Article

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ELAVL1 Regulates NLRP3 Inflammasome Activation and Contributes to Diabetic Hypoglycemia-Related Myocardial Injury

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Background: Recurrent hypoglycemia is a common complication of intensive glycemic control in patients with type 2 diabetes mellitus and may contribute to cardiovascular injury. However, the molecular mechanisms underlying hypoglycemia-induced myocardial inflammation remain incompletely understood. This study aimed to elucidate the function and mechanistic pathways by which the RNA-binding protein ELAV-like RNA binding protein 1 (ELAVL1, also termed Hu antigen R / HuR) modulates NLRP3 pathway-associated inflammatory responses in the context of myocardial injury exacerbated by recurrent hypoglycemia in type 2 diabetes mellitus.

Methods: A total of eighteen male C57BL/6J mice were allocated at random into three experimental groups (n = 6 per group): a control group, a type 2 diabetes mellitus group generated by high-fat feeding followed by streptozotocin administration, and a diabetic hypoglycemia group, in which diabetic mice received daily intraperitoneal insulin treatment for five consecutive days to establish recurrent hypoglycaemic episodes. Hypoglycemia was defined as a blood glucose concentration of ≤3.5 mmol/L maintained for 1 h. Cardiac function was assessed by echocardiography. Myocardial histopathology was examined by hematoxylin–eosin staining. Serum concentrations of cardiac troponin I (cTnI), cTnT, interleukin-1β (IL-1β), IL-18, and insulin were quantified using enzyme-linked immunosorbent assay (ELISA). Myocardial ELAVL1 mRNA expression was quantified by quantitative real-time PCR (qPCR). An intermittent low-glucose/recovery model was established in HL-1 atrial cardiomyocytes. ELAVL1 expression was silenced (sh-ELAVL1) or overexpressed (oe-ELAVL1) using lentiviral vectors. Cell injury was evaluated by CCK-8 assay and lactate dehydrogenase (LDH) release. The transcript and protein abundance of ELAVL1, NLRP3, and cleaved caspase-1 (p20) were determined using qPCR and immunoblot analysis, whereas GSDMD-N (p30) expression was visualized by immunofluorescence.

Results: HDM mice demonstrated greater reductions in left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) than diabetes mellitus (DM) controls ($p < 0.05$), with more severe myocardial fiber disorganization, interstitial edema, and inflammatory cell infiltration. The HDM group displayed markedly higher circulating concentrations of cTnI, cTnT, IL-1β, and IL-18, together with increased myocardial ELAVL1 transcript abundance ($p < 0.05$). *In vitro*, recurrent hypoglycemic stimulation reduced cell viability and increased LDH release ($p < 0.05$), accompanied by upregulation of ELAVL1 and NLRP3 expression. ELAVL1 silencing restored cell viability, reduced LDH release, and decreased the expression of NLRP3, cleaved Caspase-1 (p20), and GSDMD-N (p30) ($p < 0.05$), whereas ELAVL1 overexpression exerted the opposite effects ($p < 0.05$).

Conclusion: Recurrent hypoglycemia exacerbates myocardial injury in diabetic mice and is accompanied by enhanced inflammatory responses and increased ELAVL1 levels. *In vitro* functional studies further demonstrated that ELAVL1 positively regulates NLRP3/Caspase-1/gasdermin D (GSDMD)-associated pyroptotic signaling and aggravates hypoglycemia-induced cardiomyocyte injury. These findings suggest that ELAVL1 may contribute to the pathogenesis of diabetes-associated hypoglycemic myocardial injury and represents a potential therapeutic target.

Keywords: diabetic cardiomyopathy; hypoglycemia; NLRP3 inflammasome; pyroptosis; ELAVL1

Introduction

Diabetic cardiomyopathy (DCM) represents a diabetes-associated myocardial disease marked by progressive cardiac structural abnormalities and contractile dysfunction in the absence of overt coronary, hypertensive, or valvular pathology, ultimately predisposing affected individuals to heart failure and increased mortality [1,2,3]. In contemporary clinical practice, achieving intensive glycaemic control often requires insulin or insulin secretagogues, both of which substantially increase the risk of recurrent hypoglycaemic episodes. Reflecting this concern, the 2024 American Diabetes Association Standards of Care identified hypoglycemia as a critical safety issue requiring

continuous surveillance and proactive management [4]. Severe hypoglycemia has been consistently associated with heightened risks of cardiovascular complications and increased mortality in epidemiological studies; however, secondary analyses of recent randomized clinical trials have yielded inconsistent findings, suggesting that hypoglycemia may act not only as a direct pathogenic trigger but also as a marker of heightened cardiovascular vulnerability [5,6,7]. Mechanistically, hypoglycemia induces excessive sympathoadrenal activation, oxidative stress bursts, inflammatory perturbation, and haemodynamic instability, all of which provide a biologically plausible basis for aggravating diabetic myocardial injury [8,9].

Among the mechanisms implicated in DCM pathogenesis, NLRP3 inflammasome-mediated pyroptosis has emerged as a central focus of investigation [1,10]. In the canonical pyroptotic pathway, metabolic stress triggers NLRP3 inflammasome assembly, activating caspase-1 and cleaving gasdermin D (GSDMD) to release its pore-forming N-terminal fragment, thereby disrupting plasma membrane integrity and facilitating the secretion of mature interleukin-1β (IL-1β) and IL-18 [11]. Diabetic cardiac tissues and high glucose-treated human ventricular cardiomyocytes exhibited markedly elevated expression of inflammasome-associated molecules, including NLRP3, caspase-1, and IL-1β, whereas pharmacological inhibition or genetic depletion of NLRP3 alleviated myocardial inflammation, pyroptotic injury, and fibrotic remodeling, resulting in improved cardiac function in experimental models [12,13,14,15]. Consistent with these findings, recent reviews have identified the NLRP3 inflammasome as a promising therapeutic target for DCM [16]. Notably, however, nearly all of these studies have been conducted under sustained hyperglycaemic conditions. Whether the more clinically relevant composite metabolic stress of diabetes combined with recurrent hypoglycemia further amplifies NLRP3 pathway-associated inflammatory responses and thereby acts as a pivotal driver of myocardial injury remains largely unexplored.

RNA-binding proteins (RBPs) regulate RNA stability, alternative splicing, subcellular localization, and translational efficiency, thereby constituting a critical post-transcriptional regulatory layer in cardiovascular disease and pathological cardiac remodelling [17]. Among them, ELAVL1 (HuR) is one of the best-characterized RBPs and exerts broad regulatory effects by binding AU-rich elements in target mRNAs, enhancing their stability and translation, and consequently amplifying inflammatory and stress-responsive signalling pathways [18]. In cardiovascular pathology, ELAVL1 has been implicated in myocardial ischaemia–reperfusion injury, fibrosis, and inflammatory responses. In the context of metabolic cardiac injury, previous studies have shown that hyperglycaemia upregulates ELAVL1 expression and promotes cardiomyocyte pyroptosis, whereas the miR-9/ELAVL1 axis suppresses high glucose-induced pyroptotic injury in human ventricular cardiomyocytes [19]. More recent evidence further suggests that ELAVL1 silencing improves cardiac function in diabetic mice, supporting its pathological relevance in diabetic heart disease [20]. Nevertheless, whether ELAVL1 also contributes to recurrent hypoglycemia-induced myocardial injury, and more importantly whether it functions upstream of NLRP3 inflammasome-mediated inflammatory responses to promote pyroptotic amplification under this composite metabolic stress, remains unknown.

