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PCSK9 Knockdown Can Improve Myocardial Ischemia/Reperfusion Injury by Inhibiting Autophagy

³ Guangwei Huang^{1,2,3} · Xiyang Lu¹ · Zonggang Duan^{1,3} · Kai Zhang^{1,3} · Lei Xu² · Hailong Bao^{1,3} · Xinlin Xiong^{1,3} ·
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⁷ Abstract

8 This study investigates the effect and mechanism of proprotein convertase subtilisin/Kev ty, 9 (ICSK9) on myocardial g ischemia-reperfusion injury (MIRI) and provides a reference for clinical preventic. In treatment of acute myocardial 10 infarction (AMI). We established a rat model of myocardial ischemia/reperfusion, \mathcal{R}). \mathcal{A} C16 hypoxia/reoxygenation 11 (H/R) model. A total of 48 adult 7-week-old male Sprague–Dawley rats were ran $\frac{1}{2}$ only assigned to three groups (n = 16): 12 control, I/R, and I/R + SiRNA. In I/R and I/R + siRNA groups, myocardial ischemi. vas induced via occlusion of the left 13 anterior descending branch (LAD) of the coronary artery in rats in I/R group to 30 min and reperfused for 3 days. To assess 14 the myocardial injury, the rats were subjected to an electrocardiogram CG, cardiac function tests, cardiac enzymes 15 analysis, and 2,3,5-triphenyl tetrazolium chloride (TTC)/Evan Bly e (EB) st. ning. Meanwhile, differences in the expres-16 sion of autophagy-level proteins and Bcl-2/adenovirus E1B 19 Da steracting protein (Bnip3) signaling-related proteins 17 were determined by protein blotting. In vitro and in vivo expe. nental studies revealed that siRNA knockdown of PCSK9 18 reduced the expression of autophagic protein Beclin-1, light cha 3 (LC3) compared to normal control-treated cells and 19 control-operated groups. Simultaneously, the expressi n of Bnip3 pathway protein was downregulated. Furthermore, the 20 PCSK9-mediated small interfering RNA (siRNA) gout viected into the left ventricular wall significantly improved cardiac 21 function and myocardial infarct size. In ischem 'h poxic circumstances, PCSK9 expression was dramatically increased. 22 PCSK9 knockdown alleviated MIRI via Bnin3-m. Viated autophagic pathway, inhibited inflammatory response, reduced 23 myocardial infarct size, and protected car' nc) inctica.

²⁴ Keywords Autophagy · Bnip3 · Knr kdc wn · Myocardial ischemia-reperfusion injury · PCSK9 · SiRNA

²⁵ Introduction

Annually, more than a million people die due to car diovascular disease making it the leading cause of death
 worldwide [1]. Myocardial infarction (MI) significantly

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impacts cardiovascular disease-related mortality. Timely and effective myocardial reperfusion is the key to rescuing ischemic cardiomyocytes and limiting infarct size. However, the abrupt restoration of blood flow often aggravates the structural and functional damage of ischemic myocardium, leading to cardiomyocyte apoptosis and necrosis, potentially leading to reduced mitochondrial membrane heart failure, arrhythmias, and eventually cardiac systolic dysfunction [2, 3]. Although MIRI mechanism has not been fully elucidated, studies have demonstrated that it is closely associated with Ca²⁺ overload, reactive oxygen species (ROS) accumulation, reduced adenosine triphosphate (ATP) production, and reduced mitochondrial membrane potential [4, 5]. Under normal conditions, autophagy in the myocardium occurs at a basal level and participates in cellular homeostasis by removing excess or long-lived proteins as well as aging organelles [6]. Autophagy is activated by regulating Sirt3

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PCSK9 is an amino acid serine protease encoded by 52 PCSK9 gene on human chromosome 1 p32.3, mainly 53 expressed in hepatocytes [7]. PCSK9 competes with low-54 density lipoprotein cholesterol (LDL) to bind to the low-55 density lipoprotein receptor (LDLR) on the surface of 56 hepatocytes and guides LDLR internalization to lysosomal 57 degradation, reducing LDLR number on the cell membrane, 58 thereby upregulating cholesterol in the body s level [8]. As 59 research deepens, increasing evidence implies that PCSK9 60 expression may also be associated with inflammation, inde-61 pendent of low-density lipoprotein cholesterol regulation. 62

