IFI16 induces inflammation in Hepatitis B Virus-Associated Glomerulonephritis by Regulating the Caspase-1 /IL-1ß pathway

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Abstract

Aims & background

IFI16 plays an important role in innate immunity against invasive microbial infection by sensing double-stranded DNA viruses due to caspase-1-dependent inflammasome activation and subsequent maturation and secretion of IL-1β. However, the role of IFI16 in regulating the immune response to viruses in Hepatitis B Virus-Associated Glomerulonephritis(HBV-GN), especially in sensing the hepatitis B virus (HBV), has not been determined. In this study,, we investigated the inflammatory role of IFI16 in HBV-GN.

Methods

A total of 75 kidney tissues including 50 HBV-GN and 25 chronic glomerulonephritis (CCN) were collected to determine expression of IFI16, Caspase-1, and IL-1β by immunohistochemistry (IHC), and then the correlation between them was analyzed. In vitro, the overexpression or knockdown of IFI16 in regulating the immune response to HBV infection in the human glomerular mesangial (HGM) cell line and HEK-293T cell line. Quantitative Real-time PCR and western blotting were used to determine the expression of IFI16, Caspase-1 and IL-1β. The role effect of IFI16 in vivo was further investigated.

Results

IFI16 expression in HBV-GN biopsies (80.0%) was significantly higher than in CGN (24.0%) and was positively correlated with caspase-1 and IL-1β expression in HBV-GN. *In vitro*, over expression of IFI16 increased caspase-1 and IL-1β expression in HBV-infected HGM and HEK-293T cell lines, whereas knockdown of IFI16 mRNA by siRNA resulted in downregulation of the caspase-1 and IL-1β expression in both cell lines.

Conclusions

The elevation of IFI16 during HBV infection or replication may contribute to renal damage due to inflammation, thus providing a putative therapeutic target and a new avenue for studying the pathogenesis of HBV-GN.

1. Introduction

Hepatitis B virus (HBV) infection is an important public health problem worldwide, which causes both hepatic and extrahepatic organ injures. Hepatitis B virus-associated glomerulonephritis (HBV-GN) is the most common and well described extrahepatic manifestations of chronic HBV infection[1]. HBV-GN was first reported by Combes et al. in 1971[2], and since then cases have been described all over the world. The most widely accepted mechanism is the deposition of immune complexes of the viral antigen and host antibody in renal lesions by persistent HBV infection[3]. However, the immune pathogenesis of HBV-GN remains unclear.

IFI16 (Interferon-γ-inducible protein 16) belongs to the Pyrin-Hin200(HIN-200) family, and plays an important role in antiviral and immunomodulatory activities[4, 5]. As innate immune sensors, IFI16 recognizes both cytosolic and nuclear double-stranded DNA (dsDNA) from the invasion of many viruses, such as vaccinia virus (VACV), herpes simplex virus 1 (HSV-1), and Kaposi sarcoma-associated herpesvirus (KSHV)[6] [7, 8]. Following the detection of dsDNA viruses, IFI16 combines with the apoptosis-associated speck-like protein (ASC) and triggers downstream stimulator of the interferon genes-TANK-binding kinase1-interferon regulatory factor 3 (STING-TBK1-IRF3) signaling pathway[8, 9]. This response leads to the activation of caspase-1, which cleaves proinflammatory type I interferon (IFN-I) and interleukin-1ß (IL-1ß) to generate their active forms[10]. These inflammatory cytokines play critical roles in the host immunity against viral infection. In addition, overexpression of IFI16 leads to autoimmune diseases such as systemic lupus erythematosus (SLE) and Sjogren Syndrome (SjS) in which DNA is a major autoimmune target[11].

Recent evidences showed that IFI16 expression is associated with the degree of inflammation in acute and chronic hepatitis B [12, 13]. However, little is known about the roles of the DNA sensor IFI16 in HBV-GN pathogenesis. Studies showed that HBV DNA was also identified in the cells of the nephron unit and interstitial tissue from HBV-GN patients [14]. We propose that cytoplasmic and nuclear HBV-DNA in the renal tissues has high possibilities to be recognized by IFI16. The potential binding of IFI16 to HBV-DNA may lead to the activation of caspase-1 and subsequent maturation and secretion of IL-1β. This cascade of events leads to the development of the inflammasome, which may be responsible for the renal damage seen in HBV-GN patients.

To determine whether IFI16 expression is associated with HBV infection, we compared expression of IFI16 in HBV-GN and chronic glomerulonephritis (CGN). It was shown for the first time that higher IFI16 levels in HBV-GN compared with CGN, and IFI16 expression levels are associated with the HBV-GN inflammation degree. We further explored the relationship between expression of IFI16, caspase-1, and IL-1β in the HBV-GN group. Results showed that caspase-1 and IL-1β expression in HBV-GN was positively correlated with expression of IFI16.

To explore the mechanism by which IFI16 induces the inflammation in HBV-GN, we performed *in vitro* experiments using primary human glomerular mesangial (HGM) cells and HEK-293T cells. The cell lines were co-transfected with HBVDNA and overexpressed or silenced IFI16. Then the expression levels of IFI16□caspase-1, and IL-1β were investigated. It was demonstrated that overexpression of IFI16 increased expression of caspase-1 and IL-1β, while knockdown of IFI16 resulted in the efficient reduction of those inflammatory cytokine expressions in the cells transfected with HBV. Thus, IFI16 may play an important role in the development and progression of HBV-GN inflammation.

