Original Article

LncRNA KCNQ1OT1 promoted hepatitis C virus-induced pyroptosis of β-cell through mediating the miR-223-3p/NLRP3 axis

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Background: Type 2 diabetes is a well described extra-hepatic manifestation of hepatitis C virus (HCV) infection. This study aimed to explore the potential mechanism of KCNQ1 overlapping transcript 1 (KCNQ1OT1) in type 2 diabetes mellitus (T2DM) caused by HCV infection.

Methods: Min6 cells were infected with HCV to establish a vitro model, and the HCV copy number was detected by real-time quantitative PCR (RT-qPCR). The mRNA and protein expressions of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD were analyzed by RT-qPCR and Western blot. Flow cytometry and TUNEL assay were used to evaluate the pyroptosis of cells and enzyme-linked immunosorbent assay (ELISA) detected the secretion of insulin. A dual luciferase reporter gene assay then verified the targeting relationship of KCNQ1OT1, miRNA-223-3p, and NLRP3.

Results: KCNQ1OT1 was highly expressed in HCV-infected T2DM patients and HCV-infected β-cells. Silencing KCNQ1OT1 inhibited β-cell pyroptosis by regulating miR-223-3p/NLRP3, and inhibition of miR-223-3p or overexpression of NLRP3 reversed the pyroptosis by silencing KCNQ1OT1.

Conclusions: Our findings indicate KCNQ1OT1 promotes HCV-infected β-cell pyroptosis through the miRNA-223-3p/NLRP3 axis, effecting the production of insulin and accelerating the occurrence and development of T2DM.Regulating KCNQ1OT1 and its target genes will help to better understand the pathogenesis of T2DM induced by HCV infection and provide new theoretical foundations and therapeutic targets.

Keywords: β-cell; hepatitis C virus (HCV); KCNQ1 overlapping transcript 1 (KCNQ1OT1); miR-223-3p; pyroptosis

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic syndrome caused by insufficient insulin secretion and insulin resistance. The disease affects the metabolism of carbohydrates, fats, and proteins, causing hyperglycemia and related complications and disease (1-3). The inflammatory environment aggravates β-cell damage and promotes the occurrence of T2DM. Recent studies have shown that patients with hepatitis C virus (HCV) have a higher risk of developing T2DM than the general population (4). T2DM has also been confirmed as an extrahepatic manifestation of HCV infection, which affects insulin signal transduction cascade (5,6). In addition, the activation of pro-inflammatory mediators caused by chronic infection can

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interfere with insulin signal transduction, thereby reducing the sensitivity to insulin and leading to liver and peripheral insulin resistance (7,8). However, the interaction between HCV infection and T2DM remains unexplained, and it is necessary to clarify the potential mechanism of T2DM caused by HCV infection.

Many epidemiological studies have shown an association between T2DM and HCV, involving direct viral effects, insulin resistance, proinflammatory cytokines, chemokines, suppressors of cytokine signaling, and other immune-mediated mechanisms (9). Inflammation is one of importantly related to the pathogenesis of diabetes and HCV. The apoptosis and pyroptosis are the main cause of β-cell death. Apoptosis is a very tightly programmed cell death with distinct biochemical and genetic pathways (10). Pyroptosis is a kind of programmed cell necrosis characterized by cells continuing to swell until the cell membrane ruptures, leading to the uncontrolled release of cell inflammatory content and activating a strong inflammatory response (11,12). In recent years, studies have found that cell pyroptosis is related to diabetes, and β-cell pyroptosis is a new hot spot in the study of new pathogenesis related to T2DM (13,14), suggesting that inflammation may promote the occurrence and development of T2DM by pyroptosis. Additionally, HCV infection can cause pyroptosis of infected liver cancer cells and surrounding non-infected cells affecting the occurrence and development of cancer (15,16) and HCV induce the occurrence of T2DM (17). However, how the HCV infection in β-cell pyroptosis remains elusive.

Long non-coding RNA (lncRNA) is a heterogeneous non-coding RNA with a length of more than 200 nucleotides (18-20) and is involved in regulating the development of liver cancer caused by HCV infection (21,22). In addition, some studies have reported that lncRNA regulated β-cells to affect insulin synthesis and secretion (23-25). While it has been reported that lncRNA KCNQ1 overlapping transcript 1 (KCNQ1OT1) is related to many diseases and plays an important role in diabetes-related complications (26), the function of KCNQ1OT1 in HCV infection on diabetes is still unknown. The common mechanism by which lncRNA works is to act as a competitive endogenous RNA (ceRNA), scaffold, interact with proteins, or form membraneless organelles. lncRNA can bind to the complementary binding sites of microRNAs (miRNAs), which regulate the expression of genes by covering the 3'-UTR of downstream target genes through sponges. miRNAs regulate 30% of protein-coding genes in the human body and have become a research hotspot in the field of life sciences in recent years (27-29). miR-223-3p is a member of the miRNA family, not only as novel non-invasive markers for the early detection of HCV-positive cirrhosis and hepatocellular carcinoma (HCC), but also as a new screening biomarker to identify subjects with prediabetes at high risk of developing diabetes (30,31). A recent study showed that knockdown of KCNQ1OT1 ameliorates cardiomyocytes pyroptosis by regulating miR-214-3p/caspase-1 axis in diabetic mice (26). In this study, we explored the expression of KCNQ1OT1 in HCV-infected T2DM patients and β-cells, as well as the mechanism of KCNQ1OT1 regulates HCV-induced pyroptosis of β-cells by targeting miRNA-223-3p/NLRP3 axis. We found that KCNQ1OT1 was highly expressed in HCV-infected T2DM patients and β-cells, and miRNA-223-3p showed low expression in HCV-infected β-cells. At the molecular level, we verified that KCNQ1OT1 regulates HCV-induced pyroptosis of β-cells and inhibits the production of insulin through the miRNA-223-3p/NLRP3 axis. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-3862).

