Article

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LncRNA SENCR Ameliorates Hypoxia-Induced Myocardial Infarction by Targeting the miR-206/SMAD4 Axis

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Background: The long non-coding RNAs (lncRNAs) have been found crucial in the pathogenesis of cardiovascular diseases, including myocardial infarction (MI). Therefore, we aimed to investigate the role of lncRNA smooth muscle and endothelial cell-enriched migration/differentiation-associated lncRNA (SENCR) in MI.

Methods: An *in vitro* model of MI was established by treating human cardiomyocytes (HCM) with hypoxia conditions. The quantitative real-time polymerase chain reaction (RT-qPCR) and western blot analysis were used to assess the expression of genes both at RNA and protein levels. However, Cell Counting Kit-8 (CCK-8) and flow cytometry analysis were employed for the assessment of HCM viability and apoptosis. Furthermore, RNA-binding protein immunoprecipitation (RIP), RNA pull-down, and luciferase reporter assays were conducted to explore the regulatory relationship between SENCR and related molecules.

Results: The expression levels of SENCR were found to be progressively reduced in HCM under hypoxia treatment ($p < 0.01$). Furthermore, overexpression of SENCR stimulated HCM viability and reduced apoptosis rate during hypoxia conditions ($p < 0.01$). Moreover, SENCR was found to function as a competing endogenous RNA (ceRNA) to sequester miR-206, consequently modulating SMAD family member 4 (SMAD4) expression ($p < 0.01$).

Conclusions: LncRNA SENCR ameliorated hypoxia-induced MI by targeting the miR-206/SMAD4 axis. The findings of this research might offer novel insight for improving the treatment of MI.

Keywords: myocardial infarction; SENCR; miR-206; SMAD4

Introduction

As a serious cardiovascular disease (CVD), myocardial infarction (MI) is associated with a high global incidence and mortality. MI is commonly characterized by myocardial necrosis, primarily attributed to acute or continuous myocardial ischemia or hypoxia [1,2]. Despite considerable advancements in the treatment of MI, the prognosis for patients remains unfavorable [3].

Growing evidence suggests that long non-coding RNAs (lncRNAs) play a crucial role in the development of various diseases, like MI [4]. For example, the lncRNA cardiac autophagy inhibitory factor (CAIF) attenuates MI by hindering p53-mediated myocardin transcription [5]. Similarly, the lncRNA H19 improves MI-induced myocardial injury by regulating KDM3A [6]. Furthermore, lncRNA growth arrest-specific 5 (GAS5) promotes MI by modulating the miR-525-5p/CALM2 axis [7]. Lnc-PXMP4-2-4 alleviates myocardial cell damage induced by acute myocardial infarction (AMI) through activation of the JAK2/STAT3 signaling pathway [8]. Currently, lncRNA smooth muscle and endothelial cell-enriched migration/differentiation-associated lncRNA (SENCR) has been revealed to suppress abdominal aortic aneurysm formation [9]. Moreover, Zhi-Qing Zou *et al.* [10] have reported that the depletion of SENCR enhances the proliferative and migratory capabilities of smooth muscle cells in atherosclerosis. Additionally, the role of SENCR in other diseases, such as acute myeloid leukemia (AML), has also been elucidated [11]. However, whether SENCR is involved in the regulation of MI remains unclear.

Recently, microRNAs (miRNAs), a class of small non-coding RNAs approximately 22 nucleotides in length, have been uncovered to regulate the expression of downstream genes through interaction with the 3′-untranslated region (3′-UTR) of messenger RNAs (mRNAs) [12]. Increasing evidence demonstrates that miRNAs act as pivotal regulators in the development of MI. For instance, miR-124 has been shown to promote MI by targeting Dhcr24 [13]. Additionally, miR-130 exasperates myocardial injury induced by acute MI via targeting PPAR-γ [14]. miR-132 has been shown to ameliorate myocardial remodeling after MI [15]. Furthermore, miR-133a and miR-155-5p have also been associated with MI [16,17].

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In our study, we aimed to explore the expression pattern and biological function of SENCR in MI. Additionally, we investigated the potential interaction between SENCR and miRNA in MI. Our findings might offer novel insights into the molecular mechanism underlying the association between lncRNAs and miRNAs in the development of MI.

Material and Methods

Cell Culture and Treatment

Human cardiomyocytes (HCM) were isolated from the ventricles of the healthy adult human heart (A25973) supplied by Shanghai Huzhen Biotechnology Co., Ltd. (Shanghai, China). The human embryonic kidney cell line (HEK293T) was obtained from ATCC (CRL-3519, Manassas, VA, USA). These cells were cultured in DMEM containing 10% fetal bovine serum (FBS) followed by incubation at 37 °C in the presence of 5% CO_2. Moreover, to mimic the *in vitro* model of MI, HCM was incubated under hypoxia conditions for 0, 3, 6, 9, and 12 hours, respectively. The HEK293T cell underwent Mycoplasma testing through PCR and was authenticated using the STR method. Moreover, HEK293T cells at passage 3 were used for subsequent experimentations.

Cell Counting Kit-8 (CCK-8)

Transfected cells were seeded into 96-well plates followed by the addition of 10 µL of CCK-8 solution to each well. After incubation, their absorbance at 450 nm was monitored utilizing a microplate reader (HBS-1101, Nanjing Detie Experimental Equipment Co., Ltd. Nanjing, China). Finally, the survival rate of the cells was determined as follows: Cell survival rate = [(experimental wells – blank wells) / (control wells – blank wells)] × 100%.