2. Materials And Methods

2.1 Patients

Our retrospective study was approved by the ethics committee of the Jinan Infectious Disease Hospital (JCLL-2016-04). A total of 75 patients diagnosed with chronic nephritis between 2008 and 2016 at the Jinan Infectious Disease Hospital and Qilu Hospital of Shandong University, Shandong Province, China, were included in the study. The experimental group consisted of 50 HBV-GN patients and the negative control group consisted of 25 CGN patients. Each patient received kidney puncture biopsy under ultrasound guidance to attain nephridial tissue for diagnosis and subsequent study. Participation was dependent upon fulfillment of the following criteria:(1) patients must not have used an immune agent or antiviral agent in the past three months; (2) patients must not have HAV, HCV, HDV, HEV, or HIV co-infection; (3)patients must not have a history or current evidence of secondary glomerulonephritis; and (4) consent for participation must have been obtained from those who participated.

2.2 Diagnosis of HBV-GN, CGN and pathological classification of HBV-GN

The diagnostic criteria used for CGN and HBV-GN were in accordance with the 2002 Kidney Disease Outcome Quality Initiative (K/DOQI), edited by the National Kidney Foundation (NKF) [15]. The diagnosis of HBV-GN was confirmed by pathology. The pathological classification of and diagnostic criteria used for HBV-GN were in accordance with 1990 WHO classification criteria [16]. Frozen slices from biopsies of the 50 HBV-GN patients were kept in a low-temperature freezer. Monoclonal goat-anti-human HBsAg and HBcAg antibodies were purchased from Dako (Denmark), and immunohistochemical staining for HBsAg and HBcAg in renal biopsies and an electron microscope was used for detection of HBV to confirm the diagnosis (Figs. 1 and 2A). For HBV-GN patients with undetectable HBsAg or HBcAg in nephridial tissue, HBV was detected using the JCM-6000 scanning electron microscope from Jeol. Ltd (Japan). Sections from all biopsy specimens were routinely stained with hematoxylin and eosin (H&E), periodic acid-sliver methenamine (PASM), Masson's trichrome, and antibodies against IgA, IgG, IgM, C3, and C1q complement component. Fluorescently-labeled IgA, IgG, IgM, C3, and C1q rabbit-anti-human antibodies were purchased from Dako.

2.3 Immunohistochemistry and scoring

Immunohistochemistry was carried out using standard techniques. Nephridial and hepatic tissue specimens were first fixed in 10% formalin, then the tissue was cut, dehydrated, dipped in wax, embedded, and sectioned. These sections were then placed on slides, baked, placed into xylene, cleared of the wax, rehydrated using graded ethanol, and immersed in 0.3% hydrogen peroxide for five minutes to reduce non-specific background staining caused by endogenous peroxidase. The slides were then washed with PBS buffer three times for five minutes each, placed in citrate buffer solution at a pH of 6.0, and then into a high temperature pressure pot to recover the tissue antigen. After being heated, the slides were cooled and restored to room temperature, washed three more times in PBS buffer, and incubated with AIM2 (ab93015, rabbit anti-human polyclonal antibody, Abcam, USA), caspase-1 (sc-56063, mouse anti-human polyclonal anti- body, Santa Cruz Biotechnology Inc., USA), and IL-1β antibodies (ab2105, rabbit anti-human polyclonal anti-body, Abcam), respectively. The slides were then placed in a 4°C refrigerator

overnight. The next day, the slides were washed with PBS buffer three times, each time lasting longer than five minutes, then incubated with the secondary antibody PV-9000 (universal antibody) at 37°C for 10 minutes, washed with PBS buffer, and DAB stain was applied. The stain was terminated using running water, and then the slides were washed with hydrochloric acid alcohol for differentiation. Finally, the slides were washed with distilled water, cleared with xylene, and mounted. Appearance of a tan stain in the cytoplasm signaled positive expression of the protein. After staining, scores were assigned based on stain intensity and percentage of positive cells as follows. For stain intensity, a score of 0 was given for no brown staining (i.e., no cells stained), 1 for light brown, 2 for brown, and 3 for dark brown. For percentage of positive cells, a score of 0 was given for fewer than 5% positive cells, 1 for 5–30%, 2 for 30–60%, and 3 for greater than 60%. Scores for stain intensity and percent positive were then added together, and a negative sign (−) was assigned for scores totaling 0, mildly positive (+) for scores between 1 and 3, moderately positive (++) for scores between 4 and 6, and strongly positive (+++) for scores greater than 7.

2.4 Cell lines and reagents

The human glomerular mesangial (HGM) cell line and HEK-293T cell line used in this study were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (Life Technologies, Carlsbad, CA, USA), ampicillin and

streptomycin at 37°C under 5% CO_2. HBV expression plasmids were constructed with a pcDNA3.0 vector. The 1.1-fold over length HBV genome was cloned into the pcDNA3 vector to generate pcDNA3.0-1.1HBVDNA, 1.1HBV as the expression gene and ampicillin resistance for antibiotic selection (Amresco, Penn- sylvania, USA). We designed three kinds of IFI16-siRNAs for this experiment, which were synthesized by Gene Pharma (Shanghai, China).. The siRNA was synthesized accordingly:

siRNA #1: GGAAGUGGAUGCUACUUCAdTdT;

siRNA #2: GGAAUAUGAUAGUCUCCUAdTdT;

siRNA #3: GGAAGUGGAUGCUACUUCAdTdT;

siRNA negative control: GAUGAGAUUAGAUACUCUCdTdT.