Based on these considerations, we established a murine model of type 2 diabetes subjected to recurrent insulin-induced hypoglycemia, together with an intermittent low-glucose model in HL-1 atrial cardiomyocytes, to systematically investigate the ELAVL1–NLRP3 inflammasome–pyroptosis axis. Our study characterizes the molecular events through which recurrent hypoglycemia intensifies diabetic cardiac injury and provides experimental validation of the resulting pathological changes.

Materials and Methods

Experimental Animals and Establishment of the Diabetic Hypoglycemia Model

Eighteen male C57BL/6J mice (4–5 weeks old, 18–22 g), certified specific pathogen-free (SPF), were obtained from Chongqing Ensiweier Biotechnology Co., Ltd. (Chongqing, China). Animals were maintained in an SPF barrier facility under controlled conditions (22 ± 2 °C, 50%–60% humidity) with a 12-hour light/dark cycle and ad libitum access to food and water. Following a one-week acclimation period, mice were randomly allocated into three experimental groups (n = 6 per group): control, type 2 diabetes mellitus (DM), and diabetic hypoglycemia (HDM). The experimental procedures were approved by the Animal Experimental Ethics Committee of Chongqing Medical University (IACUC-CQMU-2023-0065) and conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals. All aspects of the study were reported in compliance with ARRIVE Guidelines 2.0 to ensure methodological transparency, reproducibility, and ethical rigor.

Animals assigned to the control group received a regular laboratory diet throughout the study. To establish type 2 diabetes, mice were exposed to a high-fat nutritional regimen (60% kcal from fat; Research Diets, D12492) for four weeks and subsequently challenged with streptozotocin (50 mg/kg, i.p.; Sigma-Aldrich, S0130) dissolved in citrate buffer (pH 4.5) once daily for five consecutive days following an overnight fast. Diabetic status was evaluated seven days after the final injection using tail-vein fasting glucose measurements obtained with a portable glucose meter (Accu-Chek Performa, Roche). Animals exhibiting persistent hyperglycaemia (≥16.7 mmol/L) together with

characteristic diabetic manifestations, including excessive food intake, increased urination, and body weight reduction, were classified as diabetic and remained on the high-fat feeding protocol for subsequent experiments.

To generate repeated hypoglycaemic episodes, diabetic animals assigned to the HDM cohort underwent a 12-h fasting period before receiving insulin (15 IU/kg; Novolin R, Novo Nordisk, Clayton, NC, USA) via intraperitoneal administration. Tail vein blood glucose was monitored every 20 min. Once blood glucose reached ≤3.5 mmol/L, hypoglycemia was maintained for 1 h, after which mice received an intraperitoneal injection of 20% glucose in sterile PBS (1 g/kg) to restore blood glucose levels. This procedure was repeated for 5 consecutive days.

Echocardiographic Evaluation

Cardiac performance was evaluated 24 h after the last hypoglycaemic episode. Under inhalational anaesthesia delivered by an isoflurane system (RWD Life Science, Shenzhen, China), animals were initially exposed to 3% isoflurane and subsequently maintained at 1.5–2.0% in oxygen (1 L/min). Throughout imaging, mice were maintained in the supine position on a thermostatically regulated platform (37 °C), while their physiological status was continuously monitored.

Cardiac ultrasound examinations were performed using a Vevo 3100 imaging platform (VisualSonics, Toronto, Canada) fitted with a 30-MHz MX400 probe. Parasternal long-axis views were recorded in both B-mode and M-mode. Ventricular dimensions at end-diastole and end-systole were quantified with VevoLab software (v5.6.0; VisualSonics, Fujifilm VisualSonics Inc., Toronto, Ontario, Canada), and systolic performance was derived from these measurements by the Teichholz formula, yielding left ventricular ejection fraction (LVEF) and fractional shortening (LVFS).

Sample Collection and Haematoxylin–Eosin Staining

After completing echocardiography, mice received deep anesthesia via intraperitoneal injection of sodium pentobarbital (150 mg/kg; P3761, Beijing Zongheng Scientific Co., China). Blood was drawn from the orbital venous plexus and left to clot at 25 °C for 30 minutes. Samples were then centrifuged at 3000 rpm for 15 minutes at 4 °C, and the resulting serum was stored at –80 °C for subsequent ELISA assays.

Following blood sampling, mice were sacrificed by an overdose of sodium pentobarbital (200 mg/kg, i.p.). Once cardiac and respiratory activity had ceased, the thoracic cavity was opened, and the heart was perfused through the left ventricle with ice-cold PBS before myocardial tissue harvest.

Cardiac specimens obtained from the mid-ventricular region of the left ventricle were preserved in 4% paraformaldehyde for 24 h and subsequently processed for paraffin embedding through routine dehydration and clearing procedures. Tissue slices (5 μm) were stained with hematoxylin and eosin (B1002, Baiqiandu Biotechnology, Wuhan, China). Morphological alterations were documented using a BX53 light microscope (Olympus, Tokyo, Japan) at 200× and 400× magnifications.

Enzyme-Linked Immunosorbent Assay (ELISA)

Commercial ELISA kits were employed to quantify circulating cTnI (Fine Biotech, EM1466; 7.813–500 pg/mL), cTnT (Fine Biotech, EM0928), IL-1β (BD Biosciences, #559603), IL-18 (R&D Systems, #7625), and insulin (Crystal Chem, #90080) in serum samples. All measurements were carried out following the protocols provided with each assay kit.

Cell Culture and Establishment of the Diabetic Hypoglycemia Cell Model

HL-1 atrial cardiomyocytes were obtained from ATCC (Manassas, VA, USA), verified by STR profiling, and confirmed free of mycoplasma. Cells were maintained in Claycomb medium (Sigma-Aldrich, 51800C) supplemented with 10% FBS (Gibco, 10099-141), 100 U/mL penicillin–streptomycin (Gibco, 15140-122), and 2 mM L-glutamine (Gibco, 25030-081) at 37 °C in a humidified incubator with 5% CO_2.

To mimic recurrent hypoglycemic episodes occurring in a diabetic setting, a recurrent hypoglycemia (RH) model was established under hyperglycemic conditions. High-glucose medium consisted of DMEM containing 4.5 g/L glucose with additional glucose added to achieve a final concentration of 30 mM, whereas low-glucose medium consisted of glucose-free DMEM supplemented with glucose to a final concentration of 1 mM. Following adaptation to hyperglycemic culture conditions, HL-1 cells underwent a 2-h glucose deprivation phase (1 mM glucose), after which they were returned to glucose-rich medium for 6 h. This hypoglycemia/recovery cycle was repeated three times to establish the RH model. Control cells were continuously maintained in high-glucose medium (30 mM glucose) to exclude potential effects attributable to culture conditions alone.