Flow Cytometry Analysis

The apoptosis rate of cardiomyocytes was evaluated using the eBioscience Annexin V-FITC Apoptosis Detection Kit (BMS500FI-300, Thermo Fisher Scientific, Waltham, MA, USA). For this purpose, cells were washed with cold phosphate-buffered saline followed by resuspension in binding buffer. After this, 100 µL of FITC Annexin V and 15 µL of PI were added to the cell suspension and incubated for 30 minutes in the dark. Finally, flow cytometry was conducted using a flow cytometer (Beckman Coulter; Kraemer Boulevard Brea, CA, USA) and analyzed through FlowJo software (Version 10.5.3, Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Western Blot

After extraction, proteins were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were subsequently transferred onto polyvinylidene fluoride (PVDF) membranes. After blocking, the membranes underwent overnight incubation with the specific primary antibodies at 4 °C. The next day, the membranes were washed and incubated with secondary antibodies for one hour. In the next step, the protein bands were developed using an enhanced chemiluminescence (ECL) reagent, and the bands were observed employing a gel imaging instrument (Amersham ImageQuant 500, Cytiva, Marlborough, MA, USA) to assess protein levels. The gray value of each protein band was determined using Image J software (version 2, National Institutes of Health, Bethesda, MD, USA). The primary antibodies used in this assay were as follows: anti-Bcl-2 (1/1,000, ab182858), anti-Bax (1/1000, ab32503), anti-Cleaved caspase 3 (1/10000, ab32042), anti-Caspase 3 (1/5,000, ab32351), and anti-GAPDH (1/10000, ab181602). The Cleaved caspase 9 (1/1000, #9505) and Caspase 9 (1/1000, #9502) were provided by Cell Signaling Technology (Danvers, MA, USA), whereas other primary antibodies were obtained from Abcam, Cambridge, MA, USA. GAPDH served as internal control. Furthermore, the secondary antibodies (Cell Signaling Technology, Danvers, MA, USA) used in this assay included Anti-rabbit IgG, HRP-linked Antibody (#7074) or Anti-mouse IgG, HRP-linked Antibody (#7076).

Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

After the isolation of total RNA, the complementary DNA (cDNA) was synthesized using a commercially available reverse transcription kit (Applied Biosystems; Foster City, CA, USA). Using cDNA as a template, qPCR was performed utilizing an SYBR® Green Premix Pro Taq HS qPCR Kit (Accurate, Changsha, China) in the Roche LightCycler® 480 System (Roche, Indianapolis, IN, USA). *U6* and *GAPDH* were used as internal references for miRNAs and SENCR/mRNAs, individually. The primers used in RT-qPCR were as follows: *SENCR* (forward: CGCTTTGGACTTGCTCACTT; reverse: AGTTGGAGTCCTTTCTGGCTG), *miR-206* (forward: CCGAGTGGAATGTAAGGAAGT; reverse: CTCAACTGGTGTCGTGGA), *SMAD4* (forward: ATGCTCAGTGGCTTCTCGAC; reverse: CCTAGGGGAGAGCAGGAAGG). *U6* (forward: CTCGCTTCGGCAGCACA; reverse: ACGCTTCACGAATTTGCGT), *GAPDH* (forward: GGTGAAGGTCGGAGTCAACG; reverse: CAAAGTTGTCATGGATGHACC). The relative gene expression was calculated based on $2^{-\Delta\Delta Ct}$ method.

Cell Transfection

To overexpress SENCR and SMAD family member 4 (SMAD4), pcDNA3.1/SENCR and pcDNA3.1/SMAD4 procured from GenePharma (Shanghai, China) were severally transfected into cells. The empty pcDNA3.1 vector was used as a control. Additionally, miR-206 mimic sequence (UGGAAUGUAAGGAAGUGUGUGG) and control NC mimic sequence (UCACCGGGUGUAAAUCAGCUUG)