IFI16-siRNA#2 was selected as the most effective silencer compared with the others, which was used for the following experiment (Fig. 4a). IFI16, Caspase-1, and IL- 1β antibodies were obtained from Cell Signaling Tech Abcam (Cambridge, MA, USA).

2.5 Cell transfection

The HGM cell line and HEK-293T cell line were seeded into 12-well plates, then the cells were transfected with either pcDNA3.0-1.1HBVDNA- IFI16 and negative control for overexpression studies, or with siIFI16

and a scrambled siRNA for knockdown studies. Lipofectamine 2000 (Invitrogen) was used according to the manufacturer's instructions with minor modifications for transfection studies. IFI16 overexpression and knockdown were confirmed by qRT-PCR and Western blot 48 h post transfection.

2.6 Western blot

According to the manufacturer's instructions, whole cell protein extracts were prepared and were separated using 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis. Proteins were then transferred to a polyvinylidene difluoride membrane (Millipore, Bed- ford, MA, USA), according to the instruction manual. Membranes were blocked overnight in 5% w/v low- fat dry milk in 10 mmol/L Tris-HCl, pH 7.5, 0.1 mol/L NaCl and 0.1% Tween- 20 and incubated with primary antibodies overnight at 4°C. After washing with TBST buffer, the blots were then incubated with HRP- conjugated secondary antibody for 2 hours at room temperature. After washing with TBST buffer, immunoreactive bands were visualized using the ECL- Plus reagent (Millipore, Billerica, MA, USA). GAPDH was used as the loading control in Western blotting.

2.7 RNA Isolation and qRT-PCR

Total RNA was isolated using Trizol RNA reagent (Invitrogen, California, USA). Quantitative real- time PCR was performed, and the expression levels of IFI16, caspase-1, and Il-1ß mRNA were normalized to GAPDH for gene expression. The primers are listed in Table 3.

Table 3
Primers' sequences used in qRT-PCR in this study

ID	Sequence (5'-3')
IFI16 F	AGCTCAGAACCCGAAAACAG TCTGTGTAGCCACTGTAGCA
IFI16 R	GTTCCATGGGTGAAGGTACA GACATTCCCTTCTGAGCCTG
caspase-1 F	AGCTACGAATCTCCGACCAC CGTTATCCCATGTGTCGAAGAA
caspase-1 R	TGTTCGTCATGGGTGTGAAC
Il-1ß F	ATGGCATGGACTGTGGTCAT
Il-1ß R	
GAPDH F	
GAPDH R	

F: forward primer; R: reversed primer.

2.8 Statistical Analysis

The SPSS program (version 19.0) and GraphPad Prism (version 8.0) were used for analysis. Measurement data are described as mean ± standard deviation. Background factors were compared using the Student's-test (numerical data) or the Chi-square test (categorical data). The Spearman's two-

tailed test was used for correlation analysis, and differences were regarded as significant if the *p* value was less than 0.05 on either side.

3. Results

3.1 Expression of IFI16 was significantly higher in HBV-GN compared with CGN

To measure expression of IFI16 in the kidneys of 50 HBV-GN and 25 CGN patients, we used immunohistochemistry with anti-IFI16 to probe sections of paraffin-embedded samples. First, we found different degrees of damage in the renal tissue in all patients. and different HBV associated antigens deposition status and pathological types in HBV-GN patients, as demonstrated by H&E staining (Fig. 2a). IFI16 expression was observed in the cytoplasm of mesangial cells and glomerular endothelial cells, especially in the HBV-GN patients. Statistical analysis revealed that the positive expression rate of IFI16 in HBV-GN patients was significantly higher than in CGN patients (80% versus 24%, p□0.01) (Table1).

Table 1
Expression of IFI16 was significantly higher in HBV-GN compared with CGN

group	Tissue	Cases(n)	Age	Gender(M %)	IFI16				Positive Rate(%)
					-	+	++	+++	
HBV-GN	K	50	36.1 ± 12.7	35(70%)	10	14	26	0	80
CGN	K	25	38.2 ± 15.5*	18(72%)**	19	6	0	0	24***

HBV-GN: Hepatitis B viral associated glomerulonephritis; CGN: Chronic glomerulonephritis; K: kidney; M: Male; *compared with HBV-GN,(t = -0.072,p = 0.943); **compared with HBV-GN, (x2 = 0.032, p = 0.85); ***compared with HBV-GN, N, (x2 = 30.091, p < 0.01)

3.2. Expression of IFI16 was positively correlated with Caspase-1 and IL-1ß in HBV-GN tissue

Expression of Caspase-1 and IL-1ß in biopsied kidney tissues from the 50 HBV-GN patients was investigated, The results showed that the positive sites of caspase-1 and IL-1β were mainly located in glomeruli, with focal distribution in renal tubules and renal interstitium in the HBV-GN tissue (Fig. 2C, D). The correlation between expression of IFI16 and Caspase-1 and IL-1ß was analyzed. Statistical analysis revealed that expression of IFI16 was positively correlated with that of Caspase-1(r = 0.998, p□0.01) and IL-1ß (r = 0.953, p□0.05) (Table2). These results suggested that expression of IFI16 was correlated with inflammation in HBV-GN tissue and that the elevation of IFI16 may be responsible for the inflammatory damage of HBV-GN.

