MicroRNA-223 Downregulation Promotes HBx-Induced Podocyte Pyroptosis by Targeting the NLRP3 Inflammasome

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Research Article

Keywords: MiR-223, HBV x gene, NLRP3 inflammasome, Podocyte, Pyroptosis, mutations

Posted Date: January 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1207651/v1

Abstract

Hepatitis B virus (HBV) and its related protein, HBV X (HBx), play an important role in podocyte injury in HBV-associated glomerulonephritis (HBV-GN). MiR-223 is expressed in several diseases, including HBV-associated disease, while nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome plays a major role in pyroptosis. This study aims to determine the potential function and related mechanism of miR-223 in HBx-induced podocyte pyroptosis. We observed that the results of polymerase chain reaction indicated that miR-223 was downregulated in HBx-transfected podocytes. Transfection of miR-223 mimic eliminated the expression of NLRP3 inflammasome and its related cytokines released by NLRP3 overexpression. Moreover, the transfection of HBx and NLRP3-overexpressing plasmids increased the expression of pyroptosis-related proteins especially in the presence of miR-223 inhibitors. In conclusion, miR-223 downregulation plays an important role in HBx-induced podocyte pyroptosis by targeting the NLRP3 inflammasome, suggesting that miR-223 is a potential therapeutic target for alleviating HBV-GN inflammation.

Introduction

Hepatitis B virus (HBV) has been widely spreading in China for a long period of time, and HBV-related glomerulonephritis (HBV-GN) is one of the major secondary kidney diseases in the country [1]. Interestingly, the course of HBV-GN inevitably results in podocyte injury, which in turn aggravates disease progression. Previous studies have reported that the expression of HBV and related proteins, mainly the HBV X protein (HBx), has crucial effects in the pathogenesis of HBV-GN [2,3]. However, the specific mechanism of HBV-GN remains unclear. Therefore, it is important to further explore the specific mechanism of podocyte injury in HBV-GN.

Pyroptosis exhibits the common characteristics of programmed cell death, such as DNA breakage and cell swelling and rupture. Notably, pyroptosis is associated with the activation of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome involving caspase-1 that leads to a strong inflammatory response [4]. On the one hand, activated caspase-1 cleaves both interleukin (IL)-1β and IL-18 precursors, producing mature cytokines. On the other hand, pyroptosis leads to cytosis and release of cytoplasm contents, exacerbating the effect of inflammation [5]. Many studies showed that the NLRP3 inflammasome, a complex mainly formed by NLRP3, apoptosis-associate speck-like protein (ASC), and pro-caspase-1, is involved not only in the inflammatory progression of myocardial ischemia-reperfusion injury and sepsis-induced liver impairment, among others, but also in the renal inflammation of chronic kidney disease (CKD) [6-9]. Additionally, a cohort study of 0.5 million Chinese adults indicated that chronic HBV infection increased the risk of CKD, especially in men [10]. However, the underlying mechanism by which pyroptosis acts during the development of HBV-GN requires clarification.

MicroRNAs (miRNAs) are a class of endogenous, noncoding single-stranded molecules approximately 19-22 nucleotides in length that inhibit the translation of target mRNAs by binding to their 3′-untranslated region (UTR), thereby negatively regulating gene expression [11]. Numerous studies showed that miRNAs

control several physiological and pathological processes, such as body growth, viral infection, and cancer metastasis [12-14]. MiRNAs, as a potential biomarker and treatment strategy, have also been reported to be a key regulatory factor in injury processes, such as podocyte apoptosis and inflammation [15]. However, few studies have clarified their mechanism in HBx-induced podocyte pyropotosis.

In this study, we investigated the function and potential mechanism of miRNAs in HBx-induced podocyte pyroptosis and confirmed that miR-223 is involved in HBV-GN pathogenesis and podocyte damage by regulating the NLRP3 signaling pathway. Based on our study, miR-223 may be a potential treatment target for HBV-GN.

Materials And Methods

Cell culture and transfection

Kidney podocytes cell line (Cat#GD-C8618339) were cultured in Roswell Park Memorial Institute Medium 1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin. Podocytes were cultured at 37˚C in a saturated humidified atmosphere of 5% CO_2, with culture medium replacement every 2 d. The sequences of HBx were amplified and inserted into empty pcDNA3.1 vectors, yielding pcDNA-HBx (HBx). The same method was used to produce pcDNA-NLRP3 (NLRP3) and pcDNA-siNLRP3 (siNLRP3). Transient transfection with the indicated plasmids into podocytes was performed using Lipofectamine 2000.

Cell treatment with a miR-223 mimic or inhibitor

Podocytes were transfected with synthetic mature miR-223 (miR-223 mimic), antagomir antisense to mature miR-223 (miR-223 inhibitor), or a scrambled control transcript that served as a negative control. Subsequently, the expression levels of miR-223, NLRP3, ASC, caspase-1, IL-1β, and IL-18 were measured. In separate experiments, the podocytes were transfected and treated as described above, after which their apoptotic status was assayed.