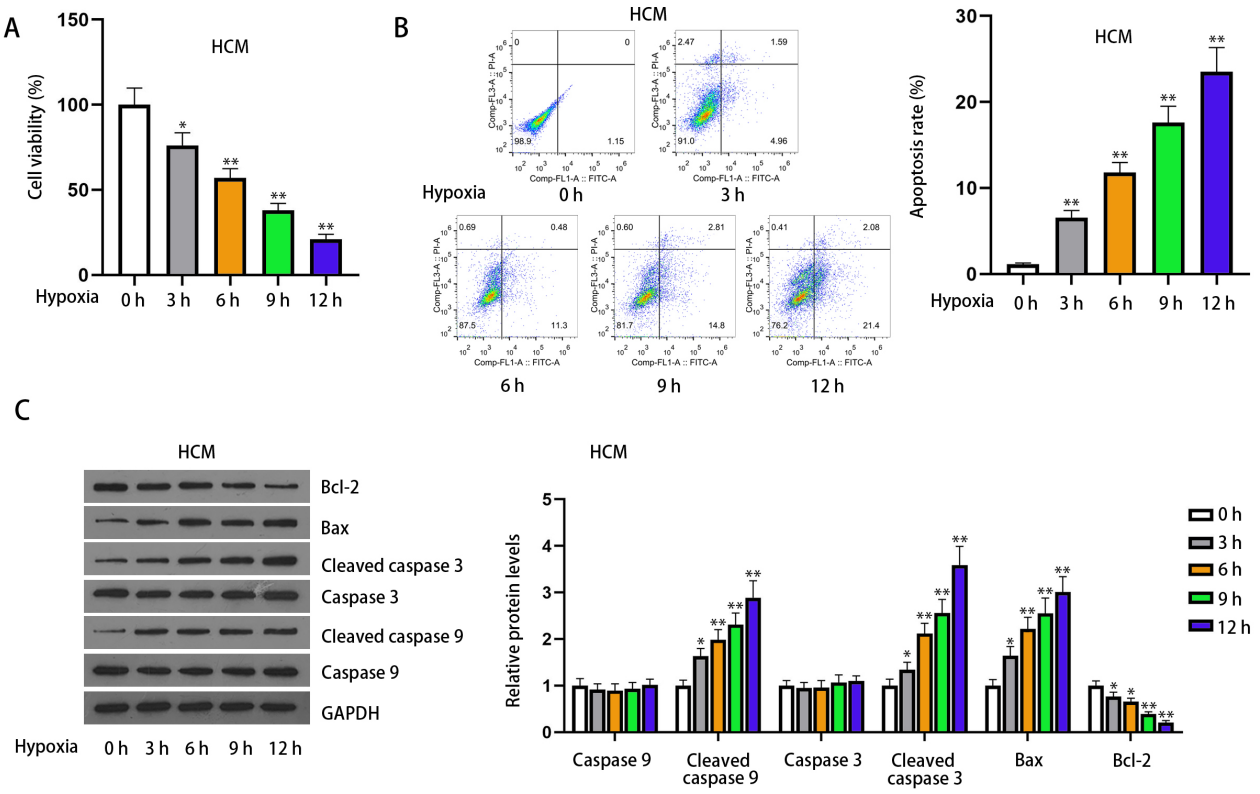

Fig. 1. Hypoxia inhibits cardiomyocyte viability and induces apoptosis. (A) Cell Counting Kit-8 (CCK-8) assay was used to determine HCM viability under hypoxia treatment for 0, 3, 6, 9, and 12 hours. (B) Flow cytometry analysis was used to assess human cardiomyocytes (HCM) apoptosis under hypoxia treatment for 0, 3, 6, 9, and 12 hours. (C) Western blot was used to examine apoptosis-related protein levels in HCM exposed to hypoxia treatment for 0, 3, 6, 9, and 12 hours. All other groups were compared with the 0-hour group. Each experiment was conducted with three biological replicates. $*p < 0.05$, $**p < 0.01$.

as well as short hairpin RNA (shRNA) targeting SMAD4 (sh/SMAD4#1 sequence: GCCATAGTGAAGGACTGTTGC; sh/SMAD4#2: GCCTCCCATTTCCAATCATCC) and negative control (sh-NC) plasmids were all synthesized and supplied by GenePharma, Shanghai, China. The cells were transfected with the corresponding plasmid using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA).

Subcellular Fractionation

The nuclear and cytoplasmic RNA fractions were isolated utilizing the PARIS Kit (AM1921, Thermo Fisher, Waltham, MA, USA) following the manufacturer's instructions. The expression levels of SENCR in nuclear or cytoplasmic RNA fraction were assessed using RT-qPCR. The U6 and GAPDH were used as nuclear and cytoplasmic references, respectively.

Fluorescent in Situ Hybridization (FISH)

This assay was conducted utilizing a FISH kit (C10910, RiboBio, Guangzhou, China), following the manufacturer's recommendations. The FISH probe for SENCR was designed and synthesized by RiboBio (Guangzhou, China). The cells were seeded onto sterile coverslips and incubated until reaching 60% confluence. Subsequently, the cells underwent washing, fixation, and permeabilization followed by incubation with the SENCR probe in a hybridization buffer. Finally, DAPI was added for nuclei staining and the fluorescence signals were captured employing a fluorescence microscope (DM2500, Leica, Wetzlar, Germany).

RNA-Binding Protein Immunoprecipitation (RIP)

This assay was performed utilizing a Magna RIP RNA-Binding Protein Immunoprecipitation Kit (17-701, Millipore, Boston, MA, USA) according to the manufacturer's protocol. The cell lysate was incubated with magnetic beads conjugated with control anti-immunoglobulin G (anti-IgG) or human anti-Ago2 antibody (abs134409; abs20032, Absin, Shanghai, China). After that, the immunoprecipitated RNAs were isolated from protein-RNA complexes and subsequently analyzed using RT-qPCR.

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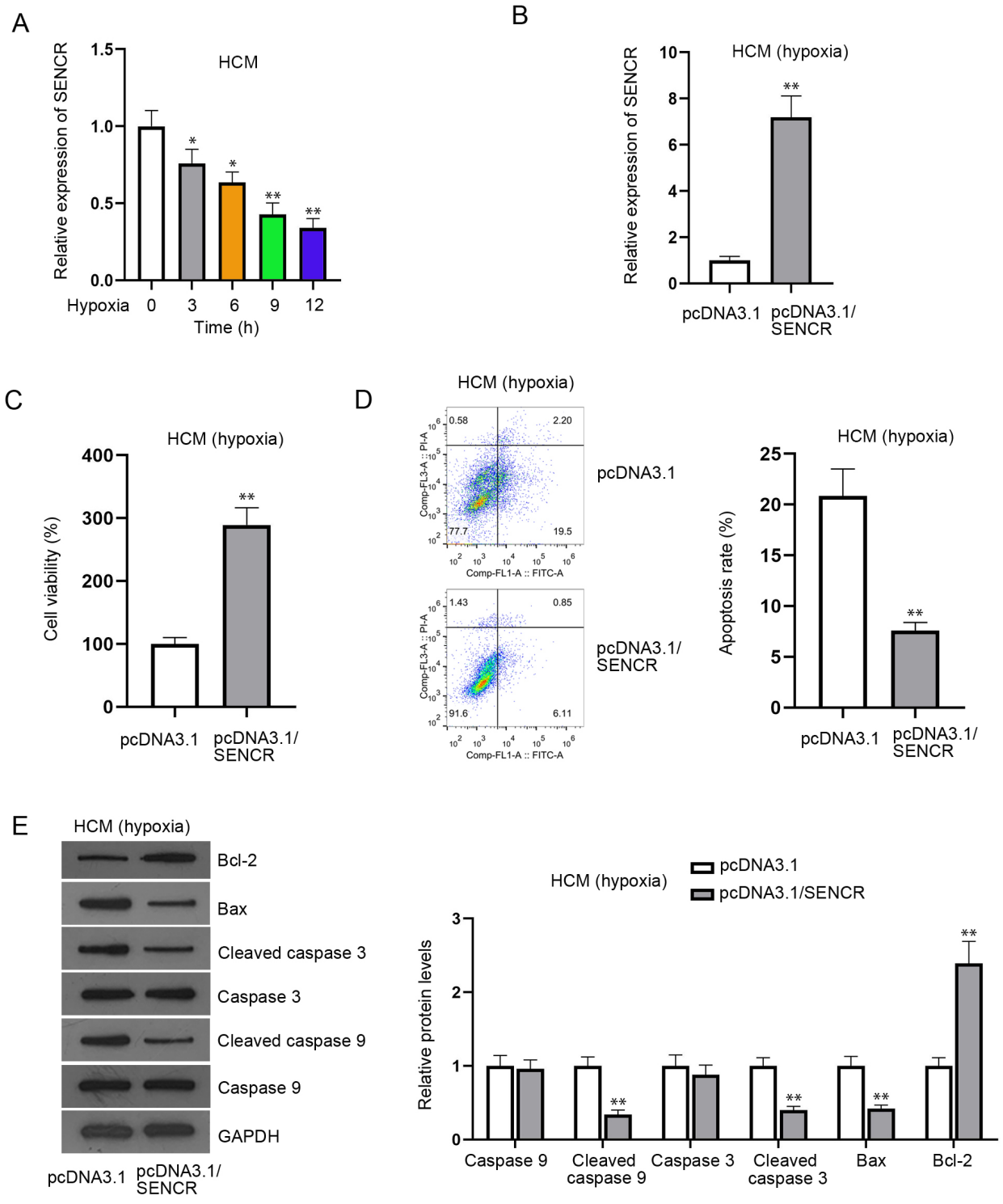