Table 2
Expression of IFI16 was positively correlated with Caspase-1 and IL-1ß in HBV-GN tissue

IFI16	Caspase-1				Il-1ß			
	-	+	++	+++	-	+	++	+++
+++	0	0	0	0	0	0	0	0
++	5	5	14	2	3	10	12	1
+	0	4	10	0	1	4	9	0
-	6	4	0	0	5	4	1	0
rs	0.998				0.953			
p	< 0.01				< 0.05			

3.3. Over expression of IFI16 promoted inflammation *in vitro*

According to the components and structure of glomerular prophase cells, HGM cells and HEK-293T cell lines were used to establish an HBV infection model *in vitro*. The cells were divided into three groups: the IFI16 and HBV DNA co-transfected group (over expression group, OE), the HBVDNA-transfected group without IFI16 (negative control group, NC), and the empty plasmids transfected group (blank group, Blank). The mRNA and protein expressions of IFI16, caspase-1, and IL-1 β were detected by Western blot and qRT-PCR. Results show that expression of IFI16, Caspase-1, and IL-1ß protein levels were significantly elevated in the OE group compared those in NC and Blank groups. There were no difference in mRNA levels. The data indicated that over expression of IFI16 resulted in upregulation of Caspase-1 and IL-1ß protein levels.(figure-3).

3.4. Knockdown of IFI16 reduced inflammation *in vitro*

To determine whether reduction in the IFI16 level could reverse the inflammation observed in the overexpression assay described above, we down-regulated the gene expression of IFI16 infected HGM cells and HEK-293T cells using siRNA (siIFI16). The cell lines were divided into three groups: the IFI16-siRNA and HBV DNA co-transfected group (si IFI16 group), HBVDNA-transfected group without siIFI16 (negative control group, NC), and the empty plasmids transfected group (blank group, Blank). By 48h post transfection, there was an approximately 90% decrease of IFI16 mRNA levels in HGM cells and 95% decrease in HEK-293T cells in the siIFI16 group. (Fig. 4b). Compared to the blank group, siRNA-mediated knockdown of IFI16 resulted in a 6.14-fold decrease of caspase-1, a 6.16-fold decrease of IL-1β in HGM cells, and a 5.21-fold decrease of caspase-1 and a 10.92-fold decrease of IL-1β in HEK-293T cells. but no reduction in their mRNA levels. (Fig. 4c).

4. Discussion

Hepatitis B virus associated glomerulonephritis (HBV-GN) is one of the most common HBV infection associated diseases, and it is present in 3–5% of patients with chronic HBV infection[17]. HBV-GN has been described and classified into four different subtypes, including membranous nephropathy, membranoproliferative glomerulonephritis, and mesangial proliferative glomerulonephritis. However, the specific pathogenesis and role of HBV in HBV-GN is not yet clear. HBV contains circular and partially double-stranded DNA (dsDNA). Increasing immunological evidence and electron microscope findings have suggested that HBV-DNA replication directly occurs in HBV-GN kidney tissue[18, 19]. HBV is not directly cytopathic in HBV-GN. The widely accepted view is that the immune complex including circulating and *in situ* immune complexes deposited in the basement membrane and/or in the mesangium induces immune damage[20]. and the autoimmune response may play an important role in the pathogenesis of HBV-GN[21, 22]. More recently, several reports have indicated that the HIN 200 family (hematopoietic interferon-inducible nuclear proteins with a 200-amino acid repeat) appear to be involved in this autoimmunity[23].

The HIN 200 family contains one or two C-terminal DNA binding HIN domains and an N-terminal homotypic protein–protein interactions pyrin domain (PYD). Currently, the HIN 200 family includes four members: AIM-2 (absent in melanoma 2), IFIX (interferon-inducible protein X), MNDA (Myeloid cell nuclear differentiation antigen), and IFI16. Studies showed that AIM2 can stimulate inflammasome activation and interact with ASC[24], and that the AIM2-ASC inflammatory signaling pathways activation may occur during HBV infection[25]. Notably, AIM2 has been shown to be a receptor for HBV DNA that regulates activation of caspase-1,which leads to the release of IFN- β[13]. Our previous work also showed that expression of AIM2 was significantly higher in HBV-GN patient kidney tissues, and the binding of HBV DNA to AIM2 appears to trigger an immune response causing renal damage[23, 26]. IFI16 is related to the DNA sensor AIM2, and its surface is similar to AIM2 allowing it to bind to dsDNA[27]. What is more, IFI16 is the only member of this family with two HIN domains, which potentially increase its DNA-binding ability[28] and the immune damage.

In this study it was found that expression of IFI16 was significantly elevated in HBV-GN kidney tissues compared with that in CCN kidney tissues. Interestingly, we found the IFI16 expression was not significantly different between the HBV parameters or the HBV-GN subtypes. The possibility is that beyond activation of IFI16, HBV DNA may have other actions. Just as HCMV has positive and negative effects on IFI 16 for HCVM tegument protein pUL83 can disrupt IFI16 and inhibit the immune reaction[29].

Further it was found that IFI16 was exclusively localized in the cytoplasm of glomerular endothelial cells and mesangial cells, not in the nucleus. This was similar to SLE in which IFI16 is localized in the cytoplasm of keratinocytes[30]. IFI16 expression in normal skin cells is restricted to the nuclei, while it is translocated to the cytoplasm in SLE and ultraviolet-induced cell injury. Furthermore, during human cytomegalovirus (HCMV) infection, IFI16 is translocated into the cytoplasm during the early stage and

improves vesicle sorting and binding ability[31].The overexpression and extranuclear appearance of IFI16 leads to the formation of inflammasomes and eventually induces a specific autoimmune reaction cascade[30]. Thus, this translocation of IFI16 is likely to enhance its protein function.