A class of PCSK9 inhibitors is used in clinical trials to 63 64 lower cholesterol levels by inhibiting the hepatic LDL receptors, raising serum LDL-c levels in the process [9]. Salvage 65 kinase, a key enzyme in insulin signaling, may negatively 66 affect susceptibility to myocardial reperfusion injury. Acti-67 vating salvage kinase or lower pH in the first phase of rep-68 erfusion after ischemia maintenance can reduce myocardial 69 infarct volume caused by a high-fat diet [10, 11]. In contrast, 70 increased myocardial infarct volume in hypercholesterolemia 71 is associated with increased ROS formation during reperfu-72 sion [12]. PCSK9 is thought to protect the myocardium by 73 preventing autophagy from occurring, which is why it is 74 upregulated in MIRI hearts [13]. Consequently, how PCSK9 75 affects ischemia-reperfusion injury, thereby improving the 76 mechanism of myocardial infarction size, has not been fully 77 understood. 78

In past studies, atherosclerotic cardiovascular disease 79 and myocardial infarction were reduced when LDL-C levels 80 were lower. The benefits of PCSK9 inhibitors in lowering 81 LDL-C and cardiovascular risk are undeniable. Therefore, in 82 August 2019, the European Society of Cardiology (ESC) and 83 the European Atherosclerosis Society (EAS) issued a joint 84 recommendation to develop stricter LDL-C level targets 85 for patients with recent MI [14]. During ischemia-reperfu-86 sion injury, PCSK9 inhibitors can reduce the incidence of 87 88 myocardial infarction and arrhythmias [15]. Notably, evolocumab may effectively reduce myocardial infarct size and 89 severity. Evolocumab is a human monoclonal immunoglob-90 91 ulin G2 (IgG2). The mechanism of action of evolocumab is to increase LDLR number that can clear LDL from the 92 blood by inhibiting PCSK9 binding to LDLR, thereby sig-93 nificantly reducing LDL-C levels and further reducing the 94 risk of myocardial infarction and stroke. Evolocumab has 95 become the only PCSK9 inhibitor approved in China for 96 treating homozygous familial hypercholesterolemia in adults 97 or adolescents over 12 years of age. It is well known that 98

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myocardial infarct size is linked to reperfusion opening time, and mortality may be further lowered if myocardial infarct size can be significantly reduced [16, 17].

Using RNA interference (RNAi) pathways to silence 102 disease-causing genes holds great promise for developing 103 therapeutics for targets that current drugs cannot address 104 [18]. siRNAs are widely used to silence target genes. This 105 process involves introducing the double-stranded RNA cor-106 responding to the target gene into the organism, resulting 107 in the corresponding mRNA degradation, thereby silencing 108 the target gene. Here, we revealed that siRNAs, when deliv-109 ered systemically in a liposomal formulation, can silence 110 the disease target PCSK9 in rodent primates with MIRI to 111 prove whether PCSK9 could be expressed in the myocardial 112 cells of rats. 113

This study seeks to answer the following questions: (1)114how does PCSK9 relate to MIRI and autophagy; (2) how115does PCSK9 affect the size and cardiac function of myo-116cardial infarction; and (3) can PCSK9 inhibitors inhibit the117inflammatory response, thereby alleviating MIRI, thereby118decreasing the size of a MI; and thereby decreasing mortal-119ity from myocardial ischemia–reperfusion injury?120

Materials and Methods

Animals

In total, 48 adult male Sprague–Dawley rats, weighing 123 250-300 g, were purchased from Suzhou Xishan Biotechnol-124 ogy Co., LTD. [License No: scxk (Xiang) 2019-0014]. All 125 rats were housed in a temperature-controlled environment 126 with a 12:12 h light-dark cycle, with free access to food and 127 water. After the experiments, the rats were sacrificed with 128 an intravenous injection of 10% chloral hydrate at 8 mL/ 129 kg. The animal experiments were approved by the Animal 130 Experimentation Committee of the Institutional Review 131 Board of Guizhou Medical University and complied with 132 the guidelines of Guizhou Medical University for the care 133 and use of animals. 134