Cell Transfection and Experimental Grouping

Lentiviral shRNA vectors targeting mouse ELAVL1 (sh-ELAVL1; 5′-CCGGCATTGGGAGAACGAATTTAATCTCGAGATTAAATTCGTTCTCCCAATGTTTTTG-3′; Clone ID: TRCN0000112087) and the scrambled control sequence (sh-NC; 5′-CAACAAGATGAAGAGCACCAA-3′) were constructed by GenePharma (Shanghai, China) using the pGLV3/H1/GFP+Puro backbone. For ELAVL1 overexpression, the full-length mouse ELAVL1 coding sequence (RefSeq NM_001001990.2) was cloned into the pLVX-CMV-MCS-puro lentiviral expression vector (Takara Bio/Clontech), and the empty vector served as the

overexpression control (oe-NC). The recombinant plasmid was verified before subsequent use.

HL-1 cells at 70%–80% confluence were infected with lentiviral particles (MOI = 30) in serum-free medium containing polybrene (8 μg/mL). After 8 h, the medium was replaced with complete growth medium. Stable transfectants were selected with puromycin (2 μg/mL; Sigma-Aldrich) starting 48 h post-infection.

Six groups were established: control, model, sh-NC, sh-ELAVL1, oe-NC, and oe-ELAVL1. The recombinant overexpression plasmid was confirmed by Sanger sequencing before use.

Cell Viability Assay

HL-1 cells were seeded into 96-well plates at 5×10^3 cells per well and exposed to the indicated treatments. Following incubation with CCK-8 reagent (10 μL/well) (Dojindo, CK04, Kumamoto, Japan) for 2 h, absorbance at 450 nm was determined using a Varioskan LUX reader (Varioskan LUX, Thermo Fisher Scientific). Relative viability was expressed as a percentage of the control group.

Lactate Dehydrogenase Release Assay

Cell culture supernatants were collected to assess cytotoxicity via lactate dehydrogenase (LDH) release using the Elabscience LDH Cytotoxicity Kit (E-BC-K046-M, Wuhan, China). Supernatants were combined with the LDH working solution and incubated in the dark at room temperature for 30 min. Absorbance at 490 nm was recorded, and LDH release was calculated relative to the control group.

Quantitative Real-Time PCR (qPCR)

Total RNA was isolated from myocardial tissue or HL-1 cells using TRIzol reagent (Invitrogen, 15596026). Purity was evaluated by A260/A280 measurement (1.8–2.0) on a NanoDrop 2000 (Thermo Fisher Scientific). One microgram of RNA was reverse-transcribed into cDNA with the PrimeScript RT kit (Takara, RR047A). Quantitative PCR was carried out using TB Green® Premix Ex Taq™ (Takara, RR420A) on a QuantStudio 7 Flex system. Cycling conditions included an initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60°C for 34 s. β-Actin served as the internal control, and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences as follows: ELAVL1, forward 5′-AAACATGGCTCTCCTCTCGC-3′ and reverse 5′-ACCCATAGGGGAGAACCTGA-3′; NLRP3, forward 5′-ACTTGCAGAAGCTGGGGTTG-3′ and reverse 5′-TCCAAGGGCATTGCTTCGTA-3′; and β-actin, forward 5′-GTGACGTTGACATCCGTAAAGA-3′ and reverse 5′-GCCGGACTCATCGTACTCC-3′.

Western Blot Analysis

Following lysis of HL-1 cells in inhibitor-containing RIPA buffer (Beyotime, P0013C; P1045) on ice, cell extracts were clarified by centrifugation (12,000 rpm, 15 min, 4 °C). Total protein levels in the resulting supernatants were subsequently quantified using a BCA assay kit (Beyotime, P0012).

Protein extracts (30 μg per sample) were resolved on 12% polyacrylamide gels under denaturing conditions and electroblotted onto PVDF membranes. Following a 1-h blocking step in 5% skim milk, membranes were exposed to antibodies against ELAVL1 (Proteintech, 11910-1-AP), NLRP3 (ABclonal, A24294), cleaved caspase-1 (p20) (Cell Signaling Technology, 89332), and β-actin (Cell Signaling Technology, 4970) at 4 °C overnight. After TBST rinsing, HRP-linked secondary antibody (Abcam, ab6721, 1:2000) was applied for 60 min before signal detection.

Chemiluminescent signals were generated using an ECL detection reagent (Tanon, 180-5001), and relative protein abundance was determined using densitometric measurement with ImageJ software (version 1.53t; National Institutes of Health, Bethesda, MD, USA).

Immunofluorescence

Following experimental intervention, HL-1 cells grown on coverslips were preserved with paraformaldehyde, rendered permeable using Triton X-100, and treated with BSA to minimize nonspecific binding. Samples were subsequently exposed to anti-GSDMD-N (p30) antibody (ABclonal, A24059; 1:200 dilution) at 4 °C overnight [21]. Following removal of unbound primary antibody, fluorescent labeling was achieved using Alexa Fluor 488-coupled goat anti-rabbit IgG (Invitrogen, A11008; 1:500). Nuclear DNA was subsequently visualized with DAPI (Sigma-Aldrich, D9542), and specimens were preserved using an antifade mounting reagent before imaging. Cellular fluorescence signals were recorded with a Leica TCS SP8 confocal imaging platform (Leica, Germany). Signal abundance was evaluated using ImageJ software, based on measurements from three independent microscopic regions per group, each containing no fewer than 20 cells.

Statistical Analysis

Experimental results are expressed as mean values with corresponding standard deviations. Data processing and statistical evaluation were conducted using GraphPad Prism 9.0 (GraphPad Software, USA). Differences across multiple experimental conditions were assessed by one-factor variance analysis with subsequent multiple-comparison correction, whereas direct comparisons between two datasets employed an independent-samples *t*-test. Statistical significance was defined at a probability threshold below 0.05.