Methods

Patients statement

Serum samples from twenty patients with HCV-infected T2DM and twenty non-HCV-infected T2DM patients were obtained from the First People's Hospital of Yunnan Province. None of the patients had coronary artery disease, hypertension, or other heart diseases. The study received approval from the ethics committee of the First People's Hospital of Yunnan Province (KHLL2020-KY059), and all participants signed informed consent forms. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

Cell culture

Mouse β-cell line min6 (ATCC, HS-C200660, Manassas, MD, USA) was maintained in RPMI 1640 medium (Gibco, CA, USA) containing 10% fetal bovine serum and 1% penicillin and streptomycin in 5% CO_2 at 37 ℃. To study the effects of KCNQ1OT1 and miRNA-223-3p on pyroptosis in HCV infection, HCV-infected (transfected with pJFH1) human liver cancer cell line Huh7.5.1 cells

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(ATCC, XY-XB-3306, Manassas, MD, USA) were cultured, the HCV particles from the culture medium were purified, and the min6 cells were incubated at a multiplicity of infection (MOI) of 1.0 for 72 hours. Huh7.5.1 cells were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco, CA, USA) containing 10% fetal bovine serum, and 1% penicillin and streptomycin in 5% CO_2 at 37 ℃.

Cell transfection

To construct the KCNQ1OT1 and NLRP3 overexpression vector. The full-length sequences of KCNQ1OT1 and NLRP3 were inserted into the pcDNA3.1 plasmid (pcDNA3.1, Invitrogen, Carlsbad, CA, USA) to obtain the KCNQ1OT1 and NLRP3 overexpression plasmid pcDNA3.1-KCNQ1OT1 and pcDNA3.1-NLRP3, and pcDNA3.1 plasmid as a negative control. Small interfering RNA targeting KCNQ1OT1 (si-KCNQ1OT1), miR-223-3p mimic/inhibitor, and negative control (NC) mimics (GenePharma, Shanghai, China) were then synthesized. Cells were transfected with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Real-time quantitative PCR (RT-qPCR)

Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from cells, and a NanoDrop 1000 spectrophotometer (NanoDrop, DE, USA) and agarose gel electrophoresis (Invitrogen, Carlsbad, CA, USA) used to detect RNA concentration and quality. The RNA was reverse transcribed using a reverse transcription kit (Promega, Madison, WI, USA). The cDNA was amplificated and detected by the Real-Time PCR system (Bio-Rad, Hercules, CA, USA) using SYBR Premix Ex Taq II (Takara, Tokyo, Japan), and the $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of genes. GAPDH and U6 served as the internal control. These primers are listed in *Table 1*.

Western blotting

Total cell protein was separated by 12% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane, then 5% fat-free milk powder was added to block for 2 h at room temperature. The primary antibodies against NLRP3 (1:1,000; ab263899, Abcam, Cambridge, UK), caspase-1 (1:1,000; ab207802, Abcam), IL-1β (1:1,000; ab283818, Abcam), IL-18 (1:5,000; ab191860, Abcam), GSDMD (1:1,000; ab219800, Abcam), and GAPDH (1:1,000; ab9485, Abcam), were then incubated at 4 ℃ overnight. After rinsing with Tris-buffered saline with 0.1% Tween 20 (TBST), the PVDF membranes were incubated with secondary antibody (1:10,000, Invitrogen, Carlsbad, CA, USA) at room temperature for 2 hours. Enhanced chemiluminescence (ECL) blotting detection reagents (BioVision, Milpitas, CA, USA) developed chemiluminescence signals and blots were quantified with ImageJ software.

Luciferase reporter assay

To identify if KCNQ1OT1 and NLRP3 were direct targets of miR-223-3p, cells were transfected with recombinant plasmid containing the firefly luciferase gene. The miR-233-3p mimic, miR-233-3p inhibitor and KCNQ1OT1-wild-type (WT)/mutant-type (MUT) recombinant plasmid were co-transfected into cells, as were the miR-233-3p and NLRP3-WT/MUT recombinant plasmids. Luciferase activity was measured 48 h after transfection using the dual luciferase reporter assay system (Promega, Madison, WI, USA).

TUNEL staining

Cells or tissues were fixed with 4% paraformaldehyde at room temperature for 10 min and permeated with 0.5% Triton X-100 in phosphate-buffered saline (PBS) for 2 min. Cells were then rinsed, and 50 µL TUNEL detection solution (Roche, Basel, Switzerland) added, and the sample was incubated at 37 ℃ for 1 hour in the dark. After washing three times with PBS, the slide was mounted with anti-fluorescence quenching mounting solution. Images were then taken by a fluorescence microscope. The nuclei were stained with DAPI and TUNEL staining was assessed.

Enzyme-linked immunosorbent assay (ELISA)

The serum obtained from the cell supernatant was preserved at −80 ℃ for subsequent analysis by an ELISA kit (MlBIO, Shanghai, China), and the levels of IL-1β, IL-18, and insulin were determined following the instruction protocols.