Fig. 2. Overexpressed SENCR reduces hypoxia-induced MI. (A) RT-qPCR measured SENCR expression levels in HCM treated with hypoxia for 0, 3, 6, 9, and 12 hours. The other groups were compared with the 0-hour group. (B) Overexpression efficiency of SENCR in hypoxia-induced HCM using RT-qPCR. (C) CCK-8 assay evaluated HCM viability after SENCR elevation under hypoxia treatment. (D) Flow cytometry analysis detected HCM apoptosis after SENCR elevation under hypoxia treatment. (E) Western blot analysis revealed the apoptosis-related protein levels following SENCR overexpression in HCM treated with hypoxia. Each experiment was conducted with three biological replicates. *$p < 0.05$, **$p < 0.01$.

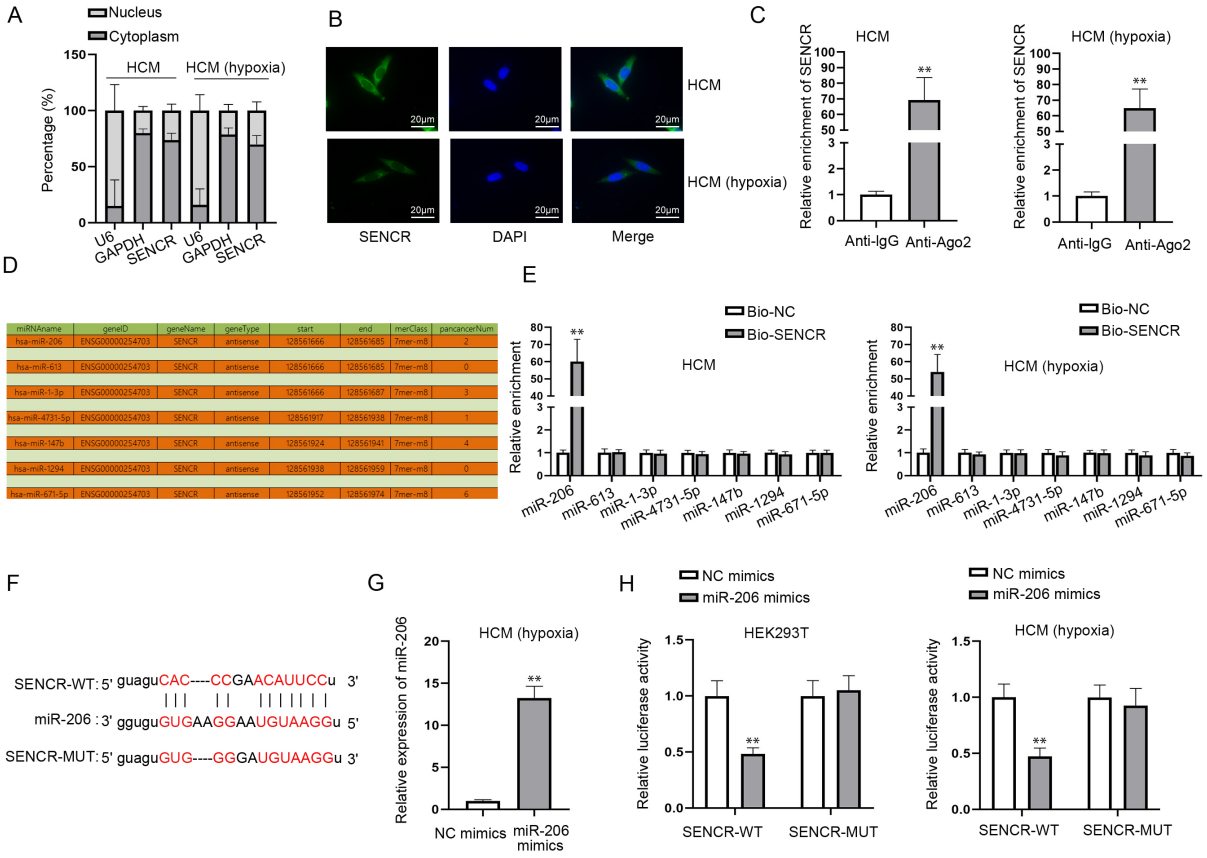

Fig. 3. SENCR binds to miR-206. (A,B) Distribution of SENCR in HCM and hypoxia-induced HCM. Scale bar = 20 μm. (C) Interaction between SENCR and Ago2. (D) Seven miRNAs combined with SENCR from starBase. (E) Correlation between seven miRNAs and SENCR. (F) Binding sites between SENCR and miR-206. (G) RT-qPCR evaluated the augmentation efficacy of miR-206 mimics. (H) Luciferase reporter assays detected the binding between SENCR and miR-206. Each experiment was conducted with three biological replicates. $^{**}p < 0.01$.

RNA Pull-Down Assay

The biotinylated SENCR and its negative control (Bio-NC) used in the RNA pull-down assay were purchased from RiboBio (Guangzhou, China). The cell lysate was incubated overnight with Dynabeads M-280 Streptavidin (11205D, Invitrogen, Carlsbad, CA, USA) conjugated with a probe at 4 °C. The RNAs present in the pull-down complex were isolated using TRIzol reagent and subsequently underwent RT-qPCR analysis.

Luciferase Reporter Assay

The wild-type or mutant sequences of SENCR (SENCR-WT/MUT) or SMAD4 3′UTR (SMAD4 3′UTR-WT/MUT), which contain the miR-206 responsive element, were subcloned into the psiCHECK-2 vector to generate luciferase reporter vectors. Subsequently, the reporter construct was co-transfected with miR-206 mimics or NC mimics for 48 hours and luciferase activity was measured using Dual-Luciferase Reporter Assay System (PR-E1910, Promega, Madison, WI, USA).