Immunofluorescence staining

Podocytes in six-well plates were fixed with 4% paraformaldehyde at room temperature for 30 min, incubated in phosphate-buffered saline (PBS) containing 0.4% Triton X-100 for 10 min, and blocked with 2% bovine serum albumin at 37°C for 60 min. The cells were then incubated with the primary antibodies anti-vimentin (1:100; Boster Biological Technology, Wuhan, China) and anti-keratin (1:100; Boster Biological Technology) at 4°C overnight. PBS was used as the negative control. Then, the cells were washed with PBS and incubated with anti-mouse IgG Alexa Fluor 488 or anti-rabbit IgG Alexa Fluor 594 (1:1000; Life Technologies, Paisley, UK) for 1 h at room temperature. The glass cover slides were installed using the installation medium with 4′,6-diamidino-2-phenylindole dye (Vector Labs, Peterborough, UK) and prepared for imaging under a fluorescence microscope under ⊆200 magnification (Olympus, Tokyo, Japan).

Bioinformatics analysis

TargetScan and miRGator were used to predict the target gene of miR-223.

Dual luciferase reporter assay

Luciferase activity was analyzed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Hoechst 33342 staining

The morphological changes of podocytes were observed by staining the nucleus with Hoechst 33342 (C0030; Solarbio, Beijing, China). The podocytes transfected with different vectors were stained with Hoechst 33342 and cultured in an incubator for 20-30 min, after which the medium was discarded, and the podocytes were washed with medium two to three times. The podocytes were then immediately observed and imaged under a fluorescence microscope.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA in cells was extracted using TRIzol reagent (Tiangen Biotech, Beijing, CHINA) and were reverse transcribed to cDNA using the SuperScript Reverse Transcription Kit (Tiangen Biotech). RT-PCR was performed using SYBR Green Master Mix (Tiangen Biotech) according to the manufacturer's instructions. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β-actin served as the internal controls, and the $2^{-\triangle\triangle Ct}$ method was used to calculate the relative gene expression. The primers sequences were listed as follows:

HBx forward primer: 5'-GCGAATTCATGGCTGCTAGGGTGTGCT-3'

HBx reverse primer: 5'-ATCTCGAGTTAGGCAGAGGTGAAAAAGTTGC-3',

NLRP3 forward primer:: 5'-CACCTGTTGTGCAATCTGAAG-3'

NLRP3 reverse primer: 5'-GCAAGATCCTGACAACATGC-3'

ASC forward primer: 5'-AGGCCTGCACTTTATAGACC-3'

ASC reverse primer: 5'-GCTGGTGTGAAACTGAAGAG-3'

Caspase-1forward primer: 5'-CCTTAATATGCAAGACTCTCAAGGA-3'

Caspase-1 reverse primer: 5'-TAAGCTGGGTTGTCCTGCACT-3'

IL-1βforward primer: 5'-TACCTGTCCTGCGTGTTGAA-3'

IL-1βreverse primer: 5'-TCTTTGGGTAATTTTTGGGATCT-3'

IL-18 forward primer: 5'-TGCATCAACTTTGTGGCAAT-3'

IL-18 reverse primer: 5'-ATAGAGGCCGATTTCCTTGG-3'

β-actin forward primer: 5'-AGCGAGCATCCCCCAAAGTT-3'

β-actin reverse primer: 5'-GGGCACGAAGGCTCATCATT-3'

GAPDH forward primer: 5'- GCTCGCTTCGGCAGCACA -3'

GAPDH reverse primer:5'- GAACGCTTCACGAATTTGCGTG-3'

Western blot (WB) analysis

Proteins were extracted from hematopoietic progenitor cells using radioimmunoprecipitation assay buffer, and their concentration was determined using the bicinchoninic acid method. After denaturation, the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). The membranes were blocked in 5% skimmed milk at room temperature for 1 h and then incubated overnight at 4˚C with the primary antibodies specific for: NLRP3 (1:1000; Abcam, Cambridge, UK), caspase-1, ASC, IL-1β, IL-18 (1:1000; ABclonal Biotechnology, Wuhan, China), and GAPDH (1:5000; Proteintech, Chicago, IL, USA). The membranes were then incubated with goat anti-rabbit or -mouse IgG (1:5000; ZSGB Biotech, Beijing, China) and quantified using ImageJ.

Pyroptosis detection

Cell pyroptosis was analyzed by flow cytometry using In Situ Cell Death Detection Kit (Immunochemistry, Bloomington, United States) according to the manufacturer's instructions. Podocytes were cultured in sixwell plates and divided into nine groups: control, empty plasmid, HBx, HBx + miR-mimic, HBx + miR-inhibitor, HBx + miR-mimic + NLRP3, HBx + miR-mimic + si-NLRP3, HBx + miR-inhibitor + NLRP3, and HBx + miR-inhibitor+ si-NLRP3 groups. After transfection, the podocytes were digested and collected in tubes. The supernatant was discarded after centrifugation followed by three washes and were double-stained with annexin V-fluorescein isothiocyanate and propidium iodide at room temperature for 15 min in the dark. The results were analyzed using flow cytometry, and pyroptosis rate was calculated as the percentage of early and late pyroptosis cells.

Measurement of caspase-1 activity

To determine the activity of caspase-1 in different groups, the cells were analyzed using a Caspase 1 Activity Assay Kit (Meilunbio, Dalian, China) according to the manufacturer's instructions.

Statistical analyses

Measurement data are expressed as the mean ± standard deviation, which were further analyzed by GraphPad Prisem 8.0. For normally distributed data, unpaired t-test was used to compare two groups. All experiments were performed at least three times. Statistical significance was set at a *P*-value < 0.05.