We found that expression of IFI16 was positively correlated with expression of Caspase-1and IL-1ß, which may then be responsible for the renal damage seen in HBV-GN patients. We infer that after sensing dsDNA, IFI16 leads to the formation of inflammasomes[7], which also causes the activation of caspase-1 and subsequently the maturation and secretion of IL-1β. IL-1β is not only crucial to innate immune defense, but is also an important mediator of adaptive immune response to viral infection[32]. Thus, the Caspase-1/IL-1β pathway is critical for the clearance of pathogens or damaged cells in HBV-GN, and IFI16 may play an important role in disease pathogenesis.

To further verify if IFI16 induces the ASC-dependent inflammasome pathway through the Caspase-1/IL-1ß pathway leading to kidney injury, we first analyzed expression of IFI16 and Caspase-1and IL-1ß in over expression IFI16 cells transfected with HBV, and the results demonstrated that protein levels of IFI16, Caspase-1, and IL-1ß were significantly elevated in the OE group compared those in NC and Blank groups, respectively. but the mRNA levels of Caspase-1and IL-1ß remained unchanged. Then we used siRNA-mediated knockdown of IFI16 cells transfected with HBV, and found that the downregulation of IFI16 in the two cell lines could directly inhibit expression of caspase-1 and IL-1β at protein levels, but not mRNA levels. The results suggested that this inflammation signal transfer pathway is related to IFI16 levels in HBV-GN, and the intrinsic mechanism of Caspase-1/ IL-1ß activation is not in the transcription level but the self-cleavage induced by IFI16. However, the specific interactions that occur between IFI16 and Caspase-1/ IL-1ß activation in HBV-GN are still unknown and need further exploration.

Conclusion

This study revealed that expression of IFI16 was significantly increased in HBV-GN patients. The elevation of IFI16 during HBV infection or replication may contribute to renal damage by regulating the Caspase-1/ IL-1β pathway, which induces the inflammatory response. Our findings suggest that IFI16 plays an important role in the pathogenesis of HBV-GN, which may serve as a target molecule for diagnosing and treating HBV-GN.

Abbreviations

HBV: Hepatitis B virus; HBV-GN: Hepatitis B virus associated glomerulonephritis; CN: chronic nephritis; CCN: chronic glomerulonephritis; PHI-200:Pyrin-Hin200 family; IFN-I: type I interferon; IFI16: Interferon-g-inducible protein 16; ASC: Apoptosis-associated speck-like protein; IL-1β: Interleukin-1β; HGM: human glomerular mesangial; VACV: vaccinia virus; HSV-1:herpes simplex virus 1; KSHV: Kaposi sarcoma-associated herpesvirus; dsDNA: double-stranded DNA; HAV: Hepatitis A virus; HCV: Hepatitis C virus; HEV: Hepatitis E virus; HIV: Human immunodeficiency virus; K/DOQI: Kidney Disease outcome quality initiative; NKF: National Kidney Foundation; H&E: Hematoxylin and eosin; PASM: Periodic acid-sliver methenamine;

MsPGN: Mesangio proliferative glomerulonephritis; MPGN: Membranoproliferative glomerulonephritis; MN: Membranous nephropathy; MCG: Minimal change glomerulopathy; FSS: Focal segmental sclerosis.

Declarations

Ethical Approval and Consent to participate

This study was approved by the ethics committee of the Jinan Infectious Disease Hospital (JCLL-2016-04).

Consent for publication

Not applicable.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None of the authors has an affiliation or conflict of interests.

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Authors' contributions

Study concept and design: DWJ, Acquisition of data: LLand XSS, Analysis and interpretation of data: GY and ZXH, Drafting of the manuscript: LL, Critical revision of the manuscript for important intellectual content: DWJ, Statistical analysis: GY and LXY, Administrative, technical or material support: XSS, GY and LQ, Study supervision: LQ. All authors read and approved the final manuscript.

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Authors' information

See information below the heading.