Determination of cell pyroptosis

Cell pyroptosis was measured using the FAM fluorescent-

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Table 1 The primers of RT-qPCR used in this study

Genes	Primer set	Primers sequence (5'-3')
KCNQ1OT1	KCNQ1OT1-F	GCACTCTGGGTCCTGTTCTC
	KCNQ1OT1-R	CACTTCCCTGCCTCCTACAC
miR-223-3p	miR-223-3p-F	CGCUAUCUUUCUAUUAACUGACCAUAA
	miR-223-3p-R	CGCUAUCUUUCUAUUAUGACUCCAUAA
IL-1β	IL-1β-F	CCCTGCAGCTGGAGAGTGTGG
	IL-1β-R	TGTGCTCTGCTTGAGAGGTGCT
IL-18	IL-18-F	ACAACCGCAGTAATACGGAGCA
	IL-18-R	TGTGCTCTGCTTGAGAGGTGCT
Caspase-1	Caspase-1-F	ACACGTCTTGCCCTCATTATCT
	Caspase-1-R	ATAACCTTGGGCTTGTCTTTCA
NLRP3	NLRP3-F	GTGGAGATCCTAGGTTTCTCTG
	NLRP3-R	CAGGATCTCATTCTCTTGGATC
GSDMD	GSDMD-F	CCATCGGCCTTTGAGAAAGTG
	GSDMD-R	ACACATGAATAACGGGGTTTCC
HCV	HCV-F	CGGACGTAGCAGTGCTCACTTC
	HCV-R	TGATGAGCTGGCCAAGGAGG
GAPDH	GAPDH-F	ATCACTGCCACCCAGAAGAC
	GAPDH-R	TTTCTAGACGGCAGGTCAGG
U6	U6-F	CTCGCTTCGGCAGCACATATACT
	U6-R	ACGCTTCACGAATTTGCGTGTC

labelled inhibitor of caspase-1 assay (FLICA) and propidium iodide (PI) according to manufacturer's instruction (Bio-Rad, Hercules, CA, USA). The fluorescent signal was detected using flow cytometry, and the percentage of pyroptosis cells was the percentage of active caspase-1-PI double-positive cells in total cells.

Statistical analysis

All experiments data were performed at least three times and values were expressed as mean ± standard deviation (SD). SPSS software was used, and the data were analyzed using the Student's *t*-test and one-way analysis of variance (ANOVA). P<0.05 was considered statistically significant.

Results

High expression of KCNQ1OT1 in T2DM patients and β-cell infected by HCV

To explore the role of KCNQ1OT1 in HCV-infected T2DM, we detected the expression of KCNQ1OT1 in HCV-infected T2DM patients and non-HCV-infected T2DM patients. Compared to patients with T2DM who were not infected with HCV, those with T2DM who were infected had higher expression of KCNQ1OT1 (*Figure 1A*). In addition, we measured higher levels of pyroptosis-related proteins IL-1β, IL-18, NLRP3, caspase-1, and GSMDD in T2DM patients infected with HCV (*Figure 1B*). We then evaluated the expression of KCNQ1OT1 and miR-223p

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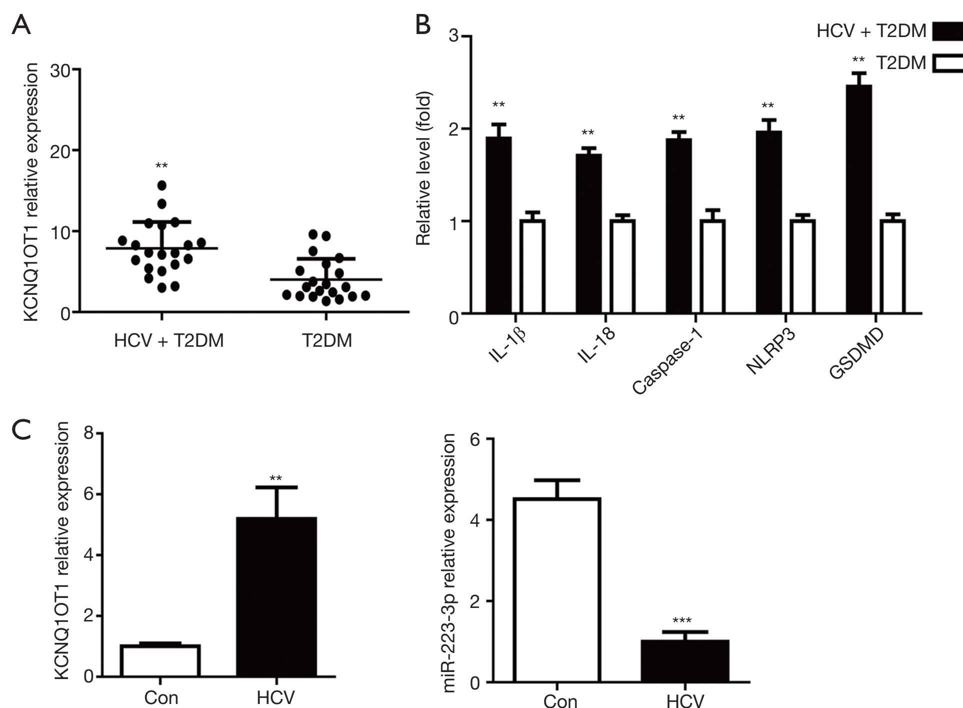

Figure 1 The expression of KCNQ1OT1 and apoptosis-related proteins increased under the induction of HCV. (A) RT-qPCR detected the expression of KCNQ1OT1. (B) ELISA detected the expression of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD. (C) The expression of KCNQ1OT1 and miR-223-3p revealed by RT-qPCR. (A,B) Compared with T2DM group, **P<0.01; (C) compared with Control (Con, min6 cells) group, **P<0.01, ***P<0.001. KCNQ1OT1, KCNQ1 overlapping transcript 1; HCV, hepatitis C virus; RT-qPCR, real-time quantitative PCR; T2DM, type 2 diabetes mellitus.

in HCV-infected min6 cells and found KCNQ1OT1 was significantly increased, and miR-223p was decreased after HCV infection (*Figure 1C*). Therefore, we determined that KCNQ1OT1 is highly expressed in HCV-infected T2DM patients and cells, and its increase may be related to pyroptosis.