Results

MiR-223 is downregulated in podocytes exposed to HBx

As the first step, HBx was successfully transfected in HBx group compared with the control group (Figure 1A). Previous studies have shown that miR-223 was downregulated in hepatitis B [16]. To evaluate its role in HBx-transfected podocytes, RT-PCR was performed to detect the expression of miR-223. As shown in Figure 1B, miR-223 was downregulated in HBx-transfected podocytes, indicating that it may play a key role in HBx-induced podocyte injury ($P < 0.05$).

HBx induces podocyte pyroptosis through NLRP3 inflammasome

To further investigate the mechanism of HBx-induced podocyte injury, immunofluorescence staining was used to observe podocytes. Immunofluorescence staining revealed that HBx increased the expression of desmin, a marker of cell necrosis, and this was notably alleviated by introduction of si-NLRP3 (Figure 2A). As expected, the opposite effect was observed in nephrin (Figure 2B). Moreover, immunofluorescence double-staining of NLRP3 (red) and nephrin (green) reached the same conclusion as before (Figure 2C). Taken together, these findings indicate the involvement of the NLRP3 inflammasome in the pathogenesis of HBx-induced pyroptosis.

NLRP3 is a direct target of miR-223

It is reported that NLRP3 inflammasomes in kidney diseases are mainly activated through the canonical pathway involving caspase-1, which results in podocyte pyroptosis [17]. To better elucidate the effect of miR-223 downregulation on podocyte pyroptosis, target prediction programs were used to predict the potential target of miR-223 and predicted NLRP3 as an miR-223 target (Figure 3A). We then investigated whether NLRP3 was a direct target of miR-223 in HBV-GN using a dual-luciferase assay. The results showed that the miR-223 agomir dramatically inhibited the luciferase activity of NLPR3 (wild-type [WT]) reporters, whereas miR-223 overexpression had little effect on luciferase activity when the binding sites of miR-223 and NLRP3 were mutated (Figure 3B). These data demonstrated that miR-223 directly bound to NLRP3 3’-UTR to attenuate its expression.

NLPR3 overexpression impaired the protective role of miR-223 in podocyte injury

To examine the effect of miR-223 on the NLRP3 inflammasome (NLRP3, Caspase-1, and ASC) and its released inflammatory factors (IL-1β and IL-18) in podocyte pyroptosis, we performed rescue experiments of miR-223 and NLRP3 inflammasome. WB was performed to analyze the expression of NLRP3, Caspase-1, ASC, IL-1β, and IL-18 in each group (Figure 4A). The results in Figure 4B showed that compared with the control group, the level of NLRP3 decreased significantly after a miR-223 mimic treatment ($P < 0.05$),

whereas the effect was aggravated after the addition of NLRP3 overexpression ($P < 0.05$). However, similar results were obtained for Caspase-1, ASC and IL-1β ($P < 0.05$; Figure 4C, D and E). In IL-18 protein expression, there was no significant difference after the addition of NLRP3 overexpression ($P > 0.05$; Figure 4F). Therefore, introduction of the NLRP3 inflammasome limited the decrease in pyroptosis-related proteins by miR-223. Taken together, our data illuminated that NLPR3 overexpression impaired the important role of miR-223 in podocyte pyroptosis.

HBx mediates podocyte pyroptosis through miR-223 and the NLRP3 inflammasome

We have already demonstrated the decreased expression of miR-223 in HBx-transfected podocytes and the negative regulatory relationship between miR-223 and NLRP3 inflammasome, but it remains unclear whether HBx mediated podocyte pyroptosis through the miR-223/NLRP3 axis. To better understand the molecular mechanism, miR-223 mimics and inhibitors were used to interfere with miR-223 expression. In addition, the level of NLRP3 was interfered by NLRP3 or si-NLRP3 plasmids and was analyzed via qRT-PCR and WB (Figure 5). As can be seen, the RT-PCR results showed that the mRNA level of NLRP3 was increased in the HBx group compared with the control and empty plasmid group. However, the expression of NLRP3 decreased in the HBx + miR-mimic and HBx + miR-mimic + si-NLRP3 group. In addition, the introduction of miR-223 inhibitors and NLRP3 upregulated NLRP3 remarkably in HBx-induced podocytes compared with HBx group ($P < 0.05$; Figure 5A). Similar results were observed for the mRNA levels of caspase-1, ASC, IL-1β, and IL-18 (Figure 5B-5E). Next, we used WB experiments to further confirm our results (Figure 5F). According to WB analysis, we found that the expression of the NLRP3 inflammasome and its inflammatory cytokines were increased in the HBx group, while the introduction of miR-223 inhibitor and NLRP3 enhanced the level of inflammatory cytokines significantiy ($P < 0.05$; Figure 5J-5K). However, there was no statistical difference in the quantitative analysis results for ASC ($P > 0.05$; Figure 5I). The gene expression of ASC in some diseases was reported to be slightly different from that of caspase-1, indicating that it may be independent of the expression of NLRP3 inflammasome [18], which may be one of the factors that affect our experimental results. Subsequently, we assessed the enzyme activity of caspase-1 and found that it was significantly increased in the HBx + miRNA inhibitor + NLRP3 transfection group compared with the HBx group ($P < 0.05$; Figure 5L).

