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RETRACTED: CXCL6 Promotes Renal Interstitial Fibrosis in Diabetic Nephropathy by Activating JAK/STAT3 Signaling Pathway

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In this study the role of CXCL6 in diabetic nephropathy (DN) was investigated. It was found to be overexpression in DN patients and DN rat model. And the expression of fibrosis-related cytokines was consistent with the expression of CXCL6. High glucose significantly increased the proliferation of rat renal fibroblasts NRK-49F cell and the expression of CXCL6. Knockdown of CXCL6 ameliorated the pro-proliferation effect of high glucose and decreased the expression of fibrosis-related cytokines, while CXCL6 overexpression exhibited the opposite phenomenon. Gene set enrichment analysis, Western blot and ELISA showed that Janus kinase-signal transducer and activator of transcription (JAK-STAT) and CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION signaling pathways were correlative with CXCL6. This data indicates that CXCL6 may promote fibrosis-related factors to accelerate the development of DN renal interstitial fibrosis by activating JAK/STAT3 signaling pathway. CXCL6 is promising to be a potential novel therapeutic target and candidate biomarker for JAK/STAT3 signaling for the treatment of DN.

Keywords: CXCL6, kidney, diabetics, fibrosis, pathway

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INTRODUCTION

Diabetic nephropathy (DN) is a well-known micro-vascular complication of diabetes, and the leading cause of ESRD (Gin et al., 2000). Over-deposition of the ECM in the renal interstitium is the main cause of renal interstitial fibrosis, which is the final common pathway of the majority of chronic progressive renal diseases, including DN (Strutz and Zeisberg, 2006). Fibroblasts are

Abbreviations: CCK-8, Counting Kit-8; CXCL6, C-X-C chemokine ligand 6; DMEM, Dulbecco's modified Eagle medium; DMSO, Dimethyl sulfoxide; DN, Diabetic Nephropathy; ECM, Extracellular matrix; ESRD, End-stage renal disease; FBS, Fetal bovine serum; GSEA, Gene Set Enrichment Analysis; JAK, Janus kinase; MMP, Matrix metalloproteinase; STAT, Signal transducer and activator of transcription; TGF-β1, Transforming Growth Factor β1; TNF-α, Tumor Necrosis Factor α.

the major cells that produce ECM in the renal interstitium and play an important role in the process of renal interstitial fibrosis (Grande and López-Novoa, 2009).

Many inflammatory factors are involved in the process of renal interstitial fibrosis in DN, such as chemokine, TGF-β_1, interleukin family, adhesion molecule, etc., which can stimulate a series of inflammatory signaling pathways, resulting in ECM thickening, interstitial fibrosis, eventually triggering renal dysfunction (Wada and Makino, 2013).

Chemokines are a class of chemotactic and inducible small molecule peptides that are ubiquitous and play an important role in acute and chronic inflammation (Wasmuth et al., 2009). Among the chemokines, Soluble CXCL6 is an important inflammatory cytokine that recruits inflammatory cells to the site of inflammation by binding to the receptors CXCR1 and CXCR2 (Sadik et al., 2011). In the cigarette-induced CXCL6-deficient mouse inflammation model, macrophage recruitment in the lungs was significantly reduced (Balamayooran et al., 2012). CXCL6 is not only significantly increased in inflammatory bowel disease, but is also higher expressed in patients with periodontitis. Immunohistochemistry has confirmed that CXCL6 is mainly distributed in gingival vascular endothelial cells (Kebschull et al., 2009). Ionizing radiation can induce a significant inflammatory reaction in the skin, this can also promote the secretion of CXCL6 in fetal foreskin and adult human dermal fibroblasts, and mast cells mediate the secretion of CXCL6 (Müller and Meineke, 2011). Microparticles, a small vesicle that accumulates in the synovial fluid of patients with rheumatoid arthritis, not only promotes the secretion of inflammatory cytokines by synovial fibroblasts but also significantly induces the secretion of the chemokine CXCL6, thereby increasing the number of vascular endothelial cell migration and thus promoting angiogenesis and aggravation of joint inflammation (Reich et al., 2011). It has also been reported that CXCL6 can also promote lung fibrosis (Besnard et al., 2013) and it is significantly increased in patients with idiopathic pulmonary fibrosis.

Anti-CXL6 treatment of 2 weeks is reported to significantly decrease the levels of IL-1β, collagen and Human Tissue Inhibitor Of Matrix Metalloprotease-1 (TIMP-1) in Bleomycin-induced lung inflammation and fibrosis mouse model together with the degree of lung inflammation and fibrosis suggesting that CXCL6 plays an important role in the process of fibrosis, however, the mechanism of action is not clearly defined (Besnard et al., 2013). The aim of this study was to investigate the potential functions and the underlying mechanism of CXCL6 in DN renal interstitial fibrosis.

MATERIALS AND METHODS

Patient Samples

All the tissue and plasma samples were obtained from The Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine between February 2015 and December 2015. 40 DN and 20 normal kidney tissue samples were harvested from DN patients with kidney interstitial fibrosis at the time of biopsy or surgical resection. Plasma samples were collected from DN patients (n = 40) with kidney interstitial fibrosis and apparently healthy subjects (n = 40) and the tissue and plasma samples were stored at -80 C until used. The following data was recorded for all participants: sex, age, and risk factors for DN (hyperlipidemia, and glycosuria), showed in **Supplementary Table S1**.

This study was approved by the Medical Ethics Committee of The Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine and written informed consent was obtained from all participants involved in this study.