KCNQ1OT1 is involved in β-cell pyroptosis induced by HCV infection

To clarify the relationship between KCNQ1OT1 and β-cell pyroptosis induced by HCV we constructed HCV-infected min6 cells and detected the HCV copy number. The HCV copy number of the HCV group was higher than the control group (*Figure 2A*), indicating that our vitro model could be used. We then applied different small infectious RNAs (siRNA), which included siRNA NC (si-NC) and siRNA-KCNQ1OT1 (si-K1/2/3), to knockdown KCNQ1OT1, and as si-K2 knockdown efficiency was the most significant (*Figure 2B*), we used it in subsequent studies. RT-qPCR and Western blot were used to detect the mRNA and protein expression levels of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD, respectively. And the results showed that while HCV infection increased the expression of these proteins related to pyroptosis, knockdown of KCNQ1OT1 significantly reduced the expression (*Figure 2C,2D*). In ELISA analysis, insulin secretion decreased after HCV infection, and insulin levels were restored after HCV-infected cells were transfected with si- KCNQ1OT1 (*Figure 2E*). Moreover, we found that HCV promoted cell pyroptosis and transfection of si-KCNQ1OT1 alleviated pyroptosis in HCV-infected cells (*Figure 2F,2G*). These results showed that KCNQ1OT1 regulated HCV-infected pyroptosis of β-cells, and knockdown of KCNQ1OT1 inhibited cell pyroptosis in HCV infection.

KCNQ1OT1 target regulates the miR-223-3p/NLRP3 axis

To explain the mechanism of KCNQ1OT1 in HCV-

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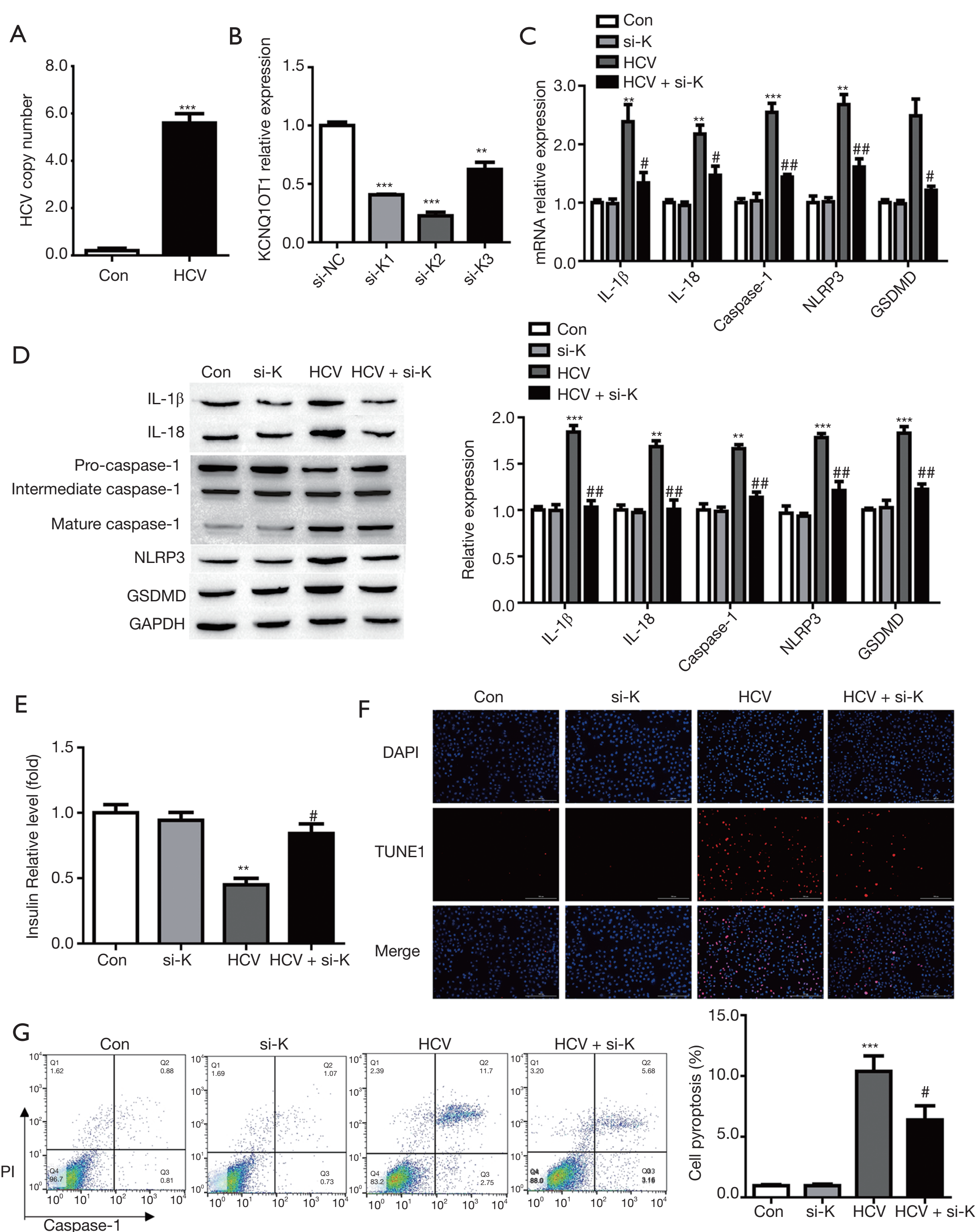

Figure 2 Knockdown of KCNQ1OT1 reduced β-cell pyroptosis induced by HCV. (A) RT-qPCR detected the HCV copy number. (B) RT-qPCR detection of knockdown efficiency. (C) The mRNA expression of *IL-1β*, *IL-18*, *NLRP3*, *caspase-1*, and *GSDMD* measured by RT-qPCR. (D) The protein expression of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD measured by Western blot. (E) ELISA to discover the level of insulin. (F) TUNEL determined cell death (scale: 200 μm). (G) Flow cytometry was used to analyze cell pyroptosis. Compared with Control (Con, min6 cells) group, **P<0.01, ***P<0.001; compared with HCV group, #P<0.05, ##P<0.01. KCNQ1OT1, KCNQ1 overlapping transcript 1; HCV, hepatitis C virus; RT-qPCR, real-time quantitative PCR; ELISA, enzyme-linked immunosorbent assay; si-K, small interfering RNA targeting KCNQ1OT1; PI, propidium iodide.