In order to further explore the relationship between the miR-223/NLRP3 axis and podocyte pyroptosis, flow cytometry-based pyroptosis detection and Hoechst 33342 staining were performed. Compared with the control group and the empty plasmid group, cell pyroptosis significantly increased in the HBx groups. However, cell pyroptosis remarkably decreased in the HBx + miR-mimic + si-NLRP3 group indicated that miR-223/NLRP3 axis play a critical role in the HBx-induced pyroptosis ($P < 0.05$; Figure 5M). Hoechst 33342 staining showed that the nuclei of podocytes were densely stained and the chromatin was condensed in the HBx group in comparison of the control group and the empty plasmid group, which were recovered in the introduction of miR-223 and si-NLRP3 (Figure 5N). These results suggest that HBx-induced pyroptosis is related to the downregulation of miR-223 via the NLRP3 pathway.

Discussion

In the present study, we found that the expression of miR-223 was downregulated in podocytes exposed to HBx. Moreover, our results confirmed that the expression of HBx protein in podocytes induced pyroptosis. HBx-induced podocyte models were established in order to demonstrate that miR-223 plays an important role in HBx-induced pyroptosis and that the activation of the NLRP3 inflammasome is closely related to the underlying mechanism.

HBV-GN is one of the most common extrahepatic manifestations in patients with chronic HBV infection [19]. A large number of studies on the HBx gene, one of the four genes of HBV, have gradually appeared since the establishment of the HBx transgenic mice [20]. However, there are various mechanisms of HBx-induced podocyte injury in human kidney diseases, such as inflammatory response and oxidative stress, and the former may be of great importance. The NLRP3 inflammasome, composed of NLRP3, ASC, and pro-caspase-1, is closely related with the innate immune system. Activated caspase-1 cleaves full-length gasdermin D (GSDMD) to form a gasdermin pore, which allows the release of proinflammatory cytokines pro-IL-1β and pro-IL-18, leading to cell swelling and dissolution [21]. It is the characteristic cascade inflammatory response of pyroptosis that sets it apart from apoptosis and necrosis. Stimulus such as CD36 and nicotine induce reactive oxygen species production and then activate the NLRP3 inflammasome to induce the inflammatory response and cell damage [22,23]. The mutations of NLRP3 inflammasome receptors trigger autoimmune diseases, and imbalanced activity is associated with the pathological process of many diseases [24]. NLRP3 inflammasome-mediated pyroptosis induced by a variety of stimulus occurs in Alzheimer's disease, atherosclerosis, diabetes mellitus, glomerular inflammatory diseases, renal ischemia reperfusion injury, and podocyte injury [25-30]. A study showed that pyroptosis can be used against cancer by induction in tumor cells [31]. Therefore, we speculate that pyroptosis may play a pivotal role in HBx-induced podocyte injury and that the NLRP3 inflammasome may be a new therapeutic target for HBV-GN.

MiRNAs are normally extended by adding stem-loop primers or 3' adenylation for traditional PCR detection [32]. Although the detection of miRNAs is very challenging, it has been one of the hotspots of our research on disease mechanisms. For example, miRNAs participate in cancer initiation and metastasis [33]. Upregulated miR-125b is related to severe liver fibrosis of patients with chronic hepatitis C [34]. MiRNAs also play an important role in kidney disease. In acute kidney injury, some miRNAs influence renal function through cell apoptosis [35]. MiR-155 influences the release of proinflammatory factors in early renal disease [36]. Previous studies confirmed that miR-223 may act as a negative regulator in the inflammatory process of viral hepatitis [37]. Therefore, we hypothesized that miR-223 has a similar regulatory effect on podocyte damage in HBV-GN. MiR-223 was first discovered in the hematopoietic system [38] and has also been proven to limit liver fibrosis and reduce urate-induced gouty inflammation and cardiovascular damage [39-41]. Interestingly, the inflammatory response of diseases caused by miR-223 down-regulation always seems to be accomplished by targeting the NLRP3 inflammasome [39,40]. In this study, we found that miR-223 downregulation in HBx-induced podocytes is associated with pyroptosis. Therefore, we tested HBx-induced pyroptosis after transfection of miR-223

mimics, and found that miR-223 upregulation mitigated the expression of NLRP3. Moreover, transfection of miR-223 mimics downregulated the expression of ASC, caspase-1, IL-1β and IL-18. These results indicate that the downregulation of miR-223 increased the expression of the NLRP3 inflammasome, which is consistent with the results of Franz et al. [42].

Studies on the mechanisms of miRNAs provide new insights for the diagnosis and treatment of many clinical diseases. To better understand the mechanism of HBV-GN, we examined the expression of pyroptosis-related proteins exposed to HBx and NLRP3 or si-NLRP3 in the presence of miR-223 mimics or inhibitors. Compared with the HBx group, HBx + miRNA inhibitor + NLRP3 transfection group exhibited more severe features of pyroptosis. It has been indicated that the detection of characteristic miRNAs in serum could be used to predict tumor recurrence and survival rates of patients with triple-negative breast cancer [43]. In treatment, miRNAs are chemically modified or delivered as vectors to reduce RNase interference [44]. MiRNAs are also packaged into multivesicular bodies with hormone-like effects and function as anticancer drug targets [45]. Based on these studies, miR-223 may be a novel diagnosis and treatment targets for HBV-GN.