Bioinformatics Analysis

CXCLs mRNA levels detected in human DN kidney samples (n = 19) and healthy kidney tissue (n = 50) were obtained from GEO DataSets (Asscess ID: GSE30122). Student's t-test was used to determine the statistical significance of CXCLs expression between human diabetic kidney samples and healthy kidney tissue.

Gene Set Enrichment Analysis gene expression profiles about fibrosis was obtained from GEO DataSets (Asscess ID: GSE28221) was analyzed by GSEA. GSEA was performed using the GSEA software, Version 3.0, obtained from the Broad Institute[1]. GSEA version 3.0 software was from the Broad Institute at MIT. The nominal P-value and normalized enrichment score (NES) was used to sort the pathways associated with CXCL6 higher expression.

Animal Model Preparation

Male SD rats (160–200 g) were obtained from Shanghai Sippr BK Laboratory Animals Ltd, (Shanghai, China). After feeding high-sugar-fat diet for 8 weeks, the rats were given a single intraperitoneal injection of 35 mg/kg STZ. After 72 h, the rats with fasting and random blood glucose reading of above 13.8 mmol/L, 16.7 mmol/L, respectively, meanwhile glycosuria showing ++++ were considered as diabetes. Four weeks after STZ injection, the DN model rat was established when the uromicroprotein exceeded 30 mg/day.

The animal experiments were carried out in accordance with the guidelines issued by Medical Ethics Committee of The Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine.

Sample Collection

After the model was successfully established, urine specimen, blood samples from rat heart, and renal tissues were collected and kept for corresponding examination. The urinary albumin concentration (U-Alb), urine albumin excretion (UAE), serum creatinine (Scr), serum urea nitrogen (BUN), uric acid (UA), and albumin (Alb) were measured by automatic biochemical analyzer. The level of IL-6, TNF-α, TGF-β_1, CollagenI, CollagenIII, MMP2 and MMP9 in blood were detected by enzyme linked immunosorbent assay (Elisa). The expression of CXCL6, CXCR1, MMP2/9, CollagenI/III, TGF-β_1 in kidney tissues was determination by quantitative real-time reverse transcription polymerase chain reaction

[1]http://software.broadinstitute.org/gsea/downloads.jsp

(qRT-PCR) and Western blotting. General histology of renal tissues was visualized by hematoxylin and eosin (H&E) and Masson staining.

Cell Culture Experiments

A normal rat kidney fibroblast cell line NRK-49F cells were provided by the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM, which were supplemented with 10% FBS,100 units/mL penicillin and 100 μg/mL streptomycin at 37°C and 5% CO2.

Cell Viability Analysis

Cells were seeded in 96-well plates at a density of 2000 cells per well and incubated overnight. The cell proliferation was quantitated using the Cell CCK-8; Dojindo Laboratories, Japan) according to the manufacturer's recommendations. Control cells were treated with cultured media containing 0.15% (v/v) DMSO.

Lentivirus Transfection

The NRK-49F cells were seeded in 6-well plates, grown overnight and transfected with CXCL6 shRNA viral or overexpression lentivirus (both from GeneChem, Shanghai, China) to investigate the regulatory role of CXCL6. All of the transfections were performed using the Lipofectamine 3000 reagent (Invitrogen®; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. A blank vector lentivirus was used as a negative control (NC). CXCL6 expression in cells following transfection was confirmed by western blot analysis and quantitative real-time PCR.

Quantitative Real-Time PCR Analysis (qRT-PCR)

Total RNA from the cells was extracted with TRIzol (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's protocol. The SYBR Green-based qRT-PCR (Thermo Fisher) was performed to examine the relative CXCL6 and CXCR1 mRNA level which was normalized with GAPDH. The CXCL6 primers were: Primer F 5′-CTTAGCTCCAAGAATTAACC-3′; Primer R 5′- GGTCAAGACAAACATTATCC-3′. CXCR1 primers were: Primer F 5′- TCTTCCGCCAGGCATATAAAC-3′; Primer R 5′-TAGCAGACCAGCATGATGAAC-3′. The GAPDH primers were: Primer F 5′- CACCCACTCCTCCACCTTTG-3′; Primer R 5′- CCACCACCCTGTTGCTGTAG -3′.

Western Blots

The bands were determined by western blotting using the enhanced chemiluminescent substrate (ECL, BioRad,