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Figures

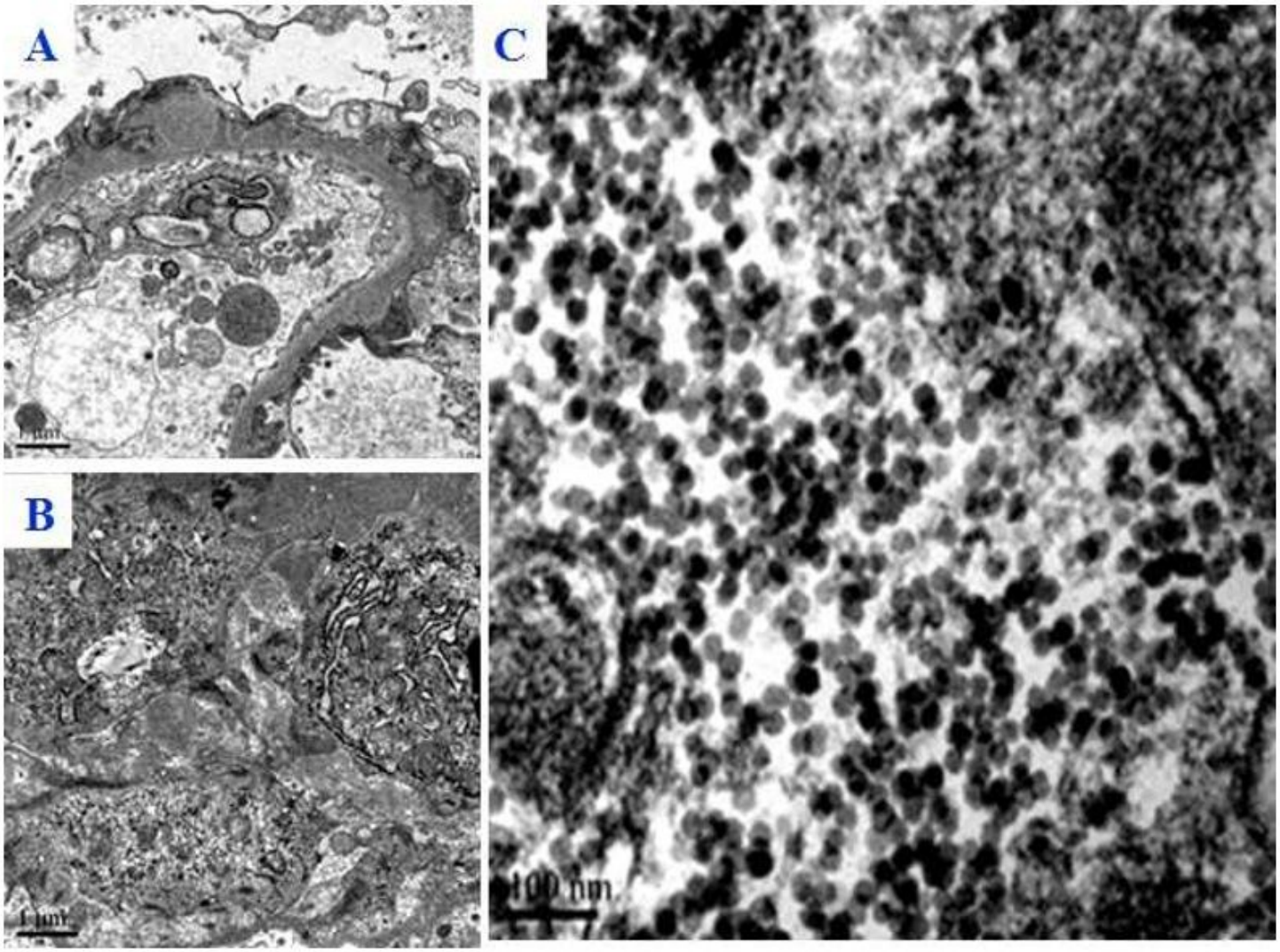

Figure 1

A; Subepithelial granular electron compact deposited in the basement membrane of capillary wall in HBV-GN. Transmission electron microscopy, 7500× B; Organelles were abundant and swollen in the cytoplasm, and more virus-like particles were deposited. Transmission electron microscopy, 7500× C; Intracytoplasmic globular virus-like particles. Transmission electron microscopy, 30000×