Our research first demonstrated that miR-223 plays a role in HBx protein-mediated podocyte pyroptosis by targeting the NLRP3 inflammasome. However, the present study has llimitations. First, we only performed relevant cellular experiments without validation in mouse models and clinical cases. Second, the regulations between HBx, miR-223, and NLRP3 inflammasome are particularly complex, and we did not analyze whether other related genes are involved. Further studies on the mechanism of HBV-GN should be performed.

In conclusion, we report that the HBx protein can induce pyroptosis of human kidney podocytes in HBV-GN and miR-223 plays a protective role in the pathogenesis of the disease. MiR-223 negatively regulates the expression of NLRP3 inflammasome, which is involved in the pathogenesis of HBV-GN. More importantly, the findings of this study may provide novel insights into the diagnosis and treatment with miRNAs in kidney disease.

Declarations

Ethics approval and consent to participate

Approval was granted by the Affiliated Hospital of Qingdao University. All methods were performed in accordance with the relevant guidelines and regulations.

Availability of data and material

All data generated or analysed during this study are included in this article.

Declarations of interest

None.

Funding

This work was supported by the National Natural Science Foundation of China [grant number NSFC 81870494], the Chinese Society of Nephrology [grant number 20010080800], and the Qingdao Outstanding Health Professional Development Fund [grant number 2020-2022].

Authors' contributions

Yani YU, Hui DONG wrote the main manuscript text. Yue ZHANG, Jingyi SUN and Baoshuang LI collected the clinical study information. Yueqi CHEN, Moxuan FENG and Xiaoqian YANG finished the formal analysis and investigation. Shengbo GAO and Wei JIANG prepared figures 1-4. All authors reviewed the manuscript.

References

1. Jiang Wei,Liu Tuo,Dong Hui et al. Relationship Between Serum DNA Replication, Clinicopathological Characteristics and Prognosis of Hepatitis B Virus-associated Glomerulonephritis with Severe Proteinuria by Lamivudine Plus Adefovir Dipivoxil Combination Therapy.[J] .Biomed Environ Sci, 2015, 28: 206-13.
2. Diao Zongli,Ding Jiaxiang,Yin Chenghong et al. Purified hepatitis B virus induces human mesangial cell proliferation and extracellular matrix expression in vitro.[J] .Virol J, 2013, 10: 300.
3. Hui D,Yan X,Wei J et al. Significance of mutations in hepatitis B virus X gene for the pathogenesis of HB-associated glomerulonephritis.[J] .Acta Virol, 2014, 58: 278-81.
4. Latz Eicke,Xiao T Sam,Stutz Andrea,Activation and regulation of the inflammasomes.[J] .Nat Rev Immunol, 2013, 13: 397-411.
5. Zhang Xiangying,Dong Peiling,Xu Lin et al. The different expression of caspase-1 in HBV-related liver disease and acts as a biomarker for acute-on-chronic liver failure.[J] .BMC Gastroenterol, 2019, 19: 148.
6. Zuo Wei,Tian Ran,Chen Qian et al. miR-330-5p inhibits NLRP3 inflammasome-mediated myocardial ischaemia-reperfusion injury by targeting TIM3.[J] .Cardiovasc Drugs Ther, 2021, 35: 691-705.
7. Zhang Huiqing,Du Yuna,Guo Yujie et al. TLR4-NLRP3-GSDMD-Mediated Pyroptosis Plays an Important Role in Aggravated Liver Injury of CD38 Sepsis Mice.[J] .J Immunol Res, 2021, 2021: 6687555.
8. Zhang Quan-Bo,Zhu Dan,Dai Fei et al. MicroRNA-223 Suppresses IL-1β and TNF-α Production in Gouty Inflammation by Targeting the NLRP3 Inflammasome.[J] .Front Pharmacol, 2021, 12: 637415.
9. Vilaysane Akosua,Chun Justin,Seamone Mark E et al. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD.[J] .J Am Soc Nephrol, 2010, 21: 1732-44.
10. Si Jiahui,Yu Canqing,Guo Yu et al. Chronic hepatitis B virus infection and risk of chronic kidney disease: a population-based prospective cohort study of 0.5 million Chinese adults.[J] .BMC Med, 2018, 16: 93.