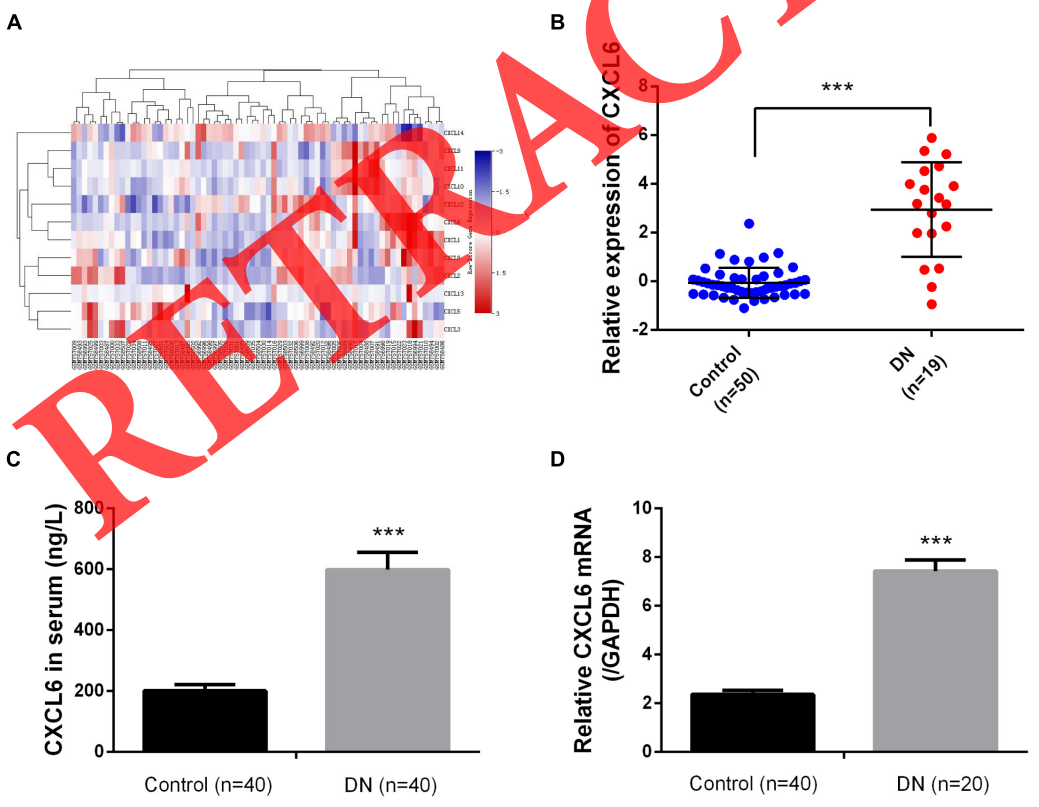

FIGURE 1 | Expression of CXCL6 was upregulated in DN patients. **(A)** CXCL mRNA expression file of 19 human DN kidney samples and 50 healthy kidney tissue were performed based on GEO DataSets (Asscess ID: GSE30122). **(B)** The expression level of CXCL6 in human DN kidney samples (n = 19) and healthy kidney tissue (n = 50) (***P < 0.001). **(C)** The Serum levels of CXCL6 in normal (n = 40) and DN patients (n = 40) with kidney interstitial fibrosis were determined using Elisa. **(D)** CXCL6 mRNA levels detected in 20 human DN kidney samples and 40 healthy kidney tissues using qRT-PCR. ***P < 0.001.