Myocardial Ischemia–Reperfusion Protocol

The rats were anesthetized with chloral hydrate (3 mL/ 136 kg), and heparin was used to avoid blood clotting during 137 the surgery. When the rats were mechanically ventilated 138 after endotracheal intubation, the tidal volume was 1.0 mL 139 per min, and the breathing rate was 100 breaths/min. A 140 left-sided chest opening was performed at the fourth inter-141 costal space, and the pericardium was opened. An 8-0 142 filament gently crossed the LAD 2/3 of the way around 143 LAD, located between the starting points near the pul-144 monary cones. LAD occlusion caused epicardial cyanosis 145

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with local hypokinesis and typical ECG changes of acute 146 myocardial infarction (marked ST-segment elevation with 147 T-wave changes). LAD was ligated for 30 min, following 148 which the ligation wires were released, and reperfusion 149 was performed for 3 days [19]. A total of 48 adult male 150 Sprague–Dawley rats were randomly divided into three 151 experimental groups using the methodology of random 152 number table, as follows: (I) the control group in which the 153 rats were subjected to the same manipulation but without 154 LAD ligation (n = 16); (II) I/R group (n = 10); and (III) 155 I/R + siRNA groups, where the left ventricular wall was 156 injected with siRNA (1 μ g/10 g) at multiple points, using 157 an insulin needle, end of ischemia at 30 min, within 3 min 158 after the start of reperfusion. For 3 days, the rats were 159 cared after the chest was closed, and they began to recover. 160 The sequence of rat-derived PCSK9 siRNA was as fol-161 lows: sense 5'-GGAGGUGUAUCUCUUAGAUTT-3' and 162 antisense 5'-AUCUAAGAGAUACACCUCCTT-3'. The 163 sequence of rat-derived scrambled siRNA was as follows: 164 sense 5'-UUCUCCGAACGUGUCACGUTT-3' and anti-165 sense 5'-ACGUGACACGUUCGGAGAATT-3'. 166

167 Assessment of the Size of Myocardial Infarct

Infarct size was estimated using Evans blue (Beijing 168 Solarbio Science & Technology Co., Ltd., China)/2,3,5-169 triphenyltetrazolium staining (Beijing Solarbio Science & 170 Technology Co., Ltd., China). Following reperfusion, the 171 rats were re-anesthetized and LAD re-ligated; they were 172 then injected with 2 mL 2% Evans blue via the tail vein. 173 After the skin of lips and distal limbs was stained blue, the 174 hearts were removed, rinsed with 4 °C phosphate-buffered 175 saline (PBS), and frozen at - 80 °C for 30 min before 176 being cut into 5-7 slices. The sections were immersed in 177 1% TTC buffer (pH7.5) for 30 min at 37 °C. The area at 178 risk (AAR) was defined as the area not stained by Evans 179 blue, and the infarct area (IA) was defined as the area not 180 stained by TTC. Images of the stained slices were captured 181 with a digital camera, quantified using Image-Pro Plus, 182 and presented as a percentage [20]. 183

184 Echocardiographic Assessment of LV Function

A VINNO6 high-resolution ultrasound system was 185 employed to perform an echocardiographic analysis on 186 day 3 after IRI to assess cardiac function in anesthetized 187 rats. Ejection fraction (EF) and shortening fraction (FS) 188 measurements were performed to assess the left ven-189 tricular (LV) systolic function of the heart. The average 190 of three consecutive cardiac cycles was used for each 191 measurement. 192

Cell Culture, Simulated Ischemia and Transfection of siRNA

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AC16 cells (American Tissue Culture Collection) were 195 cultured in Dulbecco's modified Eagle's medium (DMEM) 196 (Gibco) supplemented with 10% fetal bovine serum (FBS), 197 100 U/mL penicillin, and streptomycin. The cells were main-198 tained in a humidified 95% air/5% CO₂ incubator at 37 °C. 199 Briefly, cardiomyocytes were exposed to a glucose-free, 200 serum-free medium and transferred to a hypoxic modular 201 incubator for 10 h at 37 °C with 5% CO₂ and 95% N₂. After 202 hypoxia, the medium was replaced with a fresh oxygenated 203 normal or high glucose medium, and the dishes were trans-204 ferred to a normoxic incubator (95% air/5% CO₂) for 8-h 205 reoxygenation. All cells were starved for 12 h with serum-206 free media before being subjected to normoxia or hypoxia. 207