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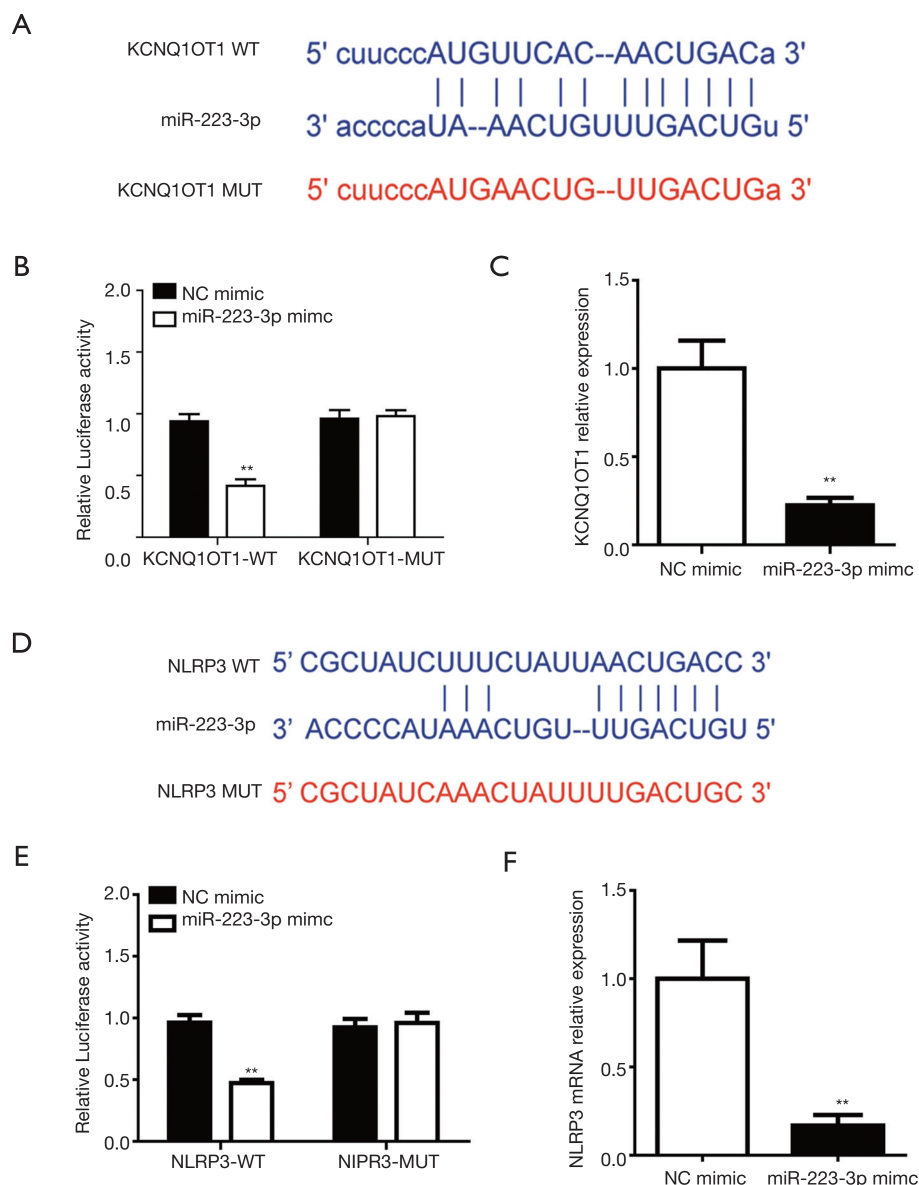

Figure 3 Targeting relationship of KCNQ1OT1, miR-223-3p, and NLRP3. (A) Sequence alignment of KCNQ1OT1, WT/MUT, and miR-223-3p. (B) Dual luciferase reporter gene assay to detect the targeting of KCNQ1OT1 and miR-223-3p. (C) The expression of KCNQ1OT1 presented by RT-qPCR. (D) Sequence alignment of NLRP3, WT/MUT, and miR-223-3p. (E) Dual luciferase reporter gene assay to detect the targeting of NLRP3 and miR-223-3p. (F) The expression of NLRP3 presented by RT-qPCR. Compared with NC group, **P<0.01. KCNQ1OT1, KCNQ1 overlapping transcript 1; WT, wild-type; MUT, mutant-type; RT-qPCR, real-time quantitative PCR; NC, negative control.

induced β-cell pyroptosis, we predicted the miRNA regulated by KCNQ1OT1 through the bioinformatics website "Starbase", and discovered miR-223-3p (*Figure 3A*). Dual luciferase reporter assay was performed to verify the interaction between KCNQ1OT1 and miR-223-3p. The KCNQ1OT1 3'-UTR WT fragments (KCNQ1OT1 WT) or MUT fragments (KCNQ1OT1 MUT) recombinant plasmids were co-transfected with the NC mimic or miR-223-3p mimic into cells, and the results showed that the luciferase activity of co-transfected KCNQ1OT1-WT and

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miR-223-3p mimics was significantly reduced (*Figure 3B*). Furthermore, the expression level of KCNQ1OT1 was evaluated after transfection with the miR-223-3p mimic and was found to have decreased (*Figure 3C*). Similarly, we found that the target gene of miR-223-3p was NLRP3, which is a key protein (*Figure 3D*). Compared with co-transfection with NLRP3-Wt and NC mimic, the luciferase activity of NLRP3-Wt and the miR-223-3p mimic decreased significantly (*Figure 3E*), and the expression of NLRP3 was down-regulated after transfection with the miR-223-3p mimic (*Figure 3F*). Therefore, we speculate that KCNQ1OT1 participates in the regulation of β-cell pyroptosis with HCV-induced through the miR-223-3p/NLRP3 axis.