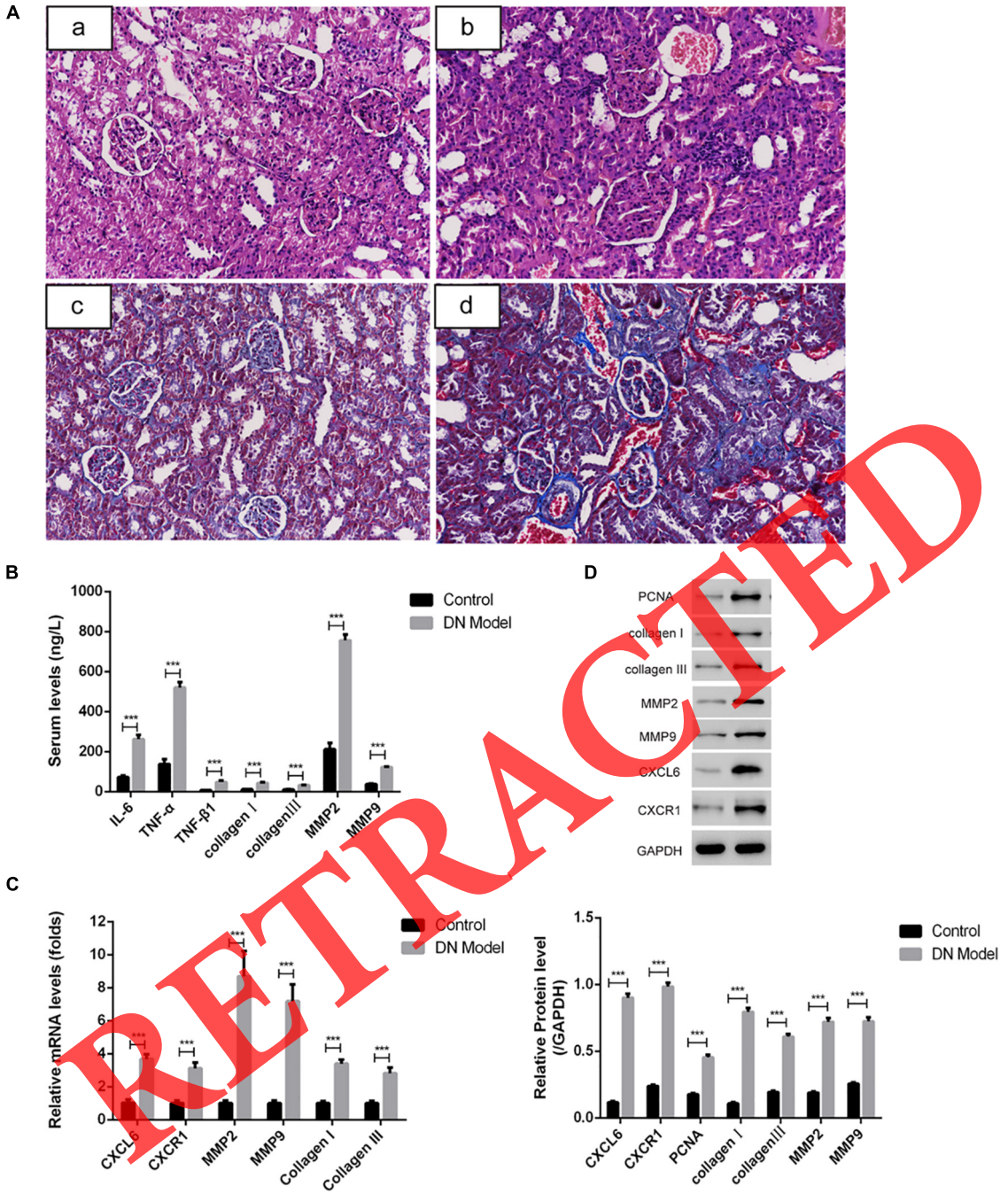

FIGURE 2 | Fibrosis related cytokines in DN rat model. **(A)** H&E and Masson staining of renal tissues. **(a)** H&E staining of renal tissues in control group **(b)** H&E staining of renal tissues model group **(c)** masson staining of renal tissues in control group **(d)** masson staining of renal tissues model group **(B)** the levels of several fibrosis related cytokines in peripheral blood were measured by Elisa. **(C)** Relative mRNA expressions of several fibrosis related cytokines were determined by qRT-PCR **(D)** the protein levels of several fibrosis related cytokines were examined by Western blotting. ***$p < 0.001$ vs. model group.

Richmond, CA, United States) in accordance with the manufacturer's instruction.

Cytokine Detection

The fibrosis-related cytokines IL-6, TNF-α, TGF-β1, CollagenI, CollagenIII, MMP2 and MMP9 were detected in cell culture supernatants and plasma samples. The following were used to measure the concentrations of cytokines: The Rat IL-6 ELISA kit, the Rat TNF-αELISA kit, the Rat TGF-β1 ELISA Kit, the Rat CollagenIII ELISA kit, the Rat Collagen ELISA kit, the Rat MMP2 ELISA kit, and the Rat MMP9 ELISA kit (Xinyu Biological Technology, Shanghai, China).

Histology

The tissue samples were fixed with 4% paraformaldehyde, dehydration and embedded in paraffin. General histology was visualized by hematoxylin and eosin (H&E) and Masson staining. The stained tissues were observed under an optical microscope, the renal fibrosis and collage deposition were estimated, respectively.

Statistical Analysis

The data are presented as the mean ± SD, and all the experiments were performance three times independently. Unpaired two-tailed student's *t*-test was used to compare the difference between the two groups. The one-way analysis of variance (ANOVA) test with Dunnet t was performed for comparisons in the multiple groups and $P < 0.05$ was considered statistically significant.

TABLE 1 | The detail data of biochemical indicators in each group (Mean ± SD, $n = 6$).

Indicator	NC	DN rat model
Blood glucose (mM)	3.98 ± 0.21	16.42 ± 0.76***
Scr (μmol/L)	129.21 ± 9.04	457.75 ± 16.06***
BUN (mmol/L)	4.44 ± 0.35	10.80 ± 0.30***
UA (μmol/L)	101.09 ± 3.59	203 ± 5.19***
Alb(g/L)	39.5 ± 0.98	23.37 ± 0.76***
U-Alb(μg/ml)	350.83 ± 44.24	1036.37 ± 25.94***
UAE(mg/L·h)	14.62 ± 1.23	43.18 ± 1.18***

****compared with control: P < 0.001.*

RESULTS

CXCL6 Is Overexpression in DN

In order to define the CXCLs expression patterns in DN, the CXCLs (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, and CXCL14) mRNA levels in GEO DataSets (Asscess ID: GSE30122) were analyzed (**Figure 1A**). It was found that CXCL6 (3.08 fold, $P < 0.001$) was significantly higher in DN human kidney than in control (**Figure 1B**). Then Elisa and qRT-PCR were used to confirm the transcript of CXCL6 and it was found that CXCL6 was higher in plasma (**Figure 1C**) and kidney tissue (**Figure 1D**) of DN than in the control samples ($P < 0.001$).

The DN rat model was established by high-sugar-fat diet combined with a single intraperitoneal injection of streptozocin (STZ). As shown in **Table 1**, the level of blood glucose, Scr, BUN, UA, U-Alb, and UAE in the DN model group was drastically increased and Alb was decreased compared with the control group. Histology staining with H&E and Masson revealed typical damage and severe collagen fibrosis of kidney tissues in the DN model group (**Figure 2A**). The results of qRT-PCR and Western