siRNA duplexes corresponding to human-derived PCSK9 208 were purchased from RiboBio Biotechnology (Guangzhou, 209 CHN). The sequence of overexpressed human-derived 210 PCSK9 siRNA1(Ps1) was CCCATGTCGACTACATCGA, 211 and for silenced siRNA2(Ps2), it was GGTCACCGACTT 212 CGAGAAT. Cardiomyocytes were transfected with 50 nM 213 of each siRNA for 48 h using siRNA transfection reagent 214 to overexpress and inhibit PCSK9 gene expression. The 215 medium was replaced with a normal medium, and the cells 216 were subsequently exposed to hypoxia for specified times. 217 As a control, the cells were transfected with sequence-dis-218 ordered siRNA control. To confirm the efficiency of protein 219 knockdown using siRNA, cell lysates were used for Q-PCR 220 or Western blot analysis. 221

Western Blot Analysis

Human-derived cardiomyocytes and infarcted tissue in the223left ventricular of the rat were extracted with RIPA lysis224buffer (Beyotime, China) and PMSF (Roche, USA) to extract225total protein. Protein content was measured using a BCA226protein assay, and protein samples were separated by electro-227phoresis on SDS–PAGE and transferred to a polyvinylidene228difluoride membrane.229

After 2-h blocking with 5% skim milk, the membranes 230 were incubated overnight at 4 °C with the primary antibody 231 at a dilution of 1:1000. After washing with PBS containing 232 0.2% Tween, the membrane was incubated with a secondary 233 antibody for 1 h at room temperature. Then, signals were 234 detected with Pierce ECL Western Blotting Substrate. Inten-235 sity quantitation of the bands was captured using Image J 236 software and normalized to β -actin. 237

Antibodies directed at PCSK9 (ab31762, ab84041), 238 BNip3 (ab109362), and Beclin-1 (ab210498) were purchased from Abcam (San Francisco, CA), LC3 (CST#4599) 240 was purchased from Cell Signaling (Danvers, MA). 241

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Real-Time Q-PCR 242

The peri-infarct area and border zone area of the left ventri-243 244 cle of the rats was removed and homogenized. Total RNA was extracted with TRizol reagent (ThermoFisher). cDNA 245 was synthesized with Hiscript III-RT Supermix for the 246 qPCR kit (Vazyme) following the manufacturer's instruc-247 tions. Relative β -actin levels were quantified for each sample 248 based on Ct (amplification cycle threshold), normalized to a 249 value of 1 as an endogenous mRNA standard, and the rela-250 tive expression level was calculated by the $2^{-\Delta\Delta CT}$ method. 251 Real-time quantitative PCR using gene-specific primers was 252 employed, as demonstrated in Table 1. 253

Histopathological Change 254

Hematoxylin-eosin (H&E) staining and Masson trichrome 255 staining were used to examine myocardial tissue's pathologi-256 257 cal and morphological changes. The hearts obtained from each group were left overnight in 4% paraformaldehyde, 258 dehydrated and embedded in paraffin blocks. Subsequently, 259 260 all myocardial tissues were cut into 5% thick slices, mounted on glass slides, dried and stained. A light microscope was 261 used to examine paraffin-embedded myocardial tissue slices 262 stained with hematoxylin for 5 min, eosin for 2 min, or Mas-263 son trichrome staining kit (Beijing Sola Biotechnology Co., 264 Ltd., China). Finally, the stained slides were immersed in 265 xylene, gradient concentrations of ethanol, according to the 266 instructions, and then sealed with resin. 267

Immunohistochemistry (IHC) 268

The left ventricular infarct tissue in the rats was par d 269 into 5-µm thick paraffin sections, dewaxe , a. J ant zeni-270 cally repaired in 1 mM EDTA (pH 9.0) to 1.5 min. The 271 slides were then incubated with 10% go. seru 1 for 1 h at 272 room temperature, with primary antibuly on straight at 4 °C 273