KCNQ1OT1 regulates pyroptosis of β-cell infected by HCV through miRNA-223-3p

We further confirmed the role of miR-223-3p in KCNQ1OT1's regulation of HCV-infected β-cell pyroptosis. After HCV induced min6 cells, overexpression of KCNQ1OT1 or miR-223-3p and KCNQ1OT1 at the same time, and transfected the empty vector (pcDNA3.1) as a negative control. Overexpression of miRNA-223-3p reduced the mRNA and protein expression levels of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD, while KCNQ1OT1 restored this reduction (*Figure 4A,4B*). In addition, we investigated insulin secretion by ELISA assay, and the results showed that overexpression of miR-223-3p increased insulin secretion, and overexpression of miR-223-3p and KCNQ1OT1 decreased insulin secretion (*Figure 4C*). In the flow cytometry analysis, we demonstrated that miRNA-223-3p played an important role in cell pyroptosis, overexpression of miRNA-223-3p obviously decreased cell pyroptosis, and the change could be reversed though the overexpression of KCNQ1OT1 (*Figure 4D*). These results indicated that KCNQ1OT1 regulated β-cell pyroptosis under HCV-infection via targeting miRNA-223-3p.

HCV induces β-cell pyroptosis by regulating KCNQ1OT1/miRNA-223-3p/NLRP3

Furthermore, we investigated the interplay among KCNQ1OT1, miR-223-3p, and NLRP3 in β-cell pyroptosis which were HCV-infected. The result of RT-qPCR showed that si-KCNQ1OT1 (si-K) significantly decreased the mRNA expressions of *IL-1β*, *IL-18*, *NLRP3*, *caspase-1*, and *GSDMD*, compared with the HCV-infected group, while co-transfection with miR-223-3p inhibitor (inh) or overexpression of NLRP3 increased the mRNA expression of these proteins (*Figure 5A*). Similarly, knockdown of KCNQ1OT1 increased insulin secretion, and inhibition of miR-223-3p or overexpression of NLRP3 reversed this effect (*Figure 5B*). Further, Western blot demonstrated the expression of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD, and showed their expression decreased in cells which were transfected with si-K. After inhibiting miR-223-3p or overexpressing NLRP3, the expression of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD increased, which was consistent with the mRNA results (*Figure 5C*). Measurement of cell pyroptosis by flow cytometry proved that knockdown of KCNQ1OT1 reduced pyroptosis, while inhibiting miR-223-3p or overexpression of NLRP3 inhibited the cell pyroptosis induced by KCNQ1OT1 (*Figure 5D*). Consequently, we concluded that the KCNQ1OT1/miR-223-3p/NLRP3 axis regulates β-cell pyroptosis infected by HCV.

Discussion

T2DM is caused by the joint participation and interaction of multiple risk factors, and its incidence has continued to rise globally. The pathogenesis mainly involves pancreatic β-cell dysfunction and insulin resistance, while inflammation plays a mediator role in its pathogenesis. Studies (32-34) have shown that HCV infection is significantly related to T2DM, and insulin resistance and T2DM are more common in patients this infection. However, the mechanism by which HCV infection is related to the occurrence and development of T2DM is still unclear. One possible mechanism may involve damage to β-cells. Pyroptosis is a newly discovered inflammatory form of programmed cell death. When the NLRP3 inflammasome is activated, caspase-1 is activated, which then mediates the activation of downstream inflammatory factors (IL-1β and IL-18) and the lysis of GSDMD to trigger the production of pyroptosis (35-37). Pyroptosis is not only related to diabetes and its complications but is closely related to HCV virus infection. Therefore, we studied the mechanism of HCV infection-induced β-cell pyroptosis leading to T2DM.

LncRNA exerts biological effects through a variety of mechanisms. LncRNA KCNQ1OT1 is abnormally expressed in a variety of diseases and plays different roles among them (38-40). Our study found that KCNQ1OT1 was highly expressed in HCV-infected T2DM patients and cells, and cell experiments proved that KCNQ1OT1