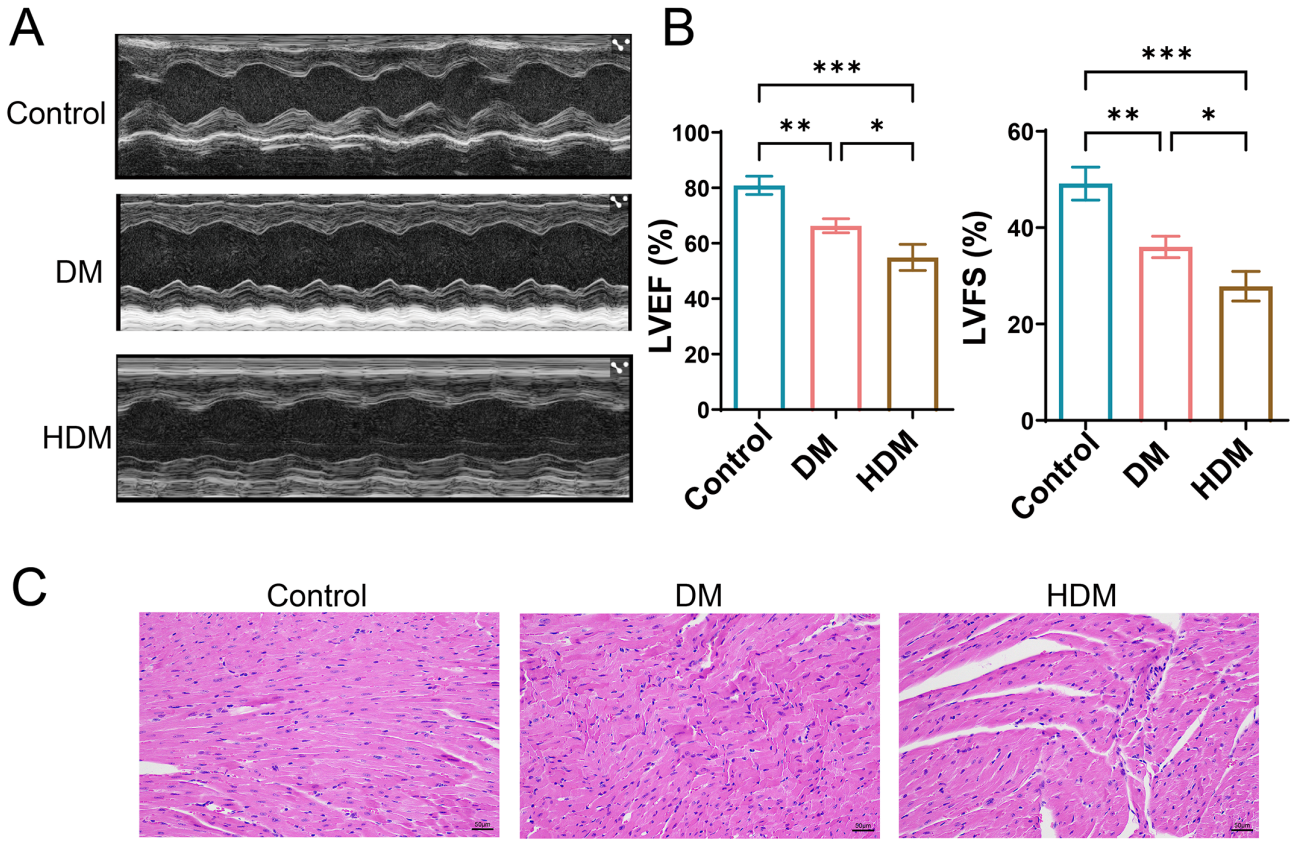

Fig. 1. Assessment of myocardial function and tissue injury under diabetic hypoglycemic conditions. (A) Echocardiographic recordings. (B) LVEF and LVFS analyses. (C) Histological appearance of cardiac tissue following H&E staining. Scale bar, 50 µm. Data are presented as mean ± SD (n = 6 biological replicates per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; H&E, hematoxylin and eosin; SD, standard deviation.

Results

Recurrent Hypoglycemia Aggravates Myocardial Structural Injury and Cardiac Dysfunction in Diabetic Mice

To evaluate cardiac damage associated with diabetic status and repeated hypoglycemic exposure, a type 2 DM model was established and subjected to repeated insulin-induced HDM. Echocardiographic analysis showed that, compared with control mice, diabetic mice exhibited significant reductions in LVEF and LVFS ($p < 0.05$). Notably, these impairments were further exacerbated in HDM mice, which showed significantly lower LVEF and LVFS than DM mice ($p < 0.05$) (Fig. 1A,B).

HE staining showed mild disorganization of myocardial fibers and partial cardiomyocyte hypertrophy in the DM group. In contrast, HDM mice exhibited more severe pathological changes, including myocardial fiber disruption, pronounced architectural disarray, marked interstitial edema, and inflammatory cell infiltration (Fig. 1C). Together, these findings indicate that recurrent hypoglycemic episodes substantially aggravate diabetes-associated myocardial structural damage and contractile dysfunction.

Diabetic Hypoglycemia Promotes NLRP3 Pathway-Associated Inflammatory Responses and Upregulates Myocardial ELAVL1 Expression

To assess the extent of myocardial injury, circulating cardiac troponins, established biomarkers released during cardiomyocyte damage, were quantified. Serum concentrations of the myocardial injury markers cTnI and cTnT were significantly elevated in diabetic mice relative to the control group, and recurrent hypoglycemic exposure produced an additional increase in both biomarkers ($p < 0.05$; Fig. 2A,B). Repeated hypoglycemic episodes further increased IL-1β and IL-18 production in diabetic mice relative to the DM group ($p < 0.05$; Fig. 2C,D), supporting intensified inflammatory signaling. Serum insulin concentrations were also increased in DM mice and further elevated in the HDM group ($p < 0.05$; Fig. 2E). qPCR analysis demonstrated significant upregulation of myocardial ELAVL1 mRNA in diabetic mice, with the highest expression observed in the HDM group ($p < 0.05$; Fig. 2F). These findings suggest that ELAVL1 may be involved in the inflammatory and injury responses associated with diabetic hypoglycemia.