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and secondary antibody for 30 min. Hematoxylin was used 274 to re-stain the nuclei for 2 min: the cells were differentiated 275 with a differentiation medium for 5 s and then returned to 276 blue with ammonia. Finally, using alcohol, the eluate was 277 dehydrated and clear, and the neutral resin was used to seal 278 the slides for fixation, microscopic observation (Lycra), and 279 analysis using Image Pro6.0. 280

Measurement of cTnT, CK-MB

Myocardial injury was evaluated by measuring the plasma 282 concentrations of cardiac troponin T (cTnT) creatine kinase-283 MB (CK-MB). At the end of the reperfusion period, blood 284 was collected and centrifuged at 1500 rg. fc. 10 min to 285 obtain plasma. CKMB and cTnT level nd, tivity were 286 measured using specific ELISA kits Quan. ou Ruixin Bio-287 logical Technology Co., LTD, Ouan, Ju, China) accordi-288 ng to manufacturer'sprotocols 289

Statistical Analysis

All experiments x reconducted three times at least (n=3). 291 All data were analy 'd using GraphPad Prism 7.0 soft-292 ware (CA. U.). Comparisons between groups were per-293 formed vi. he t-, st or one-way ANOVA test for continuous 294 numerical va. bles. Values were expressed as mean ± stand-295 ard viation, and p < 0.05 was considered statistically 296 ignific. ... 297

Results

Upregulation of PCSK9 and Autophagy Levels Under 299 Hypoxia

AC16 cells were subjected to 10-h hypoxia and 8-h reoxy-301 genation, while total cellular proteins, lysate, and protein 302

Table 1 List of primers used in this study	Quar	Species	Forward	Reverse
	PCSK9	Rat	TGGAACCTGGAGCGGATTAC	TTCCCGGTGGTCACTCTGTA
	BNIP3	Rat	GGGCTCCTGGGTAGAACT	AGACGGAAGCTGGAACG
	Beclin-1	Rat	GCGTCAGCTCTCGTCAA	GCCCGGTCTTCAGCTAC
XY	LC3	Rat	GGAGTCCTGTGTCTACGG	AAAAGCTGGGGTGTTCCT
	β-Actin	Rat	CACCCGCGAGTACAACCTC	CCCATACCCACCATCACACC
	PCSK9	Human	GCTGTGCCTTGGTTTCCT	TGTGAAGTAGGGGTGCGA
	BNIP3	Human	CCAGCCTCGGTTTCTATTT	TATCTTGGTGTCTGCGA
	Beclin-1	Human	GGATGGTGTCTCTCGCA	CAGTCTTCGGCTGAGGTT
	LC3	Human	GGCACCAACCCACCTACTC	ATCCCACCAGCCAGCAC
	β-Actin	Human	CTCGCTTCGGCAGCACA	AACGCTTCACGAAATTGCGT
	IL-1β	Rat	CCCTTGACTTGGGCTGT	CGAGATGCTGCTGTGAGA
	NLRP3	Rat	TGTTGTCAGGATCTCGCA	AGTGAAGTAAGGCCGGAAT

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buffer were extracted and prepared into upper samples, as 303 revealed in Fig. 1. PCSK9, Beclin-1, and LC3II/I expres-304 sions were upregulated in H/R group compared with the 305 normal control (NC) group (p < 0.001, n = 4), thus suggest-306 ing a potential relationship between PCSK9 and autophagy. 307 Bnip3 is known to be induced by hypoxia, and autophagy is 308 protective against Bnip3-induced autophagy and cell death 309 [21]. Additionally, PCSK9 may affect hypoxia-reoxygenated 310 cardiomyocytes by mediating autophagy through Bnip3 311 pathway. 312