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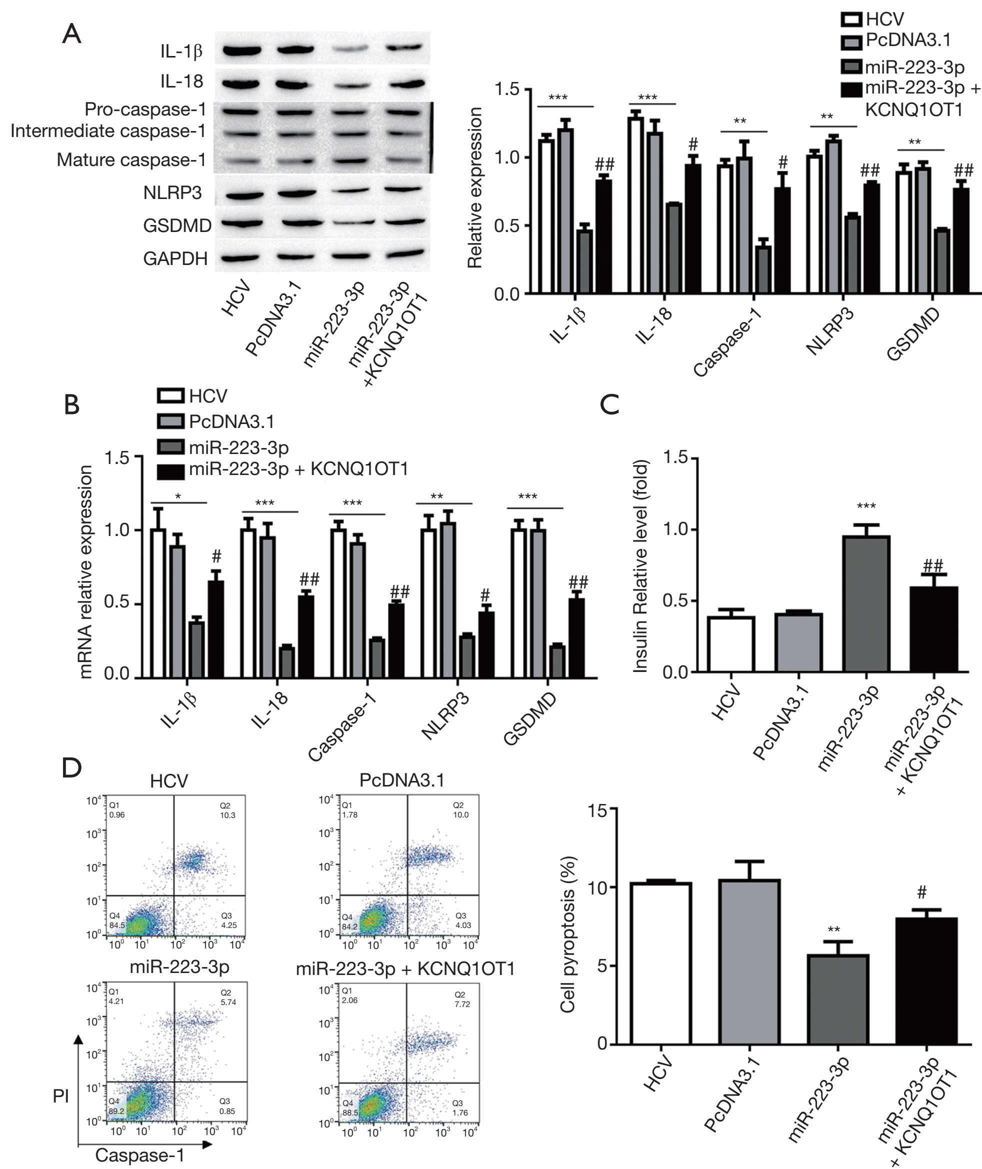

Figure 4 KCNQ1OT1 regulates β-cell pyroptosis under HCV-infected via targeting miRNA-223-3p. (A) The protein expression of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD presented by Western blot. (B) RT-qPCR detection of the mRNA expression of *IL-1β*, *IL-18*, *NLRP3*, *caspase-1*, and *GSDMD*. (C) ELISA to measure the level of insulin. (D) Flow cytometry was used to analyze cell pyroptosis. All groups were infected with HCV. Compared with HCV group, *P<0.05, **P<0.01, ***P<0.001; compared with miR-223-3p group, #P<0.05, ##P<0.01. KCNQ1OT1, KCNQ1 overlapping transcript 1; HCV, hepatitis C virus; RT-qPCR, real-time quantitative PCR; ELISA, enzyme-linked immunosorbent assay; PI, propidium iodide.

silence after HCV infection reduced β-cell pyroptosis, indicating that KCNQ1OT1 plays a considerable role in T2DM induced by HCV infection. LncRNA regulates downstream target genes by binding miRNA and is associated with pyroptosis. For example, lncRNA MALAT1/miR-23c/ELAVL1 regulated renal tubular epithelial cell pyroptosis in diabetic nephropathy (41), lncRNA MALAT1/miR-30c/NLRP3 promoted renal tubular epithelial cell pyroptosis induced by high glucose (HG) (42), and lncRNA KCNQ1OT1/miR-214-3p/NLRP3 regulated the pyroptosis