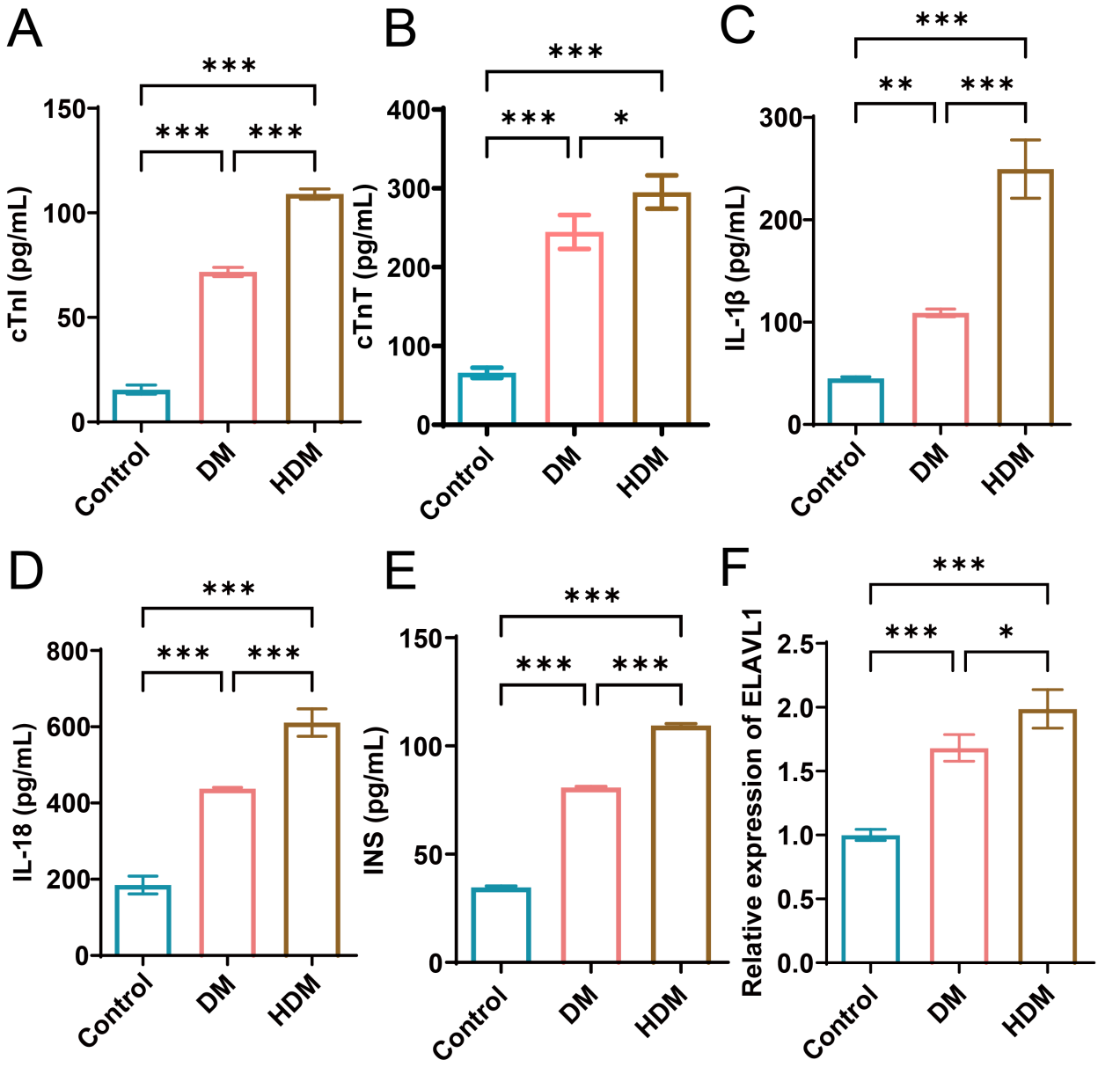

Fig. 2. Changes in myocardial injury markers, inflammatory cytokines, and ELAVL1 expression in diabetic hypoglycemic mice. (A) Serum cardiac troponin I (cTnI) levels. (B) Serum cardiac troponin T (cTnT) levels. (C) Serum interleukin-1β (IL-1β) levels. (D) Serum interleukin-18 (IL-18) levels. (E) Serum insulin levels. (F) Myocardial ELAVL1 mRNA expression. Data are presented as mean ± SD (n = 6 biological replicates per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ELAVL1, ELAV-like RNA-binding protein 1 (Hu antigen R, HuR).

ELAVL1 Regulates the NLRP3 Inflammasome Pathway and Exacerbates Low-Glucose-Induced Cardiomyocyte Pyroptosis

To elucidate the regulatory role of ELAVL1, we established an intermittent low-glucose model in HL-1 atrial cardiomyocytes to mimic diabetic hypoglycemia and performed functional interventions. Transfection efficiency verification showed that sh-ELAVL1 significantly reduced ELAVL1 levels, while oe-ELAVL1 markedly increased them ($p < 0.05$) (Fig. 3A,B). Exposure to low-glucose conditions induced marked cellular injury, as evidenced by reduced survival capacity and enhanced LDH leakage. Concurrently, transcriptional levels of ELAVL1 and NLRP3 were markedly augmented compared with untreated cells ($p < 0.05$; Fig. 3C–E). Compared with the sh-NC group, ELAVL1 knockdown significantly restored cell viability, reduced LDH release, and suppressed NLRP3 mRNA expression ($p < 0.05$). ELAVL1 augmentation aggravated the detrimental effects of glucose deprivation, as reflected by reduced viability, enhanced LDH leakage, and higher NLRP3 mRNA levels ($p < 0.05$; Fig. 3C–E).

