < 0.05).

Effects of high glucose on protein expression of HGF, TGF-β1 and c-Met

We measured the expression level of HGF protein using a commercially available ELISA kit. As shown in Table 3, the level of HGF protein reached a peak only 12 hours after high glucose induction, corresponding to nearly a 20% increase. The HGF protein level returned to baseline within 96 hours.

We also measured the protein expression level of TGF-β1 and c-Met using Western blotting. Under the high glucose condition, TGF-β1 protein increased by 2.1-fold compared with that in the control group at 48 hours (Figures 3A and 3B), an observation similar to previous studies [13], and this increase continued to 96 hours (Figure 3A). On the other hand, c-Met protein expression began to increase at 12 hours in the
high-glucose group (Figure 4A), and reached a peak at 24 hours (Figures 4A and 4B).

**Exogenous HGF inhibits TGF-β1 expression in fibroblasts**

To determine the preventive role of HGF on fibroblasts, we examined whether rhHGF affects TGF-β1 mRNA and protein expression.

The addition of various concentrations of rhHGF into the high-glucose media dose-dependently repressed the glucose-induced increase in TGF-β1, with a strong correlation coefficient ($r = -0.8726$, $p < 0.01$; Figure 5). This study also revealed that 50 ng/mL of rhHGF was sufficient to reduce TGF-β1 expression by 50% ($p < 0.05$).

Next, we examined the effect of different concentrations of rhHGF on the protein levels of TGF-β1 using Western blotting. Consistent with a decrease in TGF-β1 mRNA, suppressed upregulation of TGF-β1 was found in response to rhHGF administration.

**Exogenous HGF modulates collagen III secretion in fibroblasts**

Finally, we investigated the effect of 50 ng/mL rhHGF on ECM accumulation using type III collagen as the surrogate marker. An ELISA was used to quantify collagen III expression in fibroblasts under various culture conditions, and a significant difference was observed at 48 and 96 hours (Table 2).

**DISCUSSION**

Diabetes is the leading cause of ESRD in many developed countries and DN has emerged as an unseen epidemic worldwide [1]. Although the mechanisms accounting for kidney failure in diabetes remain controversial, clinical studies imply that tubulointerstitial lesions show the strongest correlation with renal failure in DN [5]. The tubulointerstitium of the diabetic kidney has become a major research focus. In particular, *in vitro* studies [2] have confirmed the original
observation of cellular interactions within the tubulo-interstitial compartment, and that it is an important site of action for renoprotective therapies in diabetic kidney disease. We developed a cell culture system for human kidney fibroblasts in which we could test the effects of high ambient glucose levels on growth factor biosynthesis. Fibroblasts constitute the major cell type in the interstitium, where they interconnect with tubules, vessels and each other to provide a scaffold-like structure. The aim of this research was to study the effects of high glucose on kidney interstitial fibroblast cells.

Our study shows that the expression of HGF and its receptor c-Met was increased in fibroblasts in the early stage of culture in high glucose concentrations. As a result, we began by investigating the relationship between HGF/c-Met and high glucose. Although the molecular mechanism responsible for the stimulation of HGF/c-Met expression at high glucose concentrations within fibroblasts is unknown, increased osmolarity owing to hyperglycemia appears to be one of the important contributing factors. However, a significant difference between the mannitol and glucose groups, in which osmolarity was comparable, implies
the presence of other mechanisms. One popular hypothesis is the cytokine theory, which proposes that cytokines and growth factors, including interleukin-1, interleukin-6, and HGF itself, can upregulate HGF/c-Met expression in cell culture systems [7,8].

Emerging evidence [13,14] suggests that the HGF/c-Met axis is an important component of the intercellular signaling pathway that controls the growth and differentiation of diverse types of cells. HGF not only promotes tissue repair and regeneration following acute injury, but also modulates the development and progression of chronic diseases that are characterized by progressive tissue fibrosis [15]. Autocrine HGF/c-Met signaling may also occur in other cell types within the kidney during development, as exemplified in embryogenic mesenchymal cells where the c-Met receptor and HGF are co-expressed [16]. HGF exerts mitogenic, motogenic and morphogenic effects on renal epithelial cells to promote epithelial cell regeneration and reconstitution of damaged tubules. HGF also stimulates the gene expression of fibronectin, a major component of the ECM, suggesting that it may modulate matrix remodeling and lead to the restoration of a normal extracellular environment [17]. Although the exact mechanisms underlying the preventive effects of HGF in diabetic renal fibrosis remain uncertain, some researchers have suggested that HGF might attenuate fibrosis by suppressing plasminogen activator inhibitor, an inhibitor of ECM-degrading enzymes. Consequently, tubulointerstitial fibrogenic events [14], including overexpression of TGF-β1, fibroblast hypertrophy and overactive ECM deposition, were inhibited in diabetic fibroblasts and appear to mitigate the fibrosis phenotype.