11. Ameres Stefan L,Zamore Phillip D,Diversifying microRNA sequence and function.[J] .Nat Rev Mol Cell Biol, 2013, 14: 475-88.
12. Lui Julian C,Regulation of body growth by microRNAs.[J] .Mol Cell Endocrinol, 2017, 456: 2-8.
13. Ojha Chet Raj,Rodriguez Myosotys,Dever Seth M et al. Mammalian microRNA: an important modulator of host-pathogen interactions in human viral infections.[J] .J Biomed Sci, 2016, 23: 74.
14. Cortez Maria Angelica,Bueso-Ramos Carlos,Ferdin Jana et al. MicroRNAs in body fluids–the mix of hormones and biomarkers.[J] .Nat Rev Clin Oncol, 2011, 8: 467-77.
15. Ishii Hiroki,Kaneko Shohei,Yanai Katsunori et al. MicroRNAs in Podocyte Injury in Diabetic Nephropathy.[J] .Front Genet, 2020, 11: 993.
16. Haneklaus M,Gerlic M,O'Neill L A J et al. miR-223: infection, inflammation and cancer.[J] .J Intern Med, 2013, 274: 215-26.
17. Xiang Huiling,Zhu Feng,Xu Zhifeng et al. Role of Inflammasomes in Kidney Diseases via Both Canonical and Non-canonical Pathways.[J] .Front Cell Dev Biol, 2020, 8: 106.
18. Stienstra Rinke,van Diepen Janna A,Tack Cees J et al. Inflammasome is a central player in the induction of obesity and insulin resistance.[J] .Proc Natl Acad Sci U S A, 2011, 108: 15324-9.
19. Liao Min-Tser,Chang Mei-Hwei,Lin Fu-Gong et al. Universal hepatitis B vaccination reduces childhood hepatitis B virus-associated membranous nephropathy.[J] .Pediatrics, 2011, 128: e600-4.
20. Yu D Y,Moon H B,Son J K et al. Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein.[J] .J Hepatol, 1999, 31: 123-32.
21. Fischer Fabian A,Chen Kaiwen W,Bezbradica Jelena S,Posttranslational and Therapeutic Control of Gasdermin-Mediated Pyroptosis and Inflammation.[J] .Front Immunol, 2021, 12: 661162.
22. Hou Yanjuan,Wang Qian,Han Baosheng et al. CD36 promotes NLRP3 inflammasome activation via the mtROS pathway in renal tubular epithelial cells of diabetic kidneys.[J] .Cell Death Dis, 2021, 12: 523.
23. Singh Gurinder Bir,Kshirasagar Naresh,Patibandla Sai et al. Nicotine instigates podocyte injury via NLRP3 inflammasomes activation.[J] .Aging (Albany NY), 2019, 11: 12810-12821.
24. Awad Fawaz,Assrawi Eman,Louvrier Camille et al. Inflammasome biology, molecular pathology and therapeutic implications.[J] .Pharmacol Ther, 2018, 187: 133-149.
25. Lei Bo,Liu Jiaxin,Yao Zhihui et al. viaNF-κB-Induced Upregulation of miR-146a-5p Promoted Hippocampal Neuronal Oxidative Stress and Pyroptosis TIGAR in a Model of Alzheimer's Disease.[J] .Front Cell Neurosci, 2021, 15: 653881.
26. Yin Ruihua,Zhu Xiaoyan,Wang Jing et al. MicroRNA-155 promotes the ox-LDL-induced activation of NLRP3 inflammasomes via the ERK1/2 pathway in THP-1 macrophages and aggravates atherosclerosis in ApoE-/- mice.[J] .Ann Palliat Med, 2019, 8: 676-689.
27. Xu Dan,Zhang Xiuzhen,Chen Xuan et al. Inhibition of miR-223 attenuates the NLRP3 inflammasome activation, fibrosis, and apoptosis in diabetic cardiomyopathy.[J] .Life Sci, 2020, 256: 117980.

28. Hong Jinni,Bhat Owais M,Li Guangbi et al. Lysosomal regulation of extracellular vesicle excretion during d-ribose-induced NLRP3 inflammasome activation in podocytes.[J] .Biochim Biophys Acta Mol Cell Res, 2019, 1866: 849-860.
29. Wu Haoyu,Huang Tao,Ying Liang et al. MiR-155 is Involved in Renal Ischemia-Reperfusion Injury via Direct Targeting of FoxO3a and Regulating Renal Tubular Cell Pyroptosis.[J] .Cell Physiol Biochem, 2016, 40: 1692-1705.
30. Zhang Qinghua,Conley Sabena M,Li Guangbi et al. Rac1 GTPase Inhibition Blocked Podocyte Injury and Glomerular Sclerosis during Hyperhomocysteinemia via Suppression of Nucleotide-Binding Oligomerization Domain-Like Receptor Containing Pyrin Domain 3 Inflammasome Activation.[J] .Kidney Blood Press Res, 2019, 44: 513-532.
31. Tan Yixin,Chen Quanzhu,Li Xiaoling et al. Pyroptosis: a new paradigm of cell death for fighting against cancer.[J] .J Exp Clin Cancer Res, 2021, 40: 153.
32. Lu Thomas X,Rothenberg Marc E,MicroRNA.[J] .J Allergy Clin Immunol, 2018, 141: 1202-1207.
33. Kim Jongchan,Yao Fan,Xiao Zhenna et al. MicroRNAs and metastasis: small RNAs play big roles.[J] .Cancer Metastasis Rev, 2018, 37: 5-15.
34. Sultana C, Rosca A, Ruta S. Correlation Between miR-125b Expression and Liver Fibrosis in Patients with Chronic Hepatitis C.[J] .Hepat Mon. Online ahead of Print, 2019, (1): e84615
35. Zankar Sarah,Trentin-Sonoda Mayra,Viñas Jose L et al. Therapeutic effects of micro-RNAs in preclinical studies of acute kidney injury: a systematic review and meta-analysis.[J] .Sci Rep, 2021, 11: 9100.
36. Zheng Xintong,Zhong Qiuhong,Lin Xu et al. Transforming growth factor-β1-induced podocyte injury is associated with increased microRNA-155 expression, enhanced inflammatory responses and MAPK pathway activation.[J] .Exp Ther Med, 2021, 21: 620.
37. Ye Dan,Zhang Tianbao,Lou Guohua et al. Role of miR-223 in the pathophysiology of liver diseases. [J] .Exp Mol Med, 2018, 50: 1-12.
38. Johnnidis Jonathan B,Harris Marian H,Wheeler Robert T et al. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223.[J] .Nature, 2008, 451: 1125-9.
39. Wang Xiaolin,Seo Wonhyo,Park Seol Hee et al. MicroRNA-223 restricts liver fibrosis by inhibiting the TAZ-IHH-GLI2 and PDGF signaling pathways via the crosstalk of multiple liver cell types.[J] .Int J Biol Sci, 2021, 17: 1153-1167.
40. Wang Xiaolin,Seo Wonhyo,Park Seol Hee et al. MicroRNA-223 restricts liver fibrosis by inhibiting the TAZ-IHH-GLI2 and PDGF signaling pathways via the crosstalk of multiple liver cell types.[J] .Int J Biol Sci, 2021, 17: 1153-1167.
41. Maruyama Daisuke,Kocatürk Begüm,Lee Youngho et al. MicroRNA-223 Regulates the Development of Cardiovascular Lesions in LCWE-Induced Murine Kawasaki Disease Vasculitis by Repressing the NLRP3 Inflammasome.[J] .Front Pediatr, 2021, 9: 662953.
42. Bauernfeind Franz,Rieger Anna,Schildberg Frank A et al. NLRP3 inflammasome activity is negatively controlled by miR-223.[J] .J Immunol, 2012, 189: 4175-81.