Statistical Analysis

Statistical analyses were performed utilizing GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA) and expressed in the form of mean ± standard deviation (SD). To ensure statistical significance, each experiment was conducted in triplicate. Furthermore, statistical differences between the two groups were determined using the student's *t*-test. However, for multiple group comparisons, one-way or two-way analysis of variance (ANOVA) was employed. The p-value < 0.05 was considered statistically significant.

Results

Overexpressed SENCR Reduces Hypoxia-Induced MI

Published research suggested that SENCR is a potential biomarker and possible treatment for reducing atherosclerosis [18]. However, the biological role of SENCR in MI remains unexplored. Therefore, using an *in vitro* model of MI, we assessed the viability and apoptosis

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rate in HCM following hypoxia treatment for 0, 3, 6, 9, and 12 hours. The CCK-8 assay revealed a time-dependent reduction in cell viability after hypoxia treatment ($p < 0.01$, Fig. 1A). On the contrary, the cell apoptosis rate was significantly increased with the increase in hypoxia treatment time ($p < 0.01$, Fig. 1B). Furthermore, apoptosis-related proteins were examined under hypoxia treatment using western blot analysis. There was a substantial decrease in the expression levels of Bcl-2 and an increase in the expression levels of Bax, Cleaved caspase 3, and Cleaved caspase 9 proteins with increasing duration of hypoxia treatment. These findings further support the enhancement of cell apoptosis by hypoxia treatment ($p < 0.01$, Fig. 1C). Thus, due to commendable outcomes, the hypoxia treatment duration of 12 hours was adopted in the follow-up analysis. Moreover, RT-qPCR analysis showed a progressive alleviation in the expression levels of SENCR in HCM over time under hypoxia treatment ($p < 0.01$, Fig. 2A). To assess the role of SENCR in hypoxia-induced MI, specific pcDNA3.1/SENCR plasmid was used to promote the expression of SENCR in HCM under hypoxia conditions ($p < 0.01$, Fig. 2B). It was found that overexpression of SENCR elevated cell viability and reduced apoptosis rate in HCM exposed to hypoxia conditions ($p < 0.01$, Fig. 2C,D). Furthermore, western blot analysis revealed that overexpression of SENCR led to an increase in Bcl-2 protein levels and a significant decrease in Bax, Cleaved caspase 3, and Cleaved caspase 9 protein levels, indicating a substantial reduction in apoptosis rate (Fig. 2E).

SENCR Binds to miR-206

Evaluating the distribution of SENCR in both normal HCM and hypoxia-induced HCM revealed that it was primarily localized in the cytoplasm (Fig. 3A,B). The cytoplasmic lncRNAs can work as competing endogenous RNAs (ceRNAs) to sponge miRNAs [19]. Consistent with these findings, we corroborated a significant immunoprecipitation of endogenous SENCR in Ago2 pallets ($p < 0.01$, Fig. 3C). Using the starBase (http://starbase.sysu.edu.cn) website, we predicted that SENCR could bind to 7 miRNAs (Fig. 3D). Subsequent RNA pull-down assays indicated that only miR-206 was significantly bound to SENCR ($p < 0.01$, Fig. 3E). The wild-type and mutated sequences of SENCR containing miR-206 binding sites are presented in Fig. 3F. Furthermore, to increase miR-206 expression, we transfected miR-206 mimics into HCM under hypoxia treatment ($p < 0.01$, Fig. 3G). The luciferase reporter assay indicated that miR-206 overexpression led to reduced activity of reporter vectors containing the wild-type sequence of SENCR but showed minimal impact on those containing mutant-type SENCR ($p < 0.01$, Fig. 3H).

SMAD4 is Targeted by miR-206

To explore the upstream mechanism of miR-206, we employed the starBase tool to predict targets of miR-206 across three databases, including RNA22, miRmap, and TargetScan. Consequently, seven mRNAs were predicted as candidate targets of miR-206 (Fig. 4A). Subsequently, we evaluated the expression levels of these seven candidate mRNAs in HCM after miR-206 overexpression utilizing RT-qPCR to identify significant targets. Furthermore, we observed that miR-206 overexpression specifically alleviated the expression levels of SMAD4 ($p < 0.01$, Fig. 4B). Additionally, we confirmed a gradual downregulation in the expression levels of SMAD4 within HCM exposed to hypoxia treatment ($p < 0.01$, Fig. 4C). Moreover, we predicted two binding sites between SMAD4 3′UTR and miR-206 (Site 1-WT and Site 2-WT) as well as the corresponding mutated sequences following the base pairing principle (Site 1-MUT and Site 2-MUT) (Fig. 4D). As illustrated in luciferase reporter assays, miR-206 up-regulation led to a significant decrease in luciferase activity associated with SMAD4 3′UTR containing Site 1-WT, Site 2-WT, Site 1+Site 2-WT, Site 1-MUT, and Site 2-MUT, but showed minimal impact on the activity of SMAD4 3′UTR with mutation at both Site 1 and Site 2 ($p < 0.01$, Fig. 4E). This observation suggests that the binding between miR-206 and SMAD4 3′UTR occurs at both Site 1 and Site 2. In addition, we found that SENCR negatively regulates the expression of SMAD4, which was diminished by the overexpression of miR-206 ($p < 0.01$, Fig. 4F). Furthermore, we elevated SMAD4 expression in hypoxia-induced HCM for the subsequent analysis to determine the role of SMAD4 in HCM ($p < 0.01$, Fig. 5A). As presented in Fig. 5B,C, the elevated SMAD4 expression was linked to increased cell viability and decreased cell apoptosis under hypoxic conditions ($p < 0.01$). Additionally, western blot analysis revealed that SMAD4 overexpression resulted in decreased Caspase 3, Cleaved caspase 3, and Bax protein levels and increased Caspase 9 protein level, indicating a significant reduction in the apoptosis rate ($p < 0.01$, Fig. 5D).