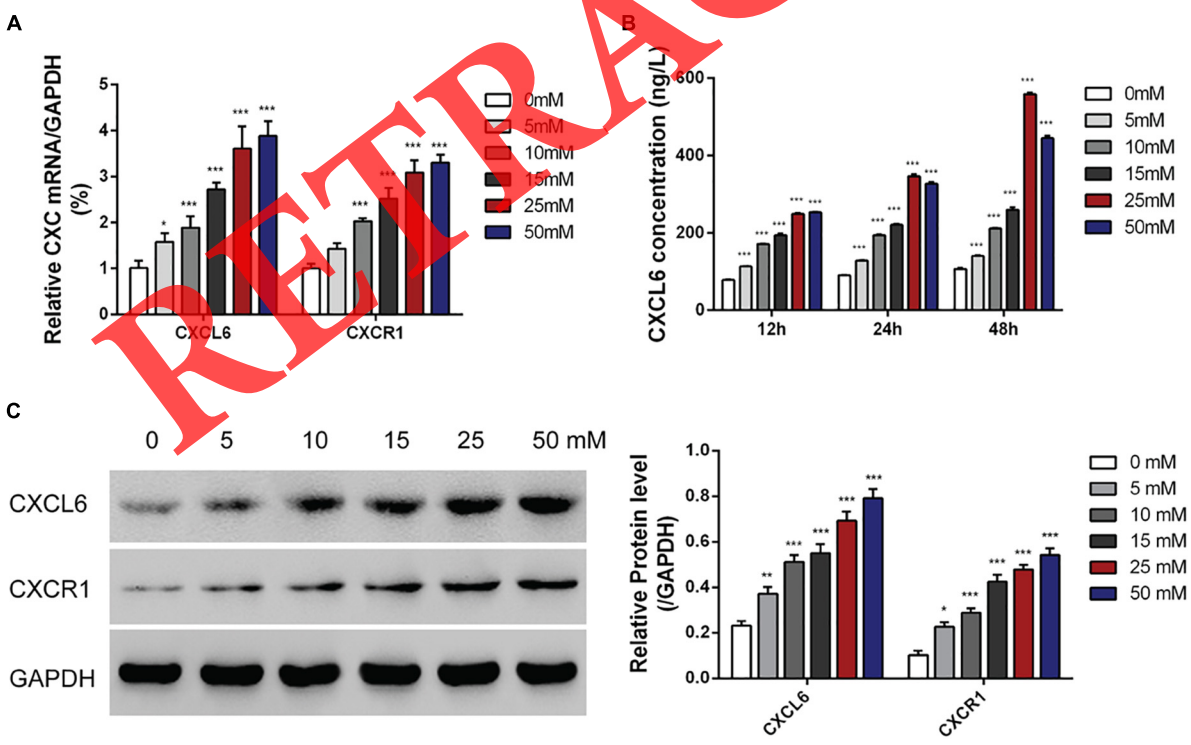

FIGURE 3 | Effect of glucose on the expression of CXCL6 in NRK-49F cells. Cells were cultured with several concentrations of glucose (0, 5, 10, 15, 25, 50 mM) for 12 h, 24 h, and 48 h. CXCL6 expression level in NRK-49F was analyzed by qRT-PCR **(A)** and WB **(B)**. CXCL6 concentration in NRK-49F cell culture supernatants was measured by Elisa **(C)**. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

blot were showed in **Figures 2C,D**. The expression of CXCL6 and its receptor CXCR1 of kidney tissues in the DN model group was significantly higher in DN rat kidney tissue than in the control group.

Fibrosis-Related Cytokines Is High Expression in DN Rat Model

The level of fibrosis related cytokines (IL-6, TNF-α, TGF-β1, CollagenI/III, MMP2/9) in serum and the expression of CollagenI/III, MMP2/9 in kidney tissues were detected by Elisa, qRT-PCR and Western blot. As displayed in **Figure 2B**, the levels of IL-6, TNF-α, TGF-β1, CollagenI, CollagenIII, MMP2 and MMP9 were increased significantly when compared with the control group. And the expression of CollagenI/III, MMP2/9 of kidney tissues were significantly upregulated at both mRNA and protein levels in the DN model group of animals compared with the control group (**Figures 2C,D**).

Glucose Increases the Expression of CXCL6 in NRK-49F Cells

Then effects of glucose on the expression of CXCL6 and its receptor CXCR1 in rat renal interstitial fibroblasts NRK-49F cells were detected by qRT-PCR and Western blotting after treatment with 5 mM, 10 mM, 15 mM, 25 mM, and 50 mM glucose, and further detected by ELISA. The results showed that 10 mM, 15 mM, 25 mM, 50 mM glucose significantly increased the expression of CXCL6 and CXCR1 both at mRNA (**Figure 3A**) and protein level (**Figure 3C**) in a concentration-dependent manner, furthermore the expression of CXCL6 increased with the treatment time of glucose (**Figure 3B**).

CXCL6 Promoted the Proliferation of NRK-49F Cell

To further investigate the function of CXCL6 in high glucose-induced NRK-49F cells, lentivirus were transfected into high glucose (25 mM) induced NRK-49F, then the expression of