43. Kleivi Sahlberg Kristine,Bottai Giulia,Naume Bjørn et al. A serum microRNA signature predicts tumor relapse and survival in triple-negative breast cancer patients.[J] .Clin Cancer Res, 2015, 21: 1207-14.
44. Lai Xin,Eberhardt Martin,Schmitz Ulf et al. Systems biology-based investigation of cooperating microRNAs as monotherapy or adjuvant therapy in cancer.[J] .Nucleic Acids Res, 2019, 47: 7753-7766.
45. Ling Hui,Fabbri Muller,Calin George A,MicroRNAs and other non-coding RNAs as targets for anticancer drug development.[J] .Nat Rev Drug Discov, 2013, 12: 847-65.

Figures

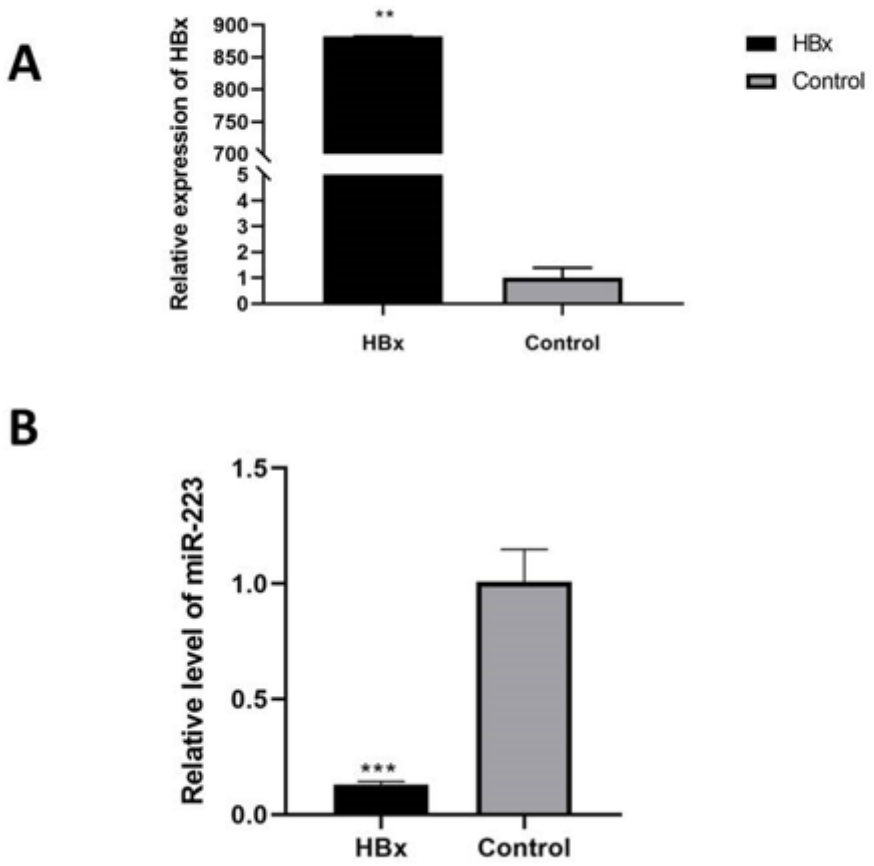

Figure 1

MiR-223 is downregulated in podocytes exposed to HBx. (A) HBx and (B) miR-223 expression were measured by RT-PCR. RT-PCR, reverse transcription polymerase chain reaction. HBx, Hepatitis virus B X protein. The data are presented as the means ± SDs. $^{**}p < 0.01$, $^{***}p < 0.001$ versus Control.

Figure 2

HBx induces podocyte pyroptosis through NLRP3 inflammasome (A) Desmin and (B) nephrin expression were detected by immunofluorescence (×200). (C) NLRP3 and nephrin double-staining were observed by immunofluorescence (×200). NC, negative control; siRNA, small interfering RNA ;N, nucleus；M, mitochondrion；RER, rough endoplasmic reticulum；LD, lipid droplet.