SENCR/miR-206/SMAD4 Axis Modulates Hypoxia-Induced MI

We further assessed the impacts of the SENCR/miR-206/SMAD4 axis on hypoxia-induced MI. We first silenced SMAD4 expression in hypoxia-induced HCM ($p < 0.01$, Fig. 6A), and then transfected the indicated plasmids into hypoxia-induced HCM for functional rescue experiments. It was found that the enhanced cell viability resulting from SENCR overexpression was significantly abrogated when miR-206 was up-regulated or SMAD4 was silenced simultaneously ($p < 0.01$, Fig. 6B). Furthermore, the inhibited cell apoptosis observed in SENCR-up-regulated HCM was restored after co-transfection with miR-206 mimics or sh/SMAD4#1 under hypoxia condi-

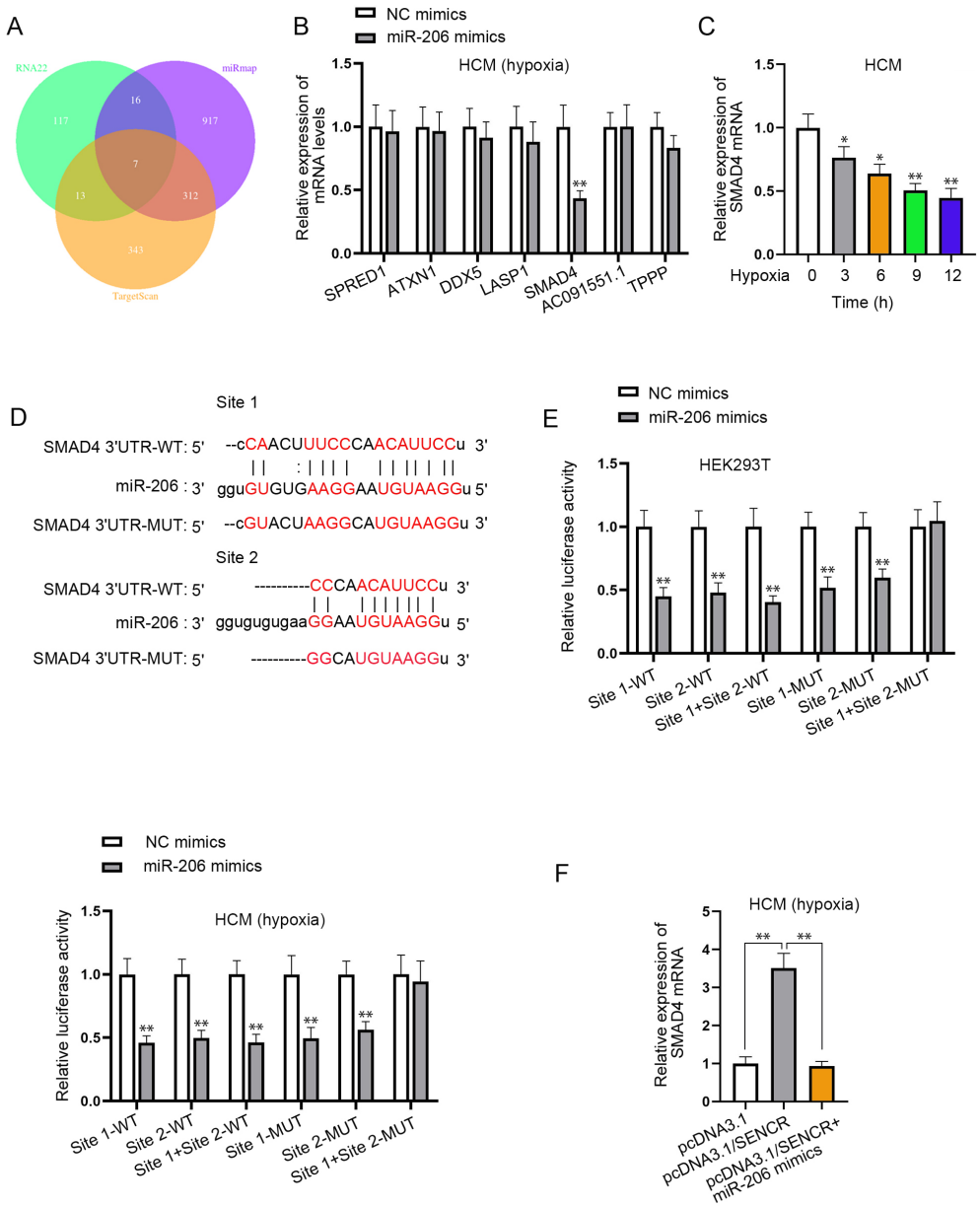

Fig. 4. SMAD4 is targeted by miR-206. (A) The overlap of the Venn diagram displays seven potential mRNAs targeted by miR-206 based on the analysis of three prediction databases (RNA22, miRmap, and TargetScan). (B) Levels of seven mRNAs in hypoxia-induced HCM after miR-206 overexpression. (C) RT-qPCR assessed SMAD4 expression in HCM treated with hypoxia for 0, 3, 6, 9, and 12 hours. The other groups were compared with the 0-hour group. (D) Binding sites between SMAD4 3′UTR and miR-206 and the sequences of SMAD4 3′UTR-MUT. (E) Luciferase reporter assays showed the interactions between SMAD4 3′UTR and miR-206. (F) RT-qPCR analysis revealed SMAD4 expression in hypoxia-induced HCM transfected with pcDNA3.1, pcDNA3.1/SENCR, or pcDNA3.1/SENCR+miR-206 mimics, respectively. Each experiment was conducted with three biological replicates. *$p < 0.05$, **$p < 0.01$.

tions ($p < 0.01$, Fig. 6C). Additionally, western blot analysis revealed that elevated Bcl-2 protein levels and reduced Bax, Cleaved caspase 3, and Cleaved caspase 9 levels caused by SENCR up-regulation were counteracted by miR-206 mimics or sh/SMAD4#1 during hypoxia treatment ($p < 0.01$, Fig. 6D).