PCSK9 Synchronization Regulate BNIP3 and Autophagy Levels in AC16 Cells

A higher or lower expression level of PCSK9 was observed in HR group than in the control group (Fig. 2A, B, p < 0.05). Furthermore, we used PCSK9 overexpression to enhance Bnip3 expression. On the contrary, Bnip3 expres-318 sion was significantly reduced after using PCSK9 knock-319 down (Fig. 2A, B, p < 0.05). Furthermore, the expression 320 level of Bnip3 increased significantly along with PCSK9 321 upregulation during HR. Additionally, the expression level 322 of Bnip3 was lower along with PCSK9 downregulation. 323 In contrast, PCSK9 knockdown in cardiomyocytes dras-324 tically reduced the expression of LC3-II, Beclin-1, and 325 autophagic flux, while it increased P62 expression, as well 326 as the expression of Bnip3 pathway proteins, which was 327 confirmed at RNA levels (Fig. 2D). 328

In vitro experiments verified the correlation between PCSK9 and the autophagic pathway, which could affect autophagy via Bnip3 pathway, thereby influencing cell death induced by hypoxic reoxygenation. We then performed in vivo experiments in SD rats to further validate this.

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Fig. 2 PCSK9 synchronization regulate BNIP3 and autophagy levels in AC16 cells. **A**, **B** The level of PCSK9 protein, BNIP3 protein in the normal control, H/R, H/R+Ps1 and H/R+Ps2 groups. **C** RNA expression and analysis of pcsk9, Bnip3, Beclin-1, lc3 and P62. Data are expressed as the mean \pm SEM, N=4. *P<0.05, **P<0.01 and ***P<0.001 vs. NC, "P<0.05, "#P<0.01, "##P<0.001 and "###P<0.001 vs. H/R

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PCSK9 is Knocked Down to Restrict MI Region 335

As depicted in the schematic diagram in Fig. 3A, MIRI 336 was induced in rats and confirmed via electrocardiogra-337 phy (Fig. 3B). Myocardial infarct size, myocardial damage 338 marker expression, cardiac function, and histomorphological 339 heart alterations were measured. TTC/EB staining revealed 340 that PCSK9 knockdown significantly reduced the myocardial 341 infarct size compared with I/R group (p < 0.001; Fig. 3C, D). 342 The findings of cardiac enzyme tests revealed that knocking 343 down PCSK9 lowered the severity of myocardial infarction 344 (*p* < 0.0001; Fig. 3E, F). 345

PCSK9 Knockdown Ameliorated MIRI in Rats 346

Echocardiography was used to investigate whether PCSK9 347 knockdown influenced cardiac function. After 3 days of 348 ischemia, the reperfused simultaneous left ventricular wall 349 350 multipoint injection of PCSK9 siRNA resulted in significantly less unfavorable remodeling as well as better ejection 351 fraction (EF) and shortening fraction (FS) compared to I/R 352 group (p < 0.001; Fig. 4A–C). Compared with the control 353 group, the values of left ventricular end-diastolic diameter 354 (LVDd) and left ventricular end-systolic diameter (LVDs) 355 in I/R group were significantly increased, and LVDd and 356 LVDs were significantly decreased after PCSK9 knockdown, 357 compared with I/R group (p < 0.05; Fig. 4D, E). Meanwhile, 358

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cardiac enzymes such as CK-MB and CTNT showed that 359 knockdown of PCSK9 resulted in a reduced degree of myocardial infarction compared to I/R group (p < 0.0001; Fig. 3E, F). 362

PCSK9 Knockdown Reduces Autophagy Expression and Improves Myocardial Fibrosis

We performed HE, Masson staining, and immunohisto-365 chemistry tests to further evaluate the effect of PCSK9 366 knockdown on myocardial histological structure and fibro-367 sis. Additionally, HE staining revealed that the cardiac 368 tissue exhibited a clear and well-organized structure with 369 little inflammatory infiltration or cardiac necrosis in con-370 trol. However, myocardial structural abnormalities and 371 histological changes, including perinuclear vacuolization, 372 necrosis, cardiac intercellular spaces, myofibrillar thinning 373 and wavy pattern consistent with infiltration and transmi-374 gration of inflammatory cells, were detected in IR group 375 (the arrow in Fig. 5A). In contrast, PCSK9 knockdown 376 reduced myocardial inflammatory infiltration with a more 377 transparent structure and less tissue necrosis (Fig. 5A). IR 378 group's Masson stain revealed a significantly increased 379 fibrosis ratio, which was also alleviated by PCSK9 380 knockdown (Fig. 5B, F). In IHC staining (Fig. 5C-E, 381 G-I), PCSK9, Bnip3, and Beclin-1 meaningfully rose in 382