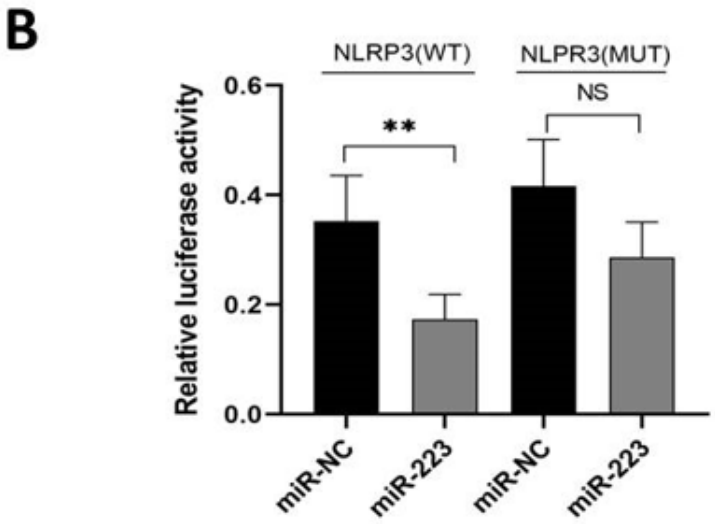

Figure 3

NLRP3 is a direct target of miR-223 (A) Binding sites between miR-223 and the 3'-untranslated regioin of NLRP3 were analyzed using bioinformatics methods. (B) The effects of miR-223 upregulation on luciferase activities of NLRP3 (WT) or NLRP3 (MUT) reporter was measured using the luciferase reporter assay system. WT, wild-type; MUT, mutant; NC, negative control. The data are presented as the means ± SDs. $^{**}p < 0.01$ versus miR-NC.

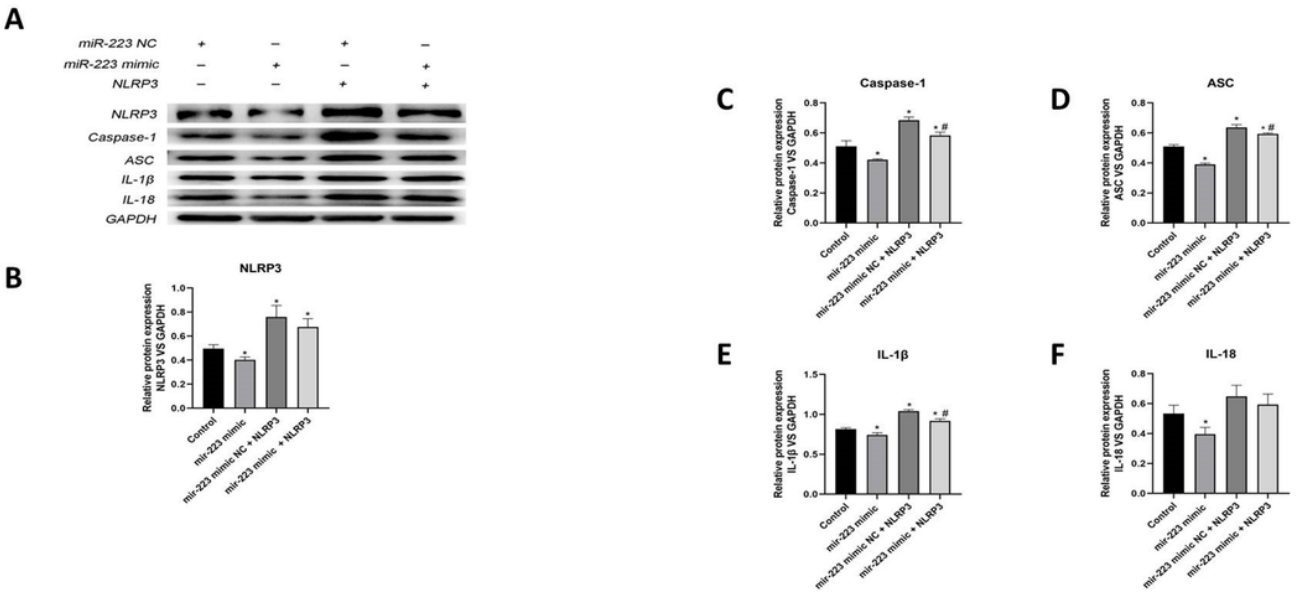

Figure 4

NLPR3 overexpression impaired the protective role of miR-223 in podocyte injury. (A) Western blot analysis showing the protein levels of pyroptosis-associated markers NLRP3, ASC, caspase-1, IL-1β, and IL-18. (B-F) Western blot of the expression of NLRP3, Caspase-1, ASC, IL-1β, and IL-18. NC, normal control. The data are presented as the means ± SDs. *$p < 0.05$ versus Control group, $^{\#}p < 0.05$ versus miR-223 mimic NC+NLRP3 group.

Figure 5

HBx mediated podocyte pyroptosis through miR-223 and the NLRP3 inflammasome. (A-E) RT-PCR assay showing the relative level of NLRP3, Caspase-1, ASC, IL-1β, and IL-18. (F-K) Western blot analysis showing the protein levels of NLRP3, Caspase-1, ASC, IL-1β, and IL-18. (L) Caspase-1 enzyme activity was examined via ELISA. (M) Flow cytometry analysis of pyroptosis in each group. (N) Fluorescence microscopy (×400) results of each group. MiRNA mimic, miR-223 mimic; miRNA inhibitor, miR-223 inhibitor. The data are presented as the means ± SDs. *$p < 0.05$ versus Control and Empty plasmid group, $^{\#}p < 0.05$ versus HBx group.