The progression of chronic kidney disease is characterized by the persistent accumulation of ECM, a predisposition of widespread tissue fibrosis. Previous studies [13,18] have revealed that high glucose concentrations can stimulate the expression of various matrix components at the mRNA and protein levels. In diabetic kidneys, the synthesis of collagen III, collagen IV, fibronectin, and laminin is increased, of which collagen IV overexpression can be abolished by HGF in cultured human mesangial cells [19]. To determine the effects of HGF on matrix protein overproduction, we added various concentrations of rhHGF to cultured renal fibroblasts exposed to hyperglycemic conditions and found decreased expression of collagen III, indicating that HGF may prevent fibrosis by suppressing ECM expression. It is known that the expression of HGF/c-Met in a high-glucose concentration environment is time-dependent. The activation of HGF/c-Met expression usually takes place within 12–24 hours of exposure to high glucose. After this period, a timing preceding and paralleling significant renal hypertrophy is found [14]. After 24 hours, the expression of HGF/c-Met is suppressed while the expression of TGF-β1 is increased. This profile is known to stimulate renal interstitial fibroblasts and glomerular mesangial cells to undergo myofibroblastic activation [20], as characterized by the induction of α-smooth muscle actin expression and overproduction of ECM components. TGF-β1 also induces the tubular epithelial–mesenchymal transition [19], a phenotypic conversion that plays a critical role in generating matrix-producing effector cells. In addition, TGF-β1 is able to induce kidney cells to undergo apoptosis [21], leading to capillary loss, and tubular atrophy. Such broad actions of TGF-β1 will eventually lead to tissue fibrosis [22]. In our study, high glucose induced the expression of steady-state mRNA and protein levels of TGF-β1 after 48 hours in the high-glucose condition.

Finally, it is important to discuss the cause-and-effect relationship between the reciprocal expression pattern of HGF and TGF-β1 in vitro. HGF-mediated inhibition of TGF-β1 expression has been consistently reported in many studies utilizing various models of chronic kidney disease, which may provide an explanation for its therapeutic efficacy [16,23,24]. Recent studies using human mesangial cells have also demonstrated that HGF can suppress TGF-β1 expression induced by a high concentration of glucose [14]. HGF also represses TGF-β1 production [25,26]. Thus, in our study, it seems that HGF alters TGF-β1 regulation at multiple levels. HGF suppressed the expression of TGF-β1 after 48 hours. Furthermore, HGF decreased TGF-β1 mRNA in a dose-dependent fashion, and HGF abolished TGF-β1 protein expression. Therefore, we believe that during the rapid progression to high glucose, the earlier increase in expression of HGF/c-Met in fibroblasts is a beneficial effect.

In vitro studies have revealed that HGF specifically counteracts many profibrotic actions of TGF-β1, suggesting that the balance between HGF and TGF-β1 may play a decisive role in the pathogenesis of chronic renal fibrosis [27]. Broadly, HGF and TGF-β1 function as the Yin and Yang of tissue fibrotic signals and elicit opposite effects [23]. Therefore, disturbance of the
balance between HGF and TGF-β1 activities will eventually cause disastrous consequences.

If the injurious stimulus is transient, such as that after an acute insult, HGF signaling will dominate and prevail, resulting in tissue repair and regeneration, perhaps leading to complete recovery. On the other hand, if the injury is chronic in nature, such as prolonged exposure to high glucose, a potential mechanism for regulating the balance may involve TGF-β1 expression, which progressively increases throughout the course of the injury, whereas HGF levels initially increase but gradually decline, possibly due to inhibition by TGF-β1. Thus, the net effect after chronic injury is to shift the balance between TGF-β1 and HGF, to favor the profibrotic TGF-β1. Accordingly, the duration of injury seems to determine the ratio of TGF-β1 and HGF, and thereby the ultimate outcome of the tissue responses. Taken together, we hypothesize that there may be reciprocal mechanisms, based on TGF-β1 and HGF expression, that regulate the progression of transforming growth factor beta is elevated in human glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice.

REFERENCES

mechanism independent of Smad signaling, J Biol Chem 2003;278:12537–45.


肝細胞生長因子抑制人腎間質成纖維細胞膠原合成和轉化生長因子－β1 表達

牟姗 王琴 施蓓莉 顧樂怡 倪兆慧
上海交通大學醫學院附屬仁濟醫院腎臟科

糖尿病腎病過程中，當腎小球濾過率下降，常常伴有腎小管間質腎變。此項研究中，採用人腎臟成纖維細胞培養系統，探討肝細胞生長因子 (HGF) 對高糖誘導的腎間質成纖維細胞損傷產生的作用。用消化法體外培養人腎間質成纖維細胞，用 D- 葡萄糖刺激細胞，造成高糖環境。另外高糖環境下外源加入 rHGF 誘導細胞，以酶聯免疫吸附法檢測 III 型膠原情況。用 RT-PCR 檢測細胞 HGF 和 TGF-β1 基因表達，Western blot 方法檢測 TGF-β1 蛋白合成。高糖處理過的細胞 III 型膠原合成增加。同時觀察到高糖作用早期 HGF 表達升高，隨著作用時間延長，其表達量下降，而 TGF-β1 出現持續高表達。同時外源加入 HGF 可以抑制 TGF-β1 基因和蛋白表達，對 III 型膠原合成產生抑制作用。體外高糖環境促進 III 型膠原合成和 TGF-β1 持續高表達。rHGF 可以部分抑制 III 型膠原和 TGF-β1 表達，從而發揮延緩間質損傷的作用。局部的 HGF/c-met 系統在糖尿病腎病過程中起重要作用。

關鍵詞：肝細胞生長因子受體，糖尿病腎病，肝細胞生長因子，間質纖維化，轉化生長因子－β1

( 高雄醫誌 2009;25:577–87)