Fig. 3 PCSK9 is knocked down to restrict the MI region. A Schematic diagram of the myocardial ischemia-reperfusion process. B Representative electrocardiograms before ischemia, during myocardial ischemia, and after reperfusion. C, D The infarct area was determined by TTC/EB staining. The ischemic area showed pale, and the viable myocardium showed red. The infarct area was quantified as

a percentage of the total slice area. The infarct area and area at risk were quantified via Evans blue and TTC double staining. Graphic representation of the infarct size expressed as a percentage of infarct area over the area at risk (n=3). Blue, non-blue and white areas represent non-ischemic, AAR and IA areas. E Serum CK-MB levels. F Serum CTNT levels

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Fig. 4 Knockdown of PCSK9 ameliorated MIRI. A Representative echocardiograms between the groups. B The percentage of ejection fraction. C The percentage of fractional shorting. D Left ventricle end-diastolic diameter (LVDd). E Left ventricle end-systolic diam-

eter (LVDs). Data are expressed as mean \pm standard deviation. N=4. **P < 0.01 and ***P < 0.001 vs. Control, ${}^{#}P < 0.05$ and ${}^{\#}P < 0.01$ vs. I/R



Fig. 5 Knockdown of PCSK9 reduces autophagy expression and improves myocardial fibrosis. Three days after the operation, the heart was isolated, and the thin paraffin section (4 μ m) were made. **A** Results of HE staining (×100). **B**, **G** Fibrosis ratio was compared among the three groups (×100). **C–E**, **H–J** PCSK9, Bnip3, and Beclin-1 were stained by immunohistochemistry, compared the three groups and quantitative analysis (×100). **F** The degree of micro-

scopic injury of the heart evaluated and graded on a scale of 0–4 with 0=no injury; 1=injury to 25% of the field; 2=injury to 50% of the field; 3=injury to 75% of the field; and 4=severe injury. Data are expressed as the mean±standard deviation (n=5). **P<0.01, ***P<0.001 and ****P<0.0001 vs. Control, *P<0.05, ##P<0.01, ###P<0.001 and ####P<0.0001 vs. I/R. *HE* hematoxylin–eosin

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IR group (p < 0.05), although both autophagy levels and Bnip3 pathway protein expression were downregulated via the knocking down of PCSK9 (p < 0.05).

PCSK9 Knockdown Inhibits Autophagy via Bnip3 Signaling Pathway

To further study the mechanism of PCSK9 in vivo, rat 388 models were constructed in this work. The knockdown 389 efficiency was validated via Western Blot and Q-PCR 390 findings. After transfection with PCSK-siRNA, the 391 protein and mRNA expression of Bnip3, Beclin-1 and 392 LC3 were significantly downregulated (p < 0.05; N = 4; 393 Fig. 6A–C). We discovered that inhibiting the Bnip3 path-394 way significantly decreased Beclin-1 expression (p < 0.05; 395 N=4; Fig. 6A–C). According to the findings, PCSK9 396 may suppress autophagy levels by activating the Bnip3 397 pathway, thereby reducing the damage caused by I/R to 398 cardiomyocytes. 399

PCSK9 Knockdown Attenuates Myocardial Inflammatory Response

The inflammatory response during myocardial ischemia-rep-402 erfusion injury is critical for cardiac healing, whereas exces-403 sive inflammation prolongs infarction and promotes poor 404 cardiac remodeling. Understanding the mechanisms underly-405 ing these uncontrolled inflammatory processes has signifi-406 cant implications during MIRI treatment [22]. It has been 407 revealed that interleukin-1 β (IL-1 β) and Nod-1-like receptor 408 protein 3 (NLRP3) are significantly elevated in ischemia. 409 and we have also performed experiments with inflammatory 410 mediators; the results are displayed in Fig. 7. Compared with 411 the control, IL-1 and NLRP3 expressions were significantly 412 upregulated in IR group (p < 0.05, n = 5; Fig. 7A); however, 413 compared with IR+PCSK9 siRNA group, expression was 414 significantly downregulated (p < 0.05; n = 5; Fig. 7), indi-415 rectly suggesting that PCSK9 knockdown could improve 416 MIRI by suppressing autophagy levels and attenuating the 417 inflammatory response. 418