Discussion

Growing evidence indicates that lncRNAs play a pivotal role in cardiovascular diseases, including MI. For instance, TUG1 contributes to cardiomyocyte apoptosis and is linked to a poor prognosis in MI [20]. XIST affects MI through its interaction with miR-130a-3p [21]. Addition-

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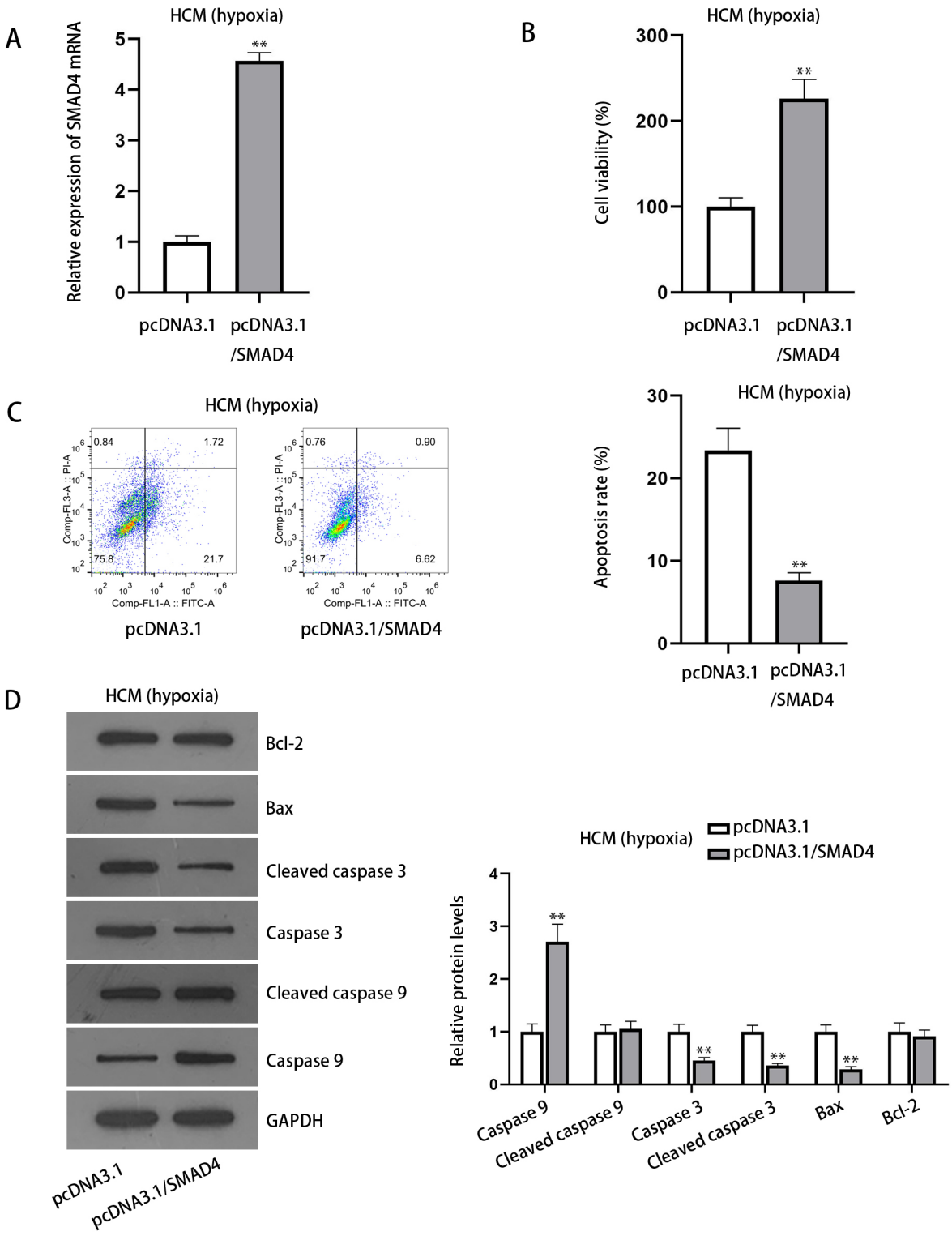

Fig. 5. Overexpressed SMAD4 protects cardiomyocyte viability and inhibits apoptosis. (A) Overexpression efficiency of SMAD4 in hypoxia-induced HCM using RT-qPCR. (B) CCK-8 assay detected HCM viability after SMAD4 elevation under hypoxia treatment. (C) Flow cytometry analysis detected HCM apoptosis following SMAD4 overexpression under hypoxia treatment. (D) Western blot analysis assessed apoptosis-related protein levels in SMAD4-overexpressed HCM exposed to hypoxia conditions. Each experiment was conducted with three biological replicates. $^{**}p < 0.01$.