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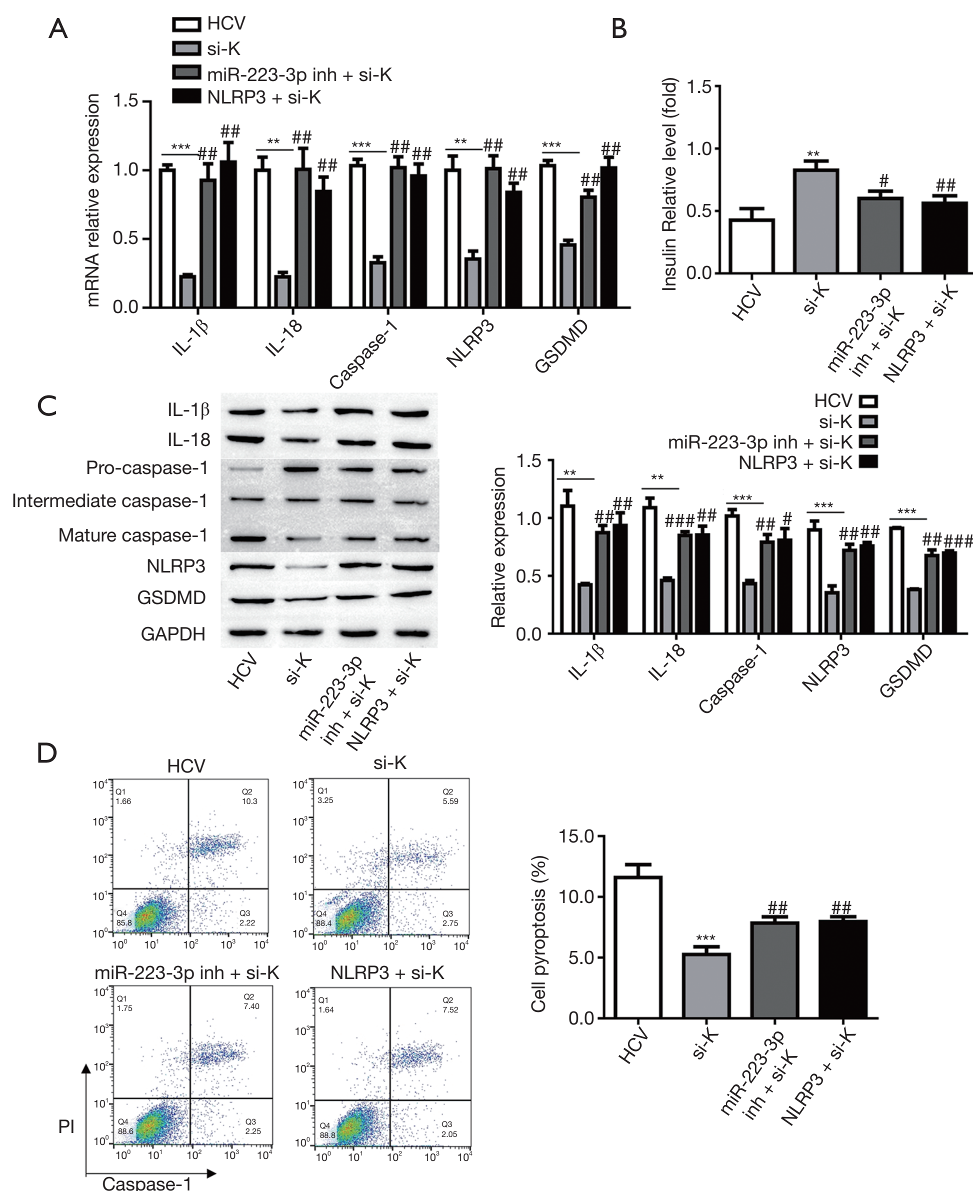

Figure 5 KCNQ1OT1/miR-223-3p/NLRP3 axis participates in HCV-induced β-cell pyroptosis. (A) RT-qPCR detection of the mRNA expression of *IL-1β*, *IL-18*, *NLRP3*, *caspase-1*, and *GSDMD*. (B) ELISA to measure the level of insulin. (C) The protein expression of IL-1β, IL-18, NLRP3, caspase-1, and GSDMD were presented by Western blot. (D) Flow cytometry was used to analyze cell pyroptosis. All groups were infected with HCV. Compared with HCV group, **P<0.01, ***P<0.001; compared with si-K group, #P<0.05, ##P<0.01, ###P<0.001. KCNQ1OT1, KCNQ1 overlapping transcript; HCV, hepatitis C virus; RT-qPCR, real-time quantitative PCR; ELISA, enzyme-linked immunosorbent assay; si-K, small interfering RNA targeting KCNQ1OT1; PI, propidium iodide; inh, inhibitor.

of cardiomyocytes in diabetic cardiomyopathy (43).

However, the molecular mechanism of lncRNA/miRNA regulating β-cell pyroptosis in T2DM induced by HCV infection has not been reported. We predicted the potential miRNA of KCNQ1OT1 through "Starbase", miR-223-3p and KCNQ1OT1 have binding sites. miR-223-3p could be used as a potential biomarker in diabetes and its complications (44), and, importantly, was closely related to HCV-infected liver cirrhosis and HCC (45). Our data indicated that miR-223-3p is lowly expressed

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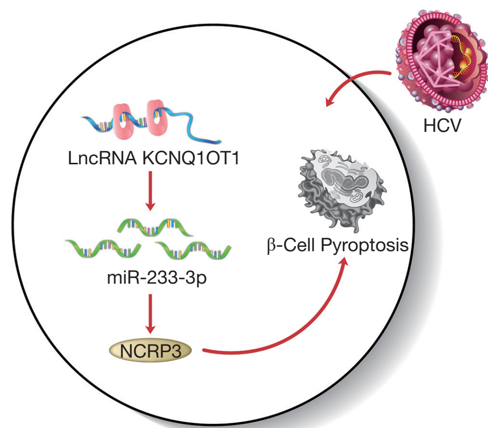

Figure 6 Schematic diagram of the mechanism of the KCNQ1OT1/miR-223-3p/NLRP3 axis involved in HCV-induced β cell pyroptosis. KCNQ1OT1, KCNQ1 overlapping transcript 1; HCV, hepatitis C virus.

in HCV-infected β-cells, and its overexpression inhibited cell pyroptosis, while KCNQ1OT1 abolished the inhibitory effect of miR-223-3p, which further proved that KCNQ1OT1 regulates HCV-infected cell pyroptosis through miR-223-3p. The pyroptosis related protein NLRP3 is a target gene of miR-223-3p. In this study, we explored NLRP3 under the induction of HCV, and found KCNQ1OT1/miR-223-3p targeted and regulated NLRP3, which affected the expression of IL-1β, IL-18, caspase-1, and GSDMD, thereby regulating β-cell pyroptosis. Taken together, these results suggest KCNQ1OT1 acts as a sponge of miR-223-3p to affect the expression of NLRP3 and participates in the pathogenesis of T2DM induced by HCV infection by regulating the apoptosis of β cells (*Figure 6*).

In summary, our study proved that KCNQ1OT1 promotes the HCV-infected pyroptosis of β-cells by regulating the miRNA-223-3p/NLRP3 axis, effecting the production of insulin and accelerating the occurrence and development of T2DM. Regulating KCNQ1OT1 and its target genes will help to better understand the pathogenesis of T2DM induced by HCV infection and provide new theoretical foundations and therapeutic targets.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study received approval from the ethics committee of the First People's Hospital of Yunnan Province (KHLL2020-KY059), and all participants signed informed consent forms. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

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