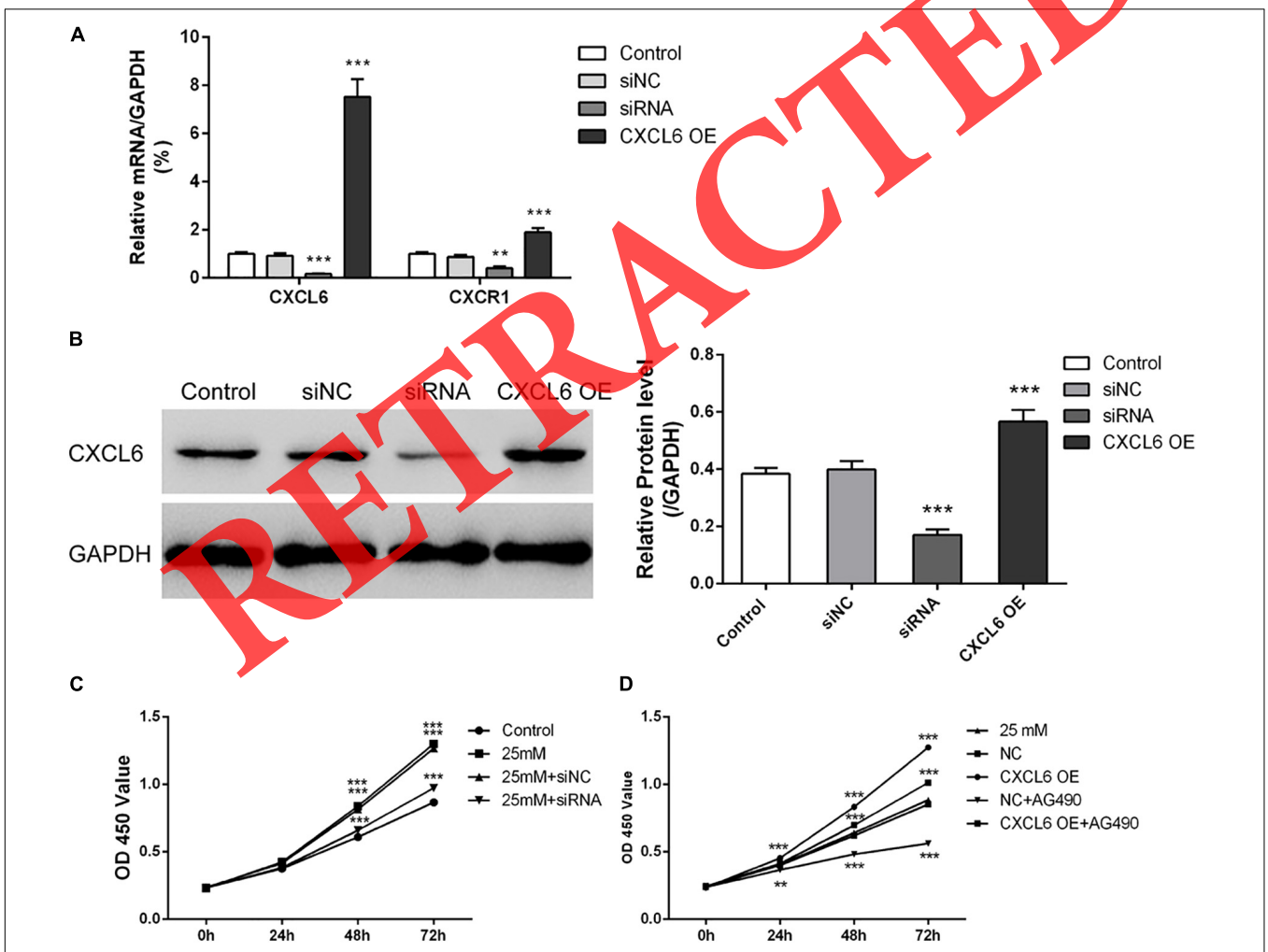

FIGURE 4 | Effect of CXCL6 on the proliferation of high glucose-induced NRK-49F. The efficiency of CXCL6 shRNA viral or overexpression lentivirus in silencing or over-expressing CXCL6 in NRK-49F cells was determined by qRT-PCR **(A)** and WB **(B)**. Following this, cck8 assays were performed to evaluate high glucose-induced NRK-49F cell viability when CXCL6 was either knocked down **(C)** or over-expressed **(D)**. $^{**}P < 0.01$, $^{***}P < 0.001$.

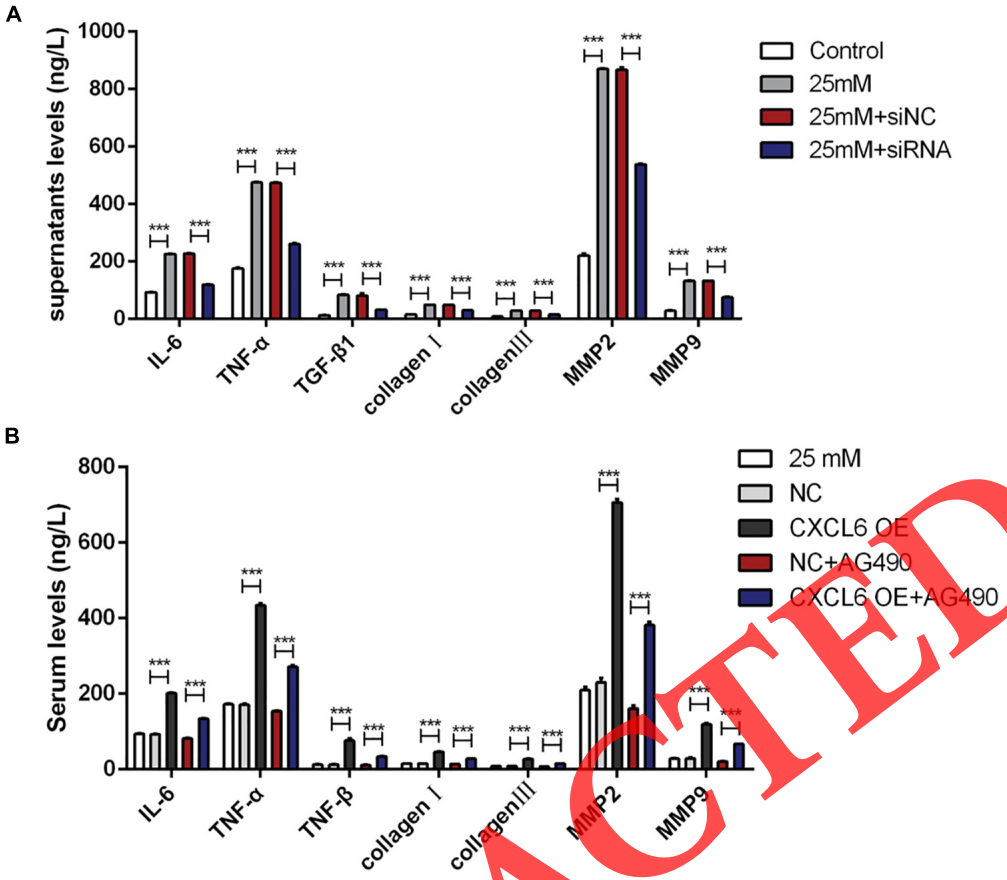

FIGURE 5 | Effects of CXCL6 on fibrosis related factors in high glucose-induced NRK-49F. The levels of IL-6, TGF-α/TGF-β1, Collagen I/III and MMP2/MMP9 in CXCL6 knocked down **(A)** and CXCL6 overexpressed **(B)** NRK-49F cell culture supernatants were determined by Elisa assay. ***$P < 0.001$.