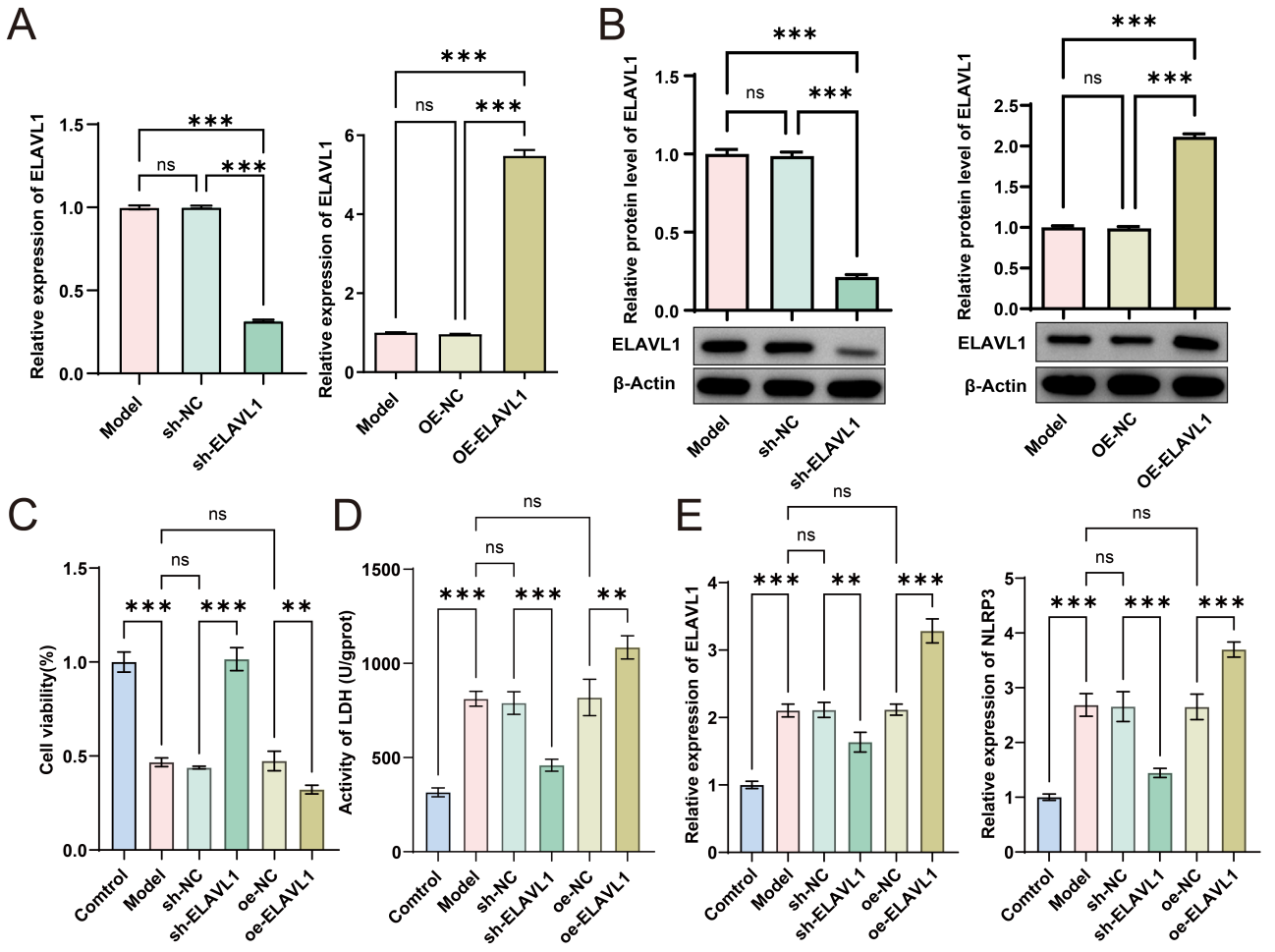

Fig. 3. Effects of ELAVL1 knockdown or overexpression on low-glucose-induced HL-1 cell injury and NLRP3 transcription. (A) Validation of ELAVL1 knockdown and overexpression efficiency in HL-1 cells by transcript quantification. (B) Corresponding alterations in ELAVL1 protein abundance following genetic manipulation. (C) Evaluation of cellular metabolic activity using the CCK-8 method. (D) Extracellular LDH levels as an indicator of membrane damage. (E) Transcriptional profiles of ELAVL1 and NLRP3 in different treatment groups. All data are presented as mean ± SD from three independent biological replicates (n = 3). $^{**}p < 0.01$; $^{***}p < 0.001$; ns, not significant. HL-1, murine atrial cardiomyocyte cell line; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; CCK-8, Cell Counting Kit-8; LDH, lactate dehydrogenase.

Immunoblotting confirmed the molecular alterations observed at the mRNA level. Low-glucose stimulation was associated with accumulation of ELAVL1, NLRP3, and cleaved caspase-1 (p20), whereas ELAVL1 knockdown attenuated these responses. In contrast, enforced ELAVL1 expression further enhanced activation of the corresponding proteins (all $p < 0.05$; Fig. 4A,B). As the pore-forming execution fragment of pyroptosis, immunofluorescence analysis of GSDMD-N (p30) demonstrated markedly enhanced green fluorescence in the model group. This signal was attenuated following ELAVL1 knockdown but further intensified upon ELAVL1 overexpression ($p < 0.05$) (Fig. 5A,B). Collectively, these data indicate that ELAVL1 is a positive contributor to the NLRP3 inflammasome pathway, and its upregulation exacerbates low-glucose-induced cardiomyocyte pyroptosis and injury.

Discussion

Episodes of profound hypoglycemia represent an important adverse consequence of glucose-lowering therapy and are associated with a greater burden of cardiovascular complications as well as an elevated risk of mortality [22]. Rana et al. [23] reported in patients with type 1 DM that acute hypoglycemia significantly reduced myocardial flow reserve and was accompanied by evidence of distal myocardial ischemia, suggesting that hypoglycemia itself may aggravate cardiac injury by inducing myocardial ischemia and autonomic stress. Similarly, Lee et al. [24] retrospectively observed that diabetic patients admitted with acute myocardial infarction (AMI) who experienced hypoglycemia had significantly higher 30-day mortality than those with well-controlled glucose levels. Our findings further sup-

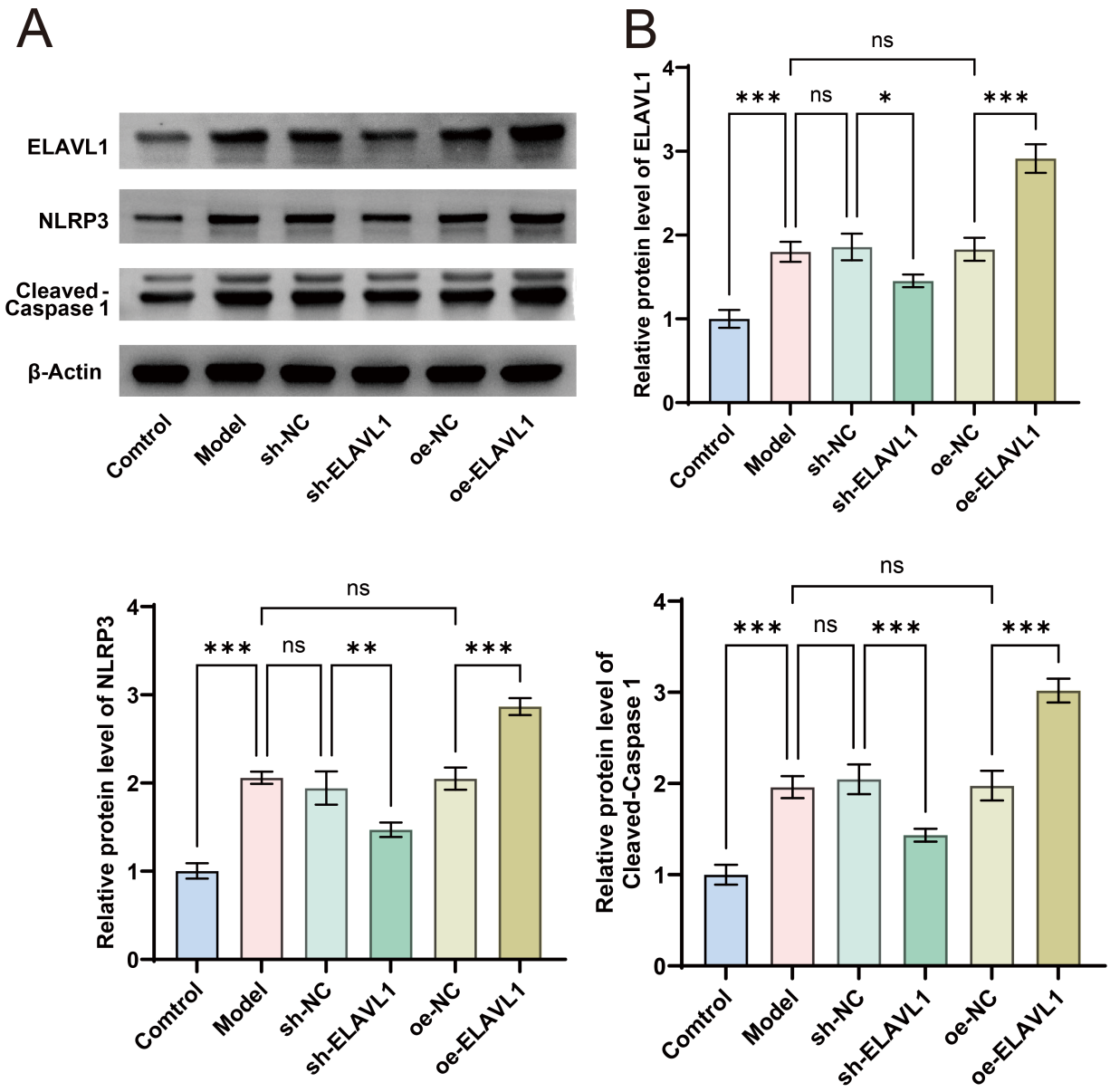

Fig. 4. ELAVL1 regulates NLRP3 inflammasome pathway protein expression under low-glucose stress. (A) Immunoblot profiles of ELAVL1, NLRP3, and active caspase-1 (p20) across experimental conditions, normalized to β-actin. (B) Relative abundance of the indicated proteins determined by band density measurements. Results are expressed as the mean ± SD from three independent assays (n = 3). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant.