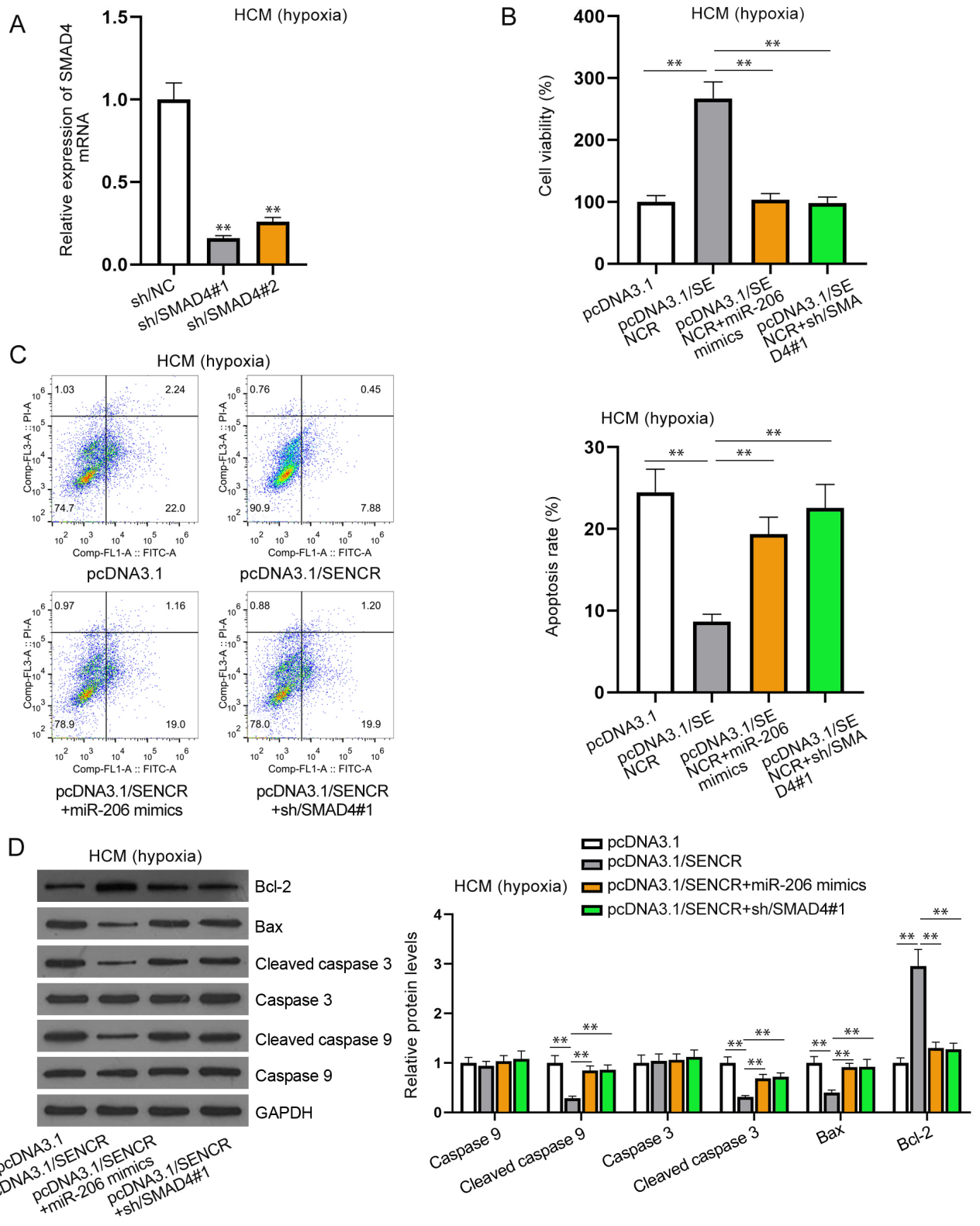

Fig. 6. Impacts of SENCR/miR-206/SMAD4 axis on hypoxia-induced MI. (A) Silencing efficiency of SMAD4 in hypoxia-induced HCM was examined using RT-qPCR. Rescue assays were performed in hypoxia-induced HCM transfected with pcDNA3.1, pcDNA3.1/SENCR, pcDNA3.1/SENCR+miR-206 mimics and pcDNA3.1/SENCR+sh/SMAD4#1, respectively. (B) Cell viability was examined. (C) Cell apoptosis was assessed. (D) Western blot analysis assessed the apoptosis-related protein levels. Each experiment was conducted with three biological replicates. $^{**}p < 0.01$.

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ally, LINC00528 promotes the progression of MI by targeting miR-143-3p/COX-2 axis [22]. MORT also contributes to the promotion of cardiomyocyte apoptosis through interaction with miR-93 in MI [23]. In this study, we observed a novel functional lncRNA in MI development. SENCR, a lncRNA primarily expressed in tissues, acts as a regulator of endothelial development [24]. Furthermore, SENCR has been proven to be linked to atherosclerosis development [25]. Herein, we uncovered the downregulation of SENCR in hypoxic HCM. Importantly, our study demonstrated that SENCR promotes cell viability and hinders cell apoptosis in HCM under hypoxia conditions.

Recently, miRNA-lncRNA interaction in MI has gained significant attention [26]. Several miRNAs have been unveiled to be implicated in MI development, such as miR-145 [27], miR-488-3p [28], and miR-29b [29]. For instance, miR-206 has been reported to induce cardiac hypertrophy [30]. Additionally, Zhi-Xin Shan *et al*. [31] proposed that miR-206 is responsible for glucose-induced cardiomyocyte apoptosis by modulating Hsp60 expression. Importantly, miR-206 expression has been documented to increase in a rat model of MI [32]. In our study, we observed an interaction between SENCR and miR-206 in hypoxia-induced HCM.

Moreover, existing evidence indicates that miRNAs can bind to the 3′-UTR region of target mRNA, thereby hampering their translation [33]. Herein, we found that miR-206 directly targets SMAD4 in hypoxia-induced HCM. SMAD4 has been identified as a genetic biomarker for the diagnosis of MI [34]. Additionally, Ying Huang *et al*. [35] have shown that miR-34a promotes fibrosis in cardiac tissue by targeting Smad4. Similarly, Yuanshi Li and colleagues [36] have elucidated that miR-130a targets Smad4, accelerating apoptosis in cardiac myocytes induced by hypoxia. The collective evidence suggests that SMAD4 might play a protective role in cardiomyocyte apoptosis. This study also found reduced expression of SMAD4 in hypoxic HCM. Moreover, overexpression of SMAD4 promotes cell viability and inhibits cell apoptosis in HCM under hypoxia treatment. Furthermore, we further observed that SENCR ameliorated hypoxia-induced MI by targeting the miR-206/SMAD4 axis. Regarding the downstream mechanism through which SMAD4 plays a role in the development of MI, further explorations are needed. Wang Jian *et al*. [37] have demonstrated that deletion of Smad4 in cardiomyocytes results in heart failure, a frequent complication of MI, and highlighted the role of intracellular signaling pathway in the pathogenesis of heart failure. Considering this, we aimed to elucidate the molecular mechanism through which SMAD4 interacts with intracellular signaling pathway to affect MI development.

Furthermore, some limitations in this research need to be addressed in future investigations. The current research predominantly focuses on *in vitro* studies, demonstrating the role and mechanism of SENCR in HCM. *In vivo* studies using animal models for MI will be performed in future research to address the major limitation of *in vitro* studies, mimicking human MI.

Conclusions

In conclusion, this study revealed that SENCR displays a reduced expression in MI. Mechanistically, SENCR up-regulates SMAD4 to regulate MI by targeting miR-206, suggesting that SENCR could be a promising biomarker for MI therapy.

Availability of Data and Materials

Research data can be obtained from the corresponding author if necessary.

Author Contributions

YW designed the research study and performed the research. YW, JYL and HH provided help and advice on the experiment. YZ and DLY analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

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This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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