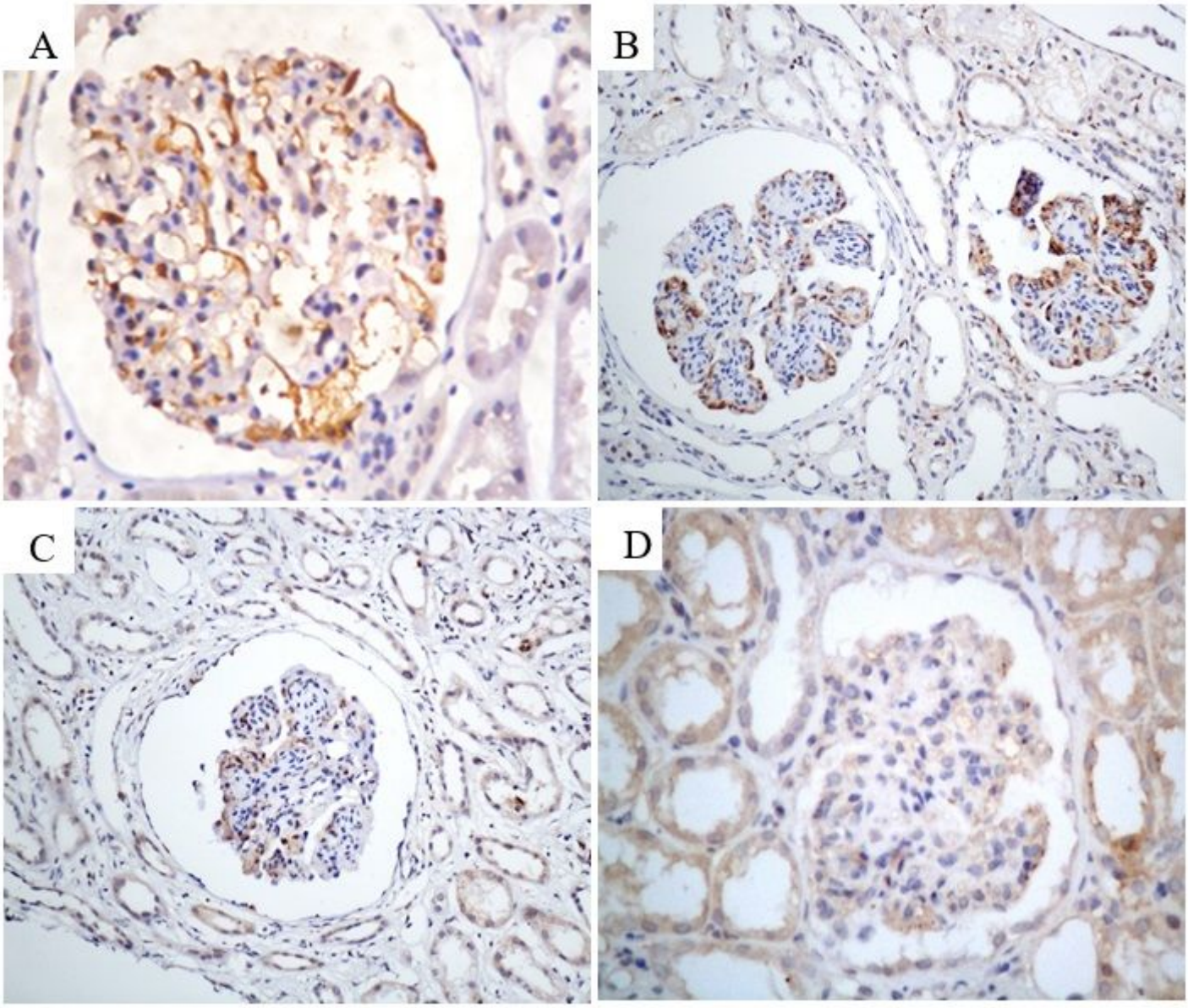

Figure 2

A; The immunohistochemical staining of HBcAg was positive, and the brown-yellow granules were distributed along the capillary wall of the glomerulus. IHC ×400× B; IFI16 was positively expressed in the cytoplasm and nucleus of glomerular cells in HBV-GN. IHC ×400× C; The positive expression of caspase-1 in HBV-GN. IHC ×400× D; The positive expression of IL-1β in HBV-GN. IHC ×400×

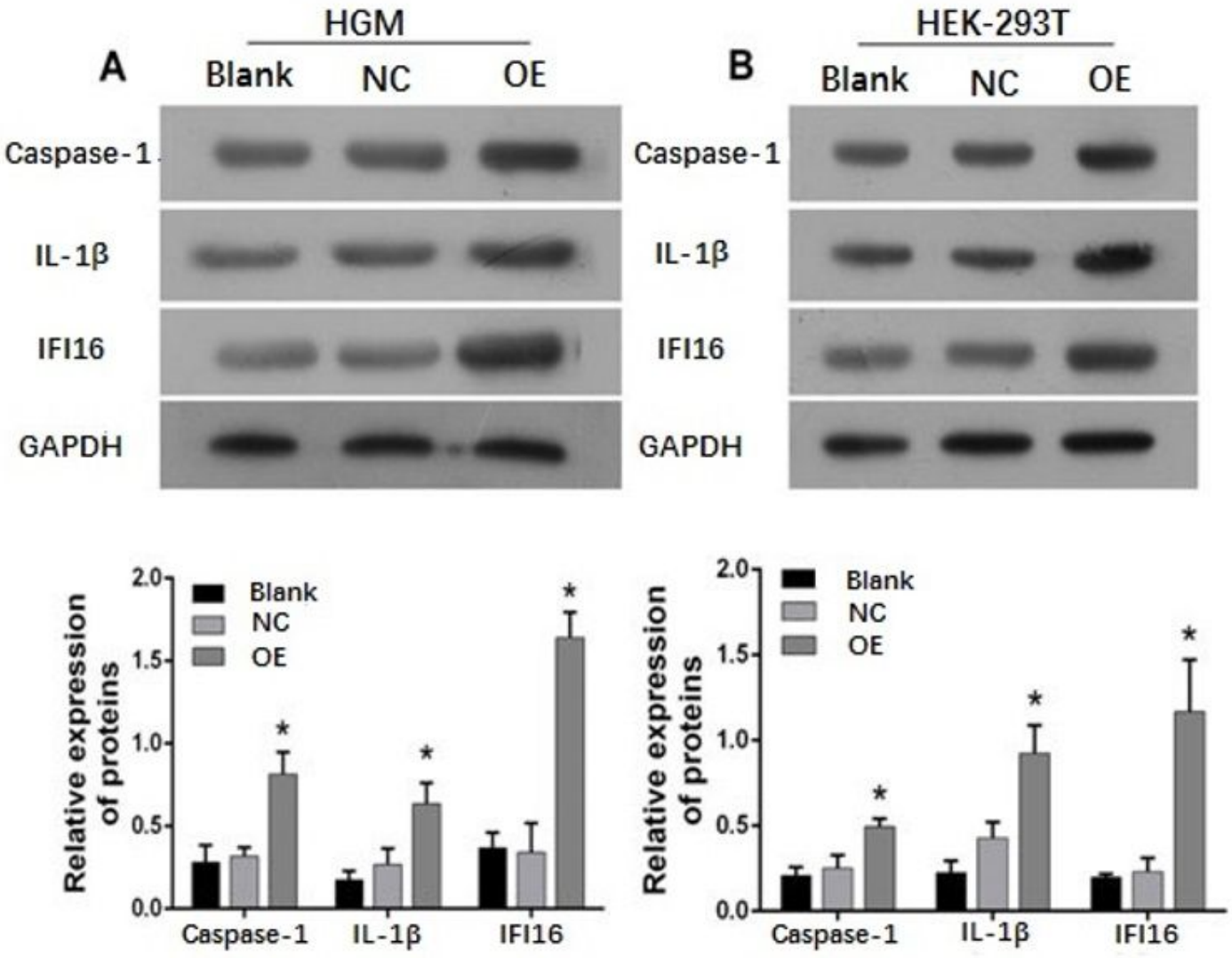

Figure 3

The expression of IFI16, Caspase-1 and IL-1ß protein levels significantly elevated in OE group compared those in NC and Blank group of the two cell lines, respectively.