port these clinical observations. Diabetic mice established through high-fat feeding and low-dose STZ administration exhibited further deterioration of cardiac systolic function when subjected to repeated hypoglycemic episodes, as evidenced by reduced LVEF and LVFS relative to animals with diabetes alone. Histological examination also revealed more severe myocardial fiber disruption and inflammatory infiltration. These results indicate that acute hypoglycemic episodes exacerbate myocardial injury in the setting of chronic hyperglycemia, potentially through mechanisms involving acute myocardial ischemia, excessive oxidative stress, and immune activation. Evidence from the ACCORD study further highlights the cardiovascular consequences of severe hypoglycemia, showing that individuals with type 2 diabetes who experienced such events had nearly a 49% higher likelihood of developing heart failure [25]. Together, these findings underscore the importance of balancing glycemic control against hypoglycemia risk in diabetes management, as treatment-induced recurrent hypoglycemia may impose additional cardiac injury beyond that caused by chronic hyperglycemia alone.

Chronic inflammatory activity plays an important role in cardiac remodeling during diabetes. In line with this concept, mice exposed to recurrent hypoglycemia exhib-

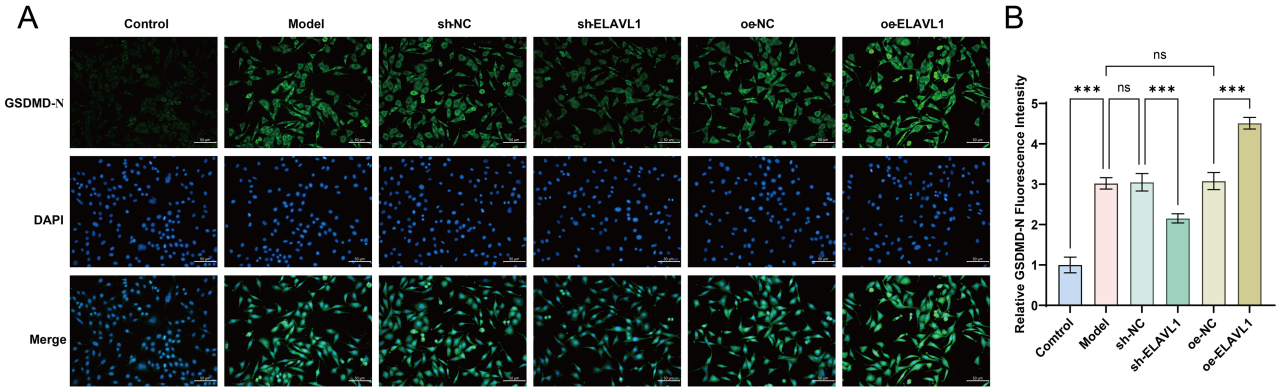

Fig. 5. ELAVL1 promotes GSDMD-N (p30)-mediated pore-forming activation in low-glucose-induced cardiomyocytes. (A) Cellular distribution of GSDMD-N (p30) detected by fluorescence microscopy. Green fluorescence indicates GSDMD-N immunoreactivity, whereas blue signals correspond to DAPI-labeled nuclei. Scale bar = 20 μm. (B) Relative intracellular GSDMD-N fluorescence levels among experimental groups. Values are shown as the mean ± SD from three independent assays (n = 3). ***$p < 0.001$; ns, not significant.

ited greater elevations of the myocardial damage markers cTnI and cTnT than diabetic animals alone, suggesting an aggravation of cardiac tissue injury. Concurrently, serum levels of IL-1β and IL-18, two canonical downstream products associated with NLRP3 inflammasome signaling, were markedly elevated in HDM mice. Increased production of these circulating cytokines is commonly regarded as indirect evidence of activation of the canonical GSDMD-dependent pyroptotic pathway [26,27]. Mechanistically, activation of the NLRP3 inflammasome triggers caspase-1 activation, leading to the release of mature IL-1β and IL-18 as well as GSDMD cleavage. The resulting membrane pores disrupt cellular integrity and ultimately induce pyroptotic cell death [27].

The present results further support a pathogenic role of NLRP3 signaling in diabetic cardiac injury. Sustained glucose overload has been reported to activate inflammasome-dependent inflammatory pathways in cardiomyocytes, promoting pyroptotic cell loss and deterioration of myocardial performance [12]. Similarly, Luo et al. [28] demonstrated that systemic NLRP3 gene silencing in a high-fat diet/STZ-induced type 2 diabetic rat model significantly attenuated myocardial pyroptosis, inflammation, and fibrosis, while improving cardiac function. Compared with diabetic mice without hypoglycemic exposure, the HDM group exhibited significantly higher circulating levels of IL-1β and IL-18. These findings indicate that recurrent hypoglycemia may intensify inflammasome-driven myocardial inflammation under diabetic conditions, which was paralleled by higher circulating cTn levels. HDM mice developed substantial hyperinsulinemia following repeated insulin exposure. In conjunction with recurrent hypoglycemia, this metabolic disturbance may intensify redox imbalance and neurohumoral responses, creating a pro-inflammatory environment that favors NLRP3 pathway-associated inflammatory responses. This observation is consistent with previous reports demonstrating excessive activation of the NLRP3–ASC–caspase-1 axis and increased myocardial IL-1β expression in diabetic animal models and human diabetic cardiac tissues [29]. Collectively, these findings suggest that recurrent hypoglycemia may aggravate myocardial injury by superimposing acute metabolic stress and amplifying NLRP3 inflammasome-associated inflammatory responses, ultimately contributing to deteriorated cardiac function and more severe histopathological damage.

The RNA-binding protein ELAVL1 (also known as HuR) regulates the stability and translation of multiple inflammatory transcripts through binding to AU-rich elements in target mRNAs [30]. Previous studies have shown that ELAVL1 expression is upregulated in hyperglycemia-induced myocardial injury and is accompanied by increased expression of caspase-1 and IL-1β. Moreover, ELAVL1 silencing suppresses NLRP3/caspase-1/IL-1β signaling and attenuates cardiomyocyte pyroptosis [19]. Myocardial ELAVL1 transcript levels increased under diabetic conditions and showed an additional rise following recurrent hypoglycemic exposure. In HL-1 atrial cardiomyocytes exposed to alternating high- and low-glucose stress, silencing ELAVL1 markedly blunted glucose fluctuation-induced upregulation of NLRP3 and caspase-1. This intervention also improved cellular survival and limited LDH leakage. Conversely, ELAVL1 overexpression enhanced NLRP3 pathway activation, promoted caspase-1 cleavage, and aggravated cardiomyocyte injury. These findings indicate that ELAVL1 positively regulates low-glucose-induced NLRP3 inflammasome activation and cardiomyocyte injury.