CXCR1 and CXCL6 were measured by RT-PCR and Western blotting. As shown in **Figures 4A,B**, compared with control, CXCL6 gene interference and over-expression resulted in a significant downregulation or upregulation of CXCL6 both at the mRNA and protein levels.

Then the effects of CXCL6 on high glucose-induced NRK-49F cells viability were investigated by CCK-8 assay. As shown in **Figure 4C**, high glucose (25 mM) increased the proliferation of NRK-49F cells strikingly. However, the proliferation of NRK-49F cell induced by high glucose was decreased markedly by knockdown CXCL6. Overexpression of CXCL6 significantly promoted the proliferation of high glucose (25 mM) induced NRK-49F cell and the addition of 30 uM JAK inhibitor AG490 obviously reduced the proliferation of NRK-49F induced by CXCL6 overexpression (**Figure 4D**).

CXCL6-Associated Signaling Pathways

To probe the CXCL6-associated pathways in fibrosis, GSEA were performed using RNA-sequencing data from the GEO DataSets (Asscess ID: GSE28221). JAK-STAT and CYTOKINE _CYTOKINE _RECEPTOR _INTERACTION signaling pathways were found to be strongly associated with CXCL6 expression (**Figure 6A**).

CXCL6 Increased the Expression of Fibrosis-Related Cytokines in NRK-49F Cells

The expression of IL-6, TNF-α, TGF-β1, CollagenI, CollageIII, MMP2 and MMP9 in the cell supernatant was detected by ELISA. As shown in **Figure 5**, the expression of these cytokines was increased by high glucose concentration (25 mM) and overexpression of CXCL6 showed the same effect. On the contrary, the expression of these cytokines was decreased sharply by knockdown of CXCL6and 30 uM JAK inhibitor AG490 significantly decreased CXCL6 overexpression-induced fibrosis-related cytokines expression. The expression of CollagenI/III, MMP2/9, PCNA, and p-STAT3 intracellular were evaluated by western blot. In **Figures 6B,C**, the results were consistent with that from ELISA. 25 mM glucose and overexpression of CXCL6 markedly promoted the expression of CollagenI/III, MMP2/9, PCNA, and p-STAT3. Knockdown of CXCL6 and treatment with 30 uM JAK inhibitor AG490 drastically decreased their expression. The expression of STAT3 was

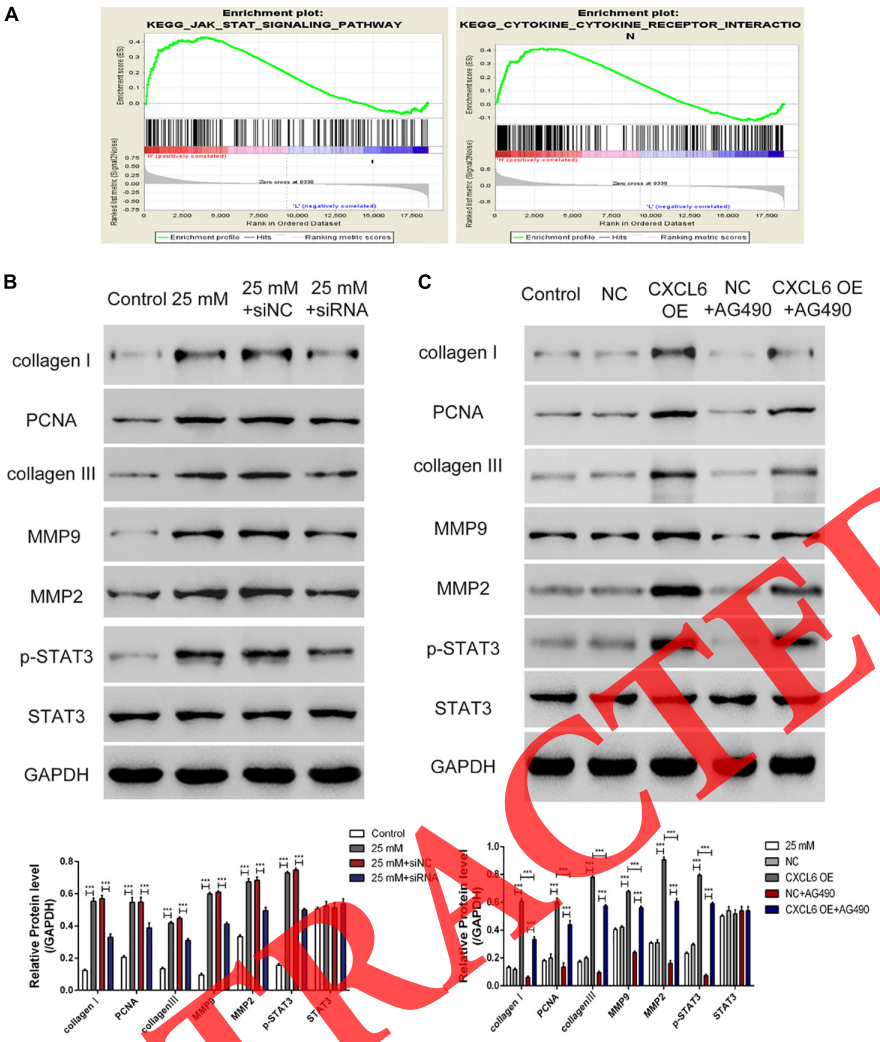

FIGURE 6 | CXCL6-associated pathways in DN. **(A)** GSEA was performed using GEO DataSets (Asscess ID: GSE28221). The JAK/STAT and CYTOKINE _CYTOKINE _RECEPTOR _INTERACTION pathways were identified with the strongest association with CXCL6 expression. **(B,C)** Key moderators were determined by western blotting. Data were based on at least three independent experiments, and shown as mean ± SD. ***$P < 0.001$.

also measured, and no significant change was observed in all the groups.