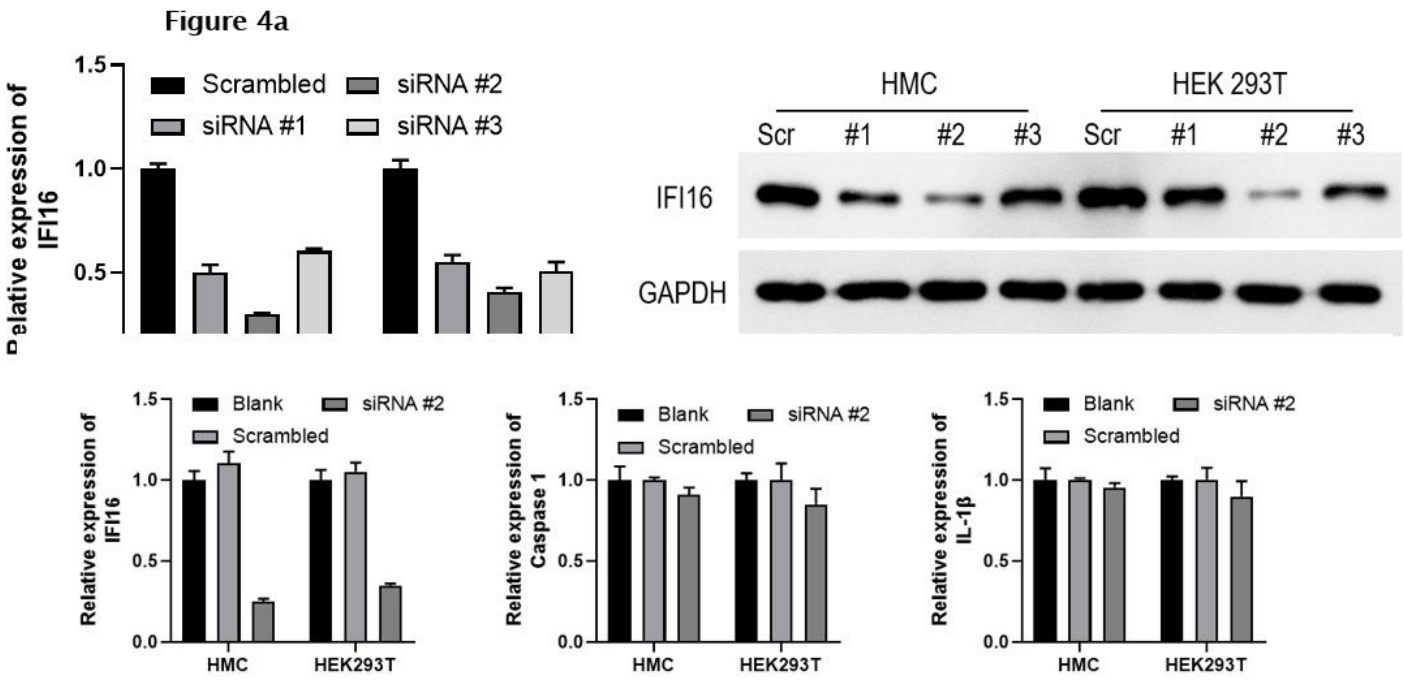

Figure 4b

Figure 4c

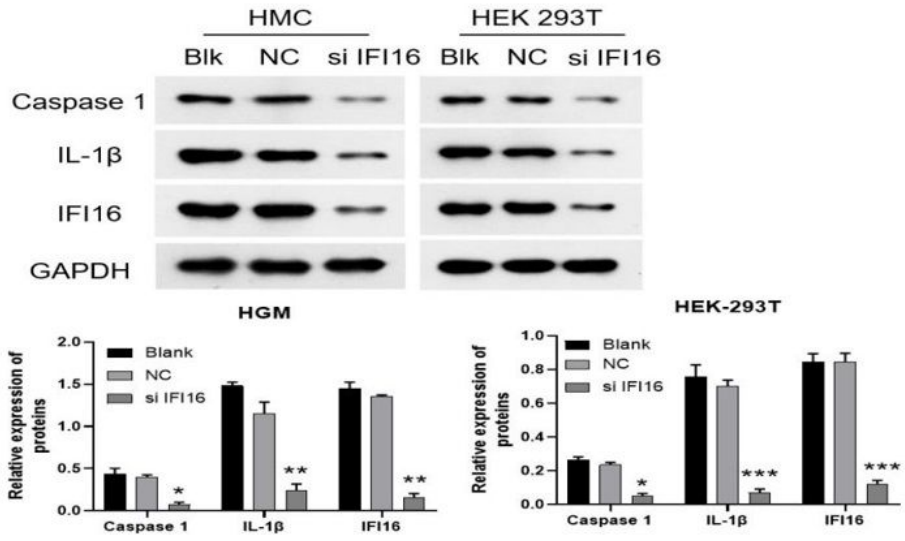

Figure 4

The cell lines were divided into three groups: the IFI16-siRNA and HBV DNA co-transfected group (si IFI16 group), HBVDNA-transfected group without siIFI16 (negative control group, NC), and the empty plasmids transfected group (blank group, Blank). By 48h post transfection, there was an approximately 90% decrease of IFI16 mRNA levels in HGM cells and 95% decrease in HEK-293T cells in the siIFI16 group. (Fig4b). Compared to the blank group, siRNA-mediated knockdown of IFI16 resulted in a 6.14-fold

decrease of caspase-1, a 6.16-fold decrease of IL-1β in HGM cells, and a 5.21-fold decrease of caspase-1 and a 10.92-fold decrease of IL-1β in HEK-293T cells. but no reduction in their mRNA levels. (figure4c).