A previous review highlighted that ELAVL1 promotes inflammatory death of cardiomyocytes under hyperglycemic conditions [31], and our data provide new experimental evidence supporting this notion. One possible explanation is that ELAVL1 facilitates inflamma-

tory signaling through post-transcriptional regulation of inflammasome-associated transcripts, leading to sustained production of key mediators such as IL-1β and caspase-1; however, this mechanism requires direct experimental verification. This is consistent with a report that the miR-9/ELAVL1 axis regulates high-glucose-induced cardiomyocyte pyroptosis, where Jeyabal et al. [19] found that ELAVL1 expression was upregulated under high-glucose conditions and regulated Caspase-1/IL-1β. Consistent with these observations, our *in vitro* data suggest that ELAVL1 functions as a positive regulator of NLRP3 inflammasome-associated signaling and pyroptotic responses under low-glucose conditions. *In vivo*, myocardial ELAVL1 mRNA expression was positively associated with increased levels of NLRP3-related inflammatory cytokines. However, direct evidence demonstrating that ELAVL1 drives pyroptosis in the diabetic hypoglycemic myocardium remains insufficient. A limitation of this work is that the proposed association between ELAVL1 and inflammasome-related transcripts remains inferential rather than experimentally verified. Nevertheless, ELAVL1 is known to regulate the stability and translation of multiple inflammation-related mRNAs involved in pyroptotic signaling pathways. Future studies employing RNA immunoprecipitation (RIP) or crosslinking immunoprecipitation (CLIP) approaches are warranted to determine whether ELAVL1 directly interacts with NLRP3, caspase-1, or other relevant transcripts in cardiomyocytes.

Taken together, our findings suggest that recurrent hypoglycemic episodes in the diabetic setting may exacerbate myocardial injury through sympathetic activation and metabolic stress, accompanied by increased ELAVL1 expression and enhanced inflammatory responses. Combined with the *in vitro* functional data, these results raise the possibility that ELAVL1 contributes to low-glucose-induced cardiomyocyte injury by regulating NLRP3/caspase-1/GSDMD-associated pyroptotic signaling. This working model provides a potential mechanistic framework linking recurrent hypoglycemia to aggravated cardiac injury in diabetes.

Nevertheless, several limitations should be acknowledged. First, neither the mouse model nor HL-1 atrial cardiomyocytes fully recapitulate the complexity of the human myocardium or the dynamic nature of glycemic fluctuations in patients. In addition, our *in vitro* model employed recurrent low-glucose exposure on a hyperglycemic background (30 mM glucose) to mimic diabetic hypoglycemia but did not include a physiological glucose control group, which limits the ability to distinguish the independent effects of hypoglycemia. Future studies incorporating cardiomyocyte-specific ELAVL1 knockout models and physiological-glucose controls will be important for establishing causality. Second, the HDM model, established through fasting and high-dose insulin injection followed by glucose administration, introduces complex confounding factors. The resulting myocardial injury may stem not only from hypoglycemia itself, but also from fasting stress, direct insulin exposure, rapid glycemic fluctuations, or hyperglycemic rebound. Due to the lack of specialized control groups (such as diabetic mice treated with insulin without developing hypoglycemia, non-diabetic hypoglycemic mice, or fasting-only controls), as well as the absence of detailed blood glucose excursion curves and model success rates in the current study, these confounding variables cannot be fully excluded. This limitation warrants cautious interpretation of the *in vivo* findings. Third, myocardial expression of NLRP3, cleaved caspase-1, and GSDMD was not directly assessed *in vivo*; thus, activation of the NLRP3/caspase-1/GSDMD pathway in HDM hearts remains to be directly validated. Fourth, although we propose that the ELAVL1→NLRP3/caspase-1/GSDMD pathway mediates myocardial injury, our functional evidence relies primarily on ELAVL1-targeted intervention, leaving the proposed causal pathway incompletely validated. Future studies incorporating rescue experiments—such as overexpressing ELAVL1 in conjunction with NLRP3 silencing (siRNA), caspase-1 inhibition, or GSDMD knockdown—are highly warranted to verify whether the myocardial injury phenotype can be reversed. Fifth, we focused primarily on NLRP3 and did not exclude alternative pyroptotic pathways (e.g., non-canonical caspase-11) or other programmed cell death modalities. Integrating transcriptomic profiling and RIP-seq will help map the comprehensive regulatory network of ELAVL1 in cardiomyocytes. Finally, the therapeutic potential of pharmacological or biological interventions targeting ELAVL1-associated signaling pathways in preventing diabetic hypoglycemia-related myocardial injury warrants further investigation.

Conclusion

The present study suggests that the RNA-binding protein ELAVL1 may play an important regulatory role in myocardial injury associated with diabetic hypoglycemia. *In vivo*, recurrent hypoglycemic episodes aggravate myocardial structural damage and impair cardiac systolic function in diabetic mice, accompanied by increased inflammatory cytokine production and elevated myocardial ELAVL1 expression. *In vitro*, ELAVL1 knockdown attenuates intermittent low-glucose-induced injury in HL-1 atrial cardiomyocytes and reduces the expression of pyroptosis-related molecules, including NLRP3, cleaved caspase-1, and GSDMD-N, whereas ELAVL1 overexpression produces the opposite effects. These findings suggest that ELAVL1 may participate in low-glucose-induced cardiomyocyte injury through modulation of NLRP3/caspase-1/GSDMD-associated pyroptotic signaling. However, because direct evidence of pyroptotic pathway activation *in vivo* is currently lacking, the precise role of ELAVL and its mechanistic contribution to diabetic hypoglycemia-related

myocardial injury require further investigation. Collectively, this study provides new experimental evidence supporting a role for post-transcriptional regulation in diabetic hypoglycemia-associated myocardial injury and offers a rationale for future exploration of ELAVL1 as a potential therapeutic target.

Availability of Data and Materials

The datasets analyzed during current study are available from the corresponding author upon reasonable request.

Author Contributions

XW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. BZ: Investigation, Data curation, Formal analysis, Writing – review & editing. SWC: Conceptualization, Investigation, Data analysis and interpretation, Methodology, Resources, Supervision, Writing – review & editing. FX: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to take public responsibility for the integrity and accuracy of all aspects of the study.

Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the Animal Experimental Ethics Committee of Chongqing Medical University (IACUC-CQMU-2023-0065). All procedures complied with the ARRIVE Guidelines 2.0.

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Conflict of Interest

The authors declare no conflict of interest.

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