DISCUSSION

CXCL6 is a member of the chemokine family and plays a crucial role in experimental bleomycin-induced pulmonary fibrosis (Besnard et al., 2013). However, previous studies did not clarify the roles of CXCL6 in DN renal interstitial fibrosis. In this study, we revealed the overexpression of CXCL6 in DN and explored the underlying mechanism of CXCL6 in DN renal interstitial fibrosis.

There is emerging evidence that renal interstitial fibrosis is the most important pathologic feature of DN (He et al., 2016). Excessive deposition of ECM is the main reason of renal interstitial fibrosis (Picard et al., 2008) and it is widely believed that renal interstitial fibroblasts are the main cells which play an important role in renal interstitial fibrosis due to producing excessive ECM (Faulkner et al., 2005). High glucose concentration, a distinctive risk factor in diabetes, is a potential stimulus for the proliferation, differentiation, and survival of certain types of cells (Lee and Han, 2010; Yuan et al., 2011; Le et al., 2012). Hence we explored the impacts of glucose on renal interstitial fibroblasts NRK-49F cell proliferation. The results from cck8 assay indicated that (10, 15, 25, 50 mM) glucose significantly promoted the proliferation of NRK-49F cells. Meanwhile, CXCL6 and CXCR1 expression were also dramatically induced by high glucose in a dose-dependent manner, demonstrating that CXCL6 may be a key factor in high glucose-induced renal fibrosis.

Then we examined the functions of CXCL6 in renal interstitial fibrosis. CXCL6 shRNA lentivirus was transfected into NRK-49F and we confirmed that CXCL6 shRNA viral transfection markedly reduced CXCL6 expression at protein

levels and partially attenuated the pro-proliferation effects of high glucose. On the contrary, ectopic expression of CXCL6 in NRK-49F significantly promoted the proliferation of NRK-49F. These data suggest that increasing the expression of CXCL6 contributes to high glucose-induced renal fibroblasts proliferation and activation.

In addition to cellular phenotype, we explored the molecular mechanism by which CXCL6 functions as an pro-fibrotic factor in DN. Overexpression of CXCL6 remarkably up-regulated the expression of pro-inflammatory cytokine (such as TNF-α and IL-6) and pro-fibrotic markers (such as TGF-β, and collagen). Moreover, The JAK/STAT signaling pathway has previously been shown to implicate in the pathophysiology of fibrotic renal disease (Matsui and Meldrum, 2012; Matsui et al., 2017). Increasing the activity of genes involved in JAK/STAT signaling pathway may mediate renal fibrosis. In the present study, GSEA data indicated that higher CXCL6 expression was positively correlated with JAK/STAT pathways in fibrosis (**Figure 5A**). The expression of JAK/STAT and CYTOKINE _CYTOKINE _RECEPTOR _INTERACTION pathway related factors was significantly suppressed by CXCL6 knockdown, while overexpression of CXCL6 in NRK-49F cells caused an inverse effect. With the addition of JAK inhibitor AG490, the proliferation of NRK-49F and the expression of these related factors induced by overexpression CXCL6 were inhibited significantly. These data suggest the roles of CXCL6 on JAK/STAT3, and may account for renal fibroblasts proliferation and renal interstitial fibrosis.

Approximately 30–40% of patients with diabetes develop nephropathy (Tesch, 2017) and the exact pathogenesis of DN is complex. Pathogenetic factors include hyperglycaemia, increased intraglomerular, the immune system, and systemic blood pressure, etc., Moreover, several cytokines and growth factors, which have complex mutual interactions, have been identified and may cause damaging effects on the kidney (Kim and Park, 2017). Renal response on these noxious effects further enhances renal damage. Although a number of therapeutic interventions have been shown to postpone the development or slow down the progression of DN, the proportion of ESRD due to DN remains high (Jin, 2015). Therefore, future interventions of other potential pathogenic factors were needed. Moreover, the use of representative animal models to defined clinical trial inclusion criteria may help to identify appropriate DN therapies.

CONCLUSION

In conclusion, this study found that CXCL6 is overexpression in DN patients and the DN rat model, and the expression of fibrosis-related cytokines was consistent with the expression of CXCL6. The results of *in vitro* experiment showed that high glucose concentration promotes renal fibroblasts proliferation and CXCL6 expression in NRK-49F. Knockdown of CXCL6 significantly inhibited the proliferation of NRK-49F and decreased the expression of pro-inflammatory and pro-fibrotic cytokines, while overexpression of CXCL6 exhibited the opposite phenomenon. In addition JAK inhibitor AG490 showed the same effect as knockdown of CXCL6 and it is likely that CXCL6 may promote fibrosis-related factors to accelerate the development of DN renal interstitial fibrosis by activating JAK/STAT3 signaling pathway. These findings not only broaden our understanding of the pathogenesis of renal DN but also provide a new attractive strategy in treating this disease. Due to the complexity and heterogeneity of the DN, further investigations of the molecular mechanisms of CXCL6 in DN are required to verify the function of CXCL6 in DN.

AUTHOR CONTRIBUTIONS

M-YS and S-JW performed qRT-PCR and western blot assays and wrote the manuscript. X-QL contributed to the animal experiments. Y-LS and X-HT contributed to cell culture and data analyses. KR revised the manuscript. J-RL and HN collected clinical samples and implemented bioinformatics analysis. HZ and L-JZ applied for grants, designed the experimental protocols, and directed manuscript writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00224/full#supplementary-material

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