Fig.7 Knockdown of PCSK9 attenuates myocardial inflammatory response. **A–C** Protein expression and analysis of NLRP3 and IL-1 β between the three groups. **D–E** Expression and analysis of mRNA of NLRP3 and IL-1 β between the three groups. Data are

expressed as the mean±standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001 and ***P < 0.0001 vs. Control, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ and $^{\#\#\#\#}P < 0.0001$ vs. I/R

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419 **Discussion**

In vitro and in vivo experimental studies revealed that 420 siRNA knockdown of PCSK9 resulted in reduced expres-421 sion of the autophagic protein Beclin-1, light chain 3 422 (LC3) compared to normal control-treated cells and con-423 trol-operated groups. Simultaneously, Bnip3 pathway pro-424 tein expression was downregulated. Furthermore, PCSK9-425 mediated small interfering RNA (siRNA) group injected 426 into the left ventricular wall significantly improved cardiac 427 function and myocardial infarct size. 428

Cardiovascular disease accounts for one-third of all 429 death, and ischemic heart disease is the primary cause 430 of death. To the best of our understanding, I/R damage 431 frequently occurs in clinical circumstances with complex 432 433 mechanisms [23]. PPCSK9 inhibitor therapy may be useful in reducing biochemical and physiological problems 434 associated with cardiac MIRI [15]. Recently, studies have 435 focused on PCSK9 effects on cholesterol and low-density 436 lipoprotein. This work demonstrated that PCSK9 knock-437 down could ameliorate MIRI by inhibiting Bnip3 pathway-438 mediated autophagy. When one suffers external damage, 439 autophagy is a self-repair mechanism; nevertheless, exces-440 sive autophagy can lead to increased cell death, reversing 441 the process. Mitophagy is a type of selective autophagy 442 involved in ischemia-reperfusion injury [24]. PCSK9 was 443 considerably upregulated in ex vivo tests after ischemia/ 444 hypoxia, whereas autophagy levels were recruited to 445 further damage mitochondrial structure and metabolic 446 function. Consistently, the results of TTC/EB staining, 447 H&E staining, Masson's trichrome staining, and cardiac 448 449 enzymes measured in this study demonstrated that the myocardial infarction area was enlarged, while structural 450 abnormalities and cardiac enzyme (cTnT, CK-MB) lev-451 els increased significantly in I/R group. However, PCSK9 452 knockdown regulated autophagy levels through Bnip3 453 pathway, and, in contrast with IR group, MIRI severity 454 was reduced, as was myocardial infarct size, thus resulting 455 in improved cardiac function and reduced mortality. These 456 results suggest that cardiac structures are protected, and 457 PCSK9 knockdown increases myocardial viability. 458

Extensive evidence indicates that autophagy is criti-459 cal in cardiomyocyte apoptosis during MIRI [25, 26]. Yu 460 et al. discovered that a lack of Mammalian STE20-like 461 kinase1 provides a pro-survival signal to the reperfused 462 heart by reversing FUN14 domain containing one related 463 mitophagy, thereby decreasing cardiomyocyte mitochon-464 drial apoptosis [27]. Furthermore, I/R and oxygen–glucose 465 deprivation/recovery damage increased hypoxia-inducible 466 factor-1 (HIF-1) expression, activated downstream Bnip3, 467 and induced mitochondria-dependent autophagy. HIF-1 α 468 upregulation and Bnip3 expression may contribute to 469

I/R-injured SD rat cardiomyocytes and OGD/R injury-470 induced autophagy in H9C2 cells. We hypothesize that 471 myocardial viability is enhanced through knockdown of 472 PCSK9 regulation associated with autophagy and Bnip3 473 pathway. To verify this idea, we investigated the relation-474 ship between PCSK9 and autophagy and Bnip3 pathway. 475 WB analysis revealed increased Bnip3, Beclin-1, and 476 LC3II/I ratios, thus indicating that PCSK9 activated the 477 Bnip3 signaling pathway and increased autophagy lev-478 els. PCSK9 siRNA was injected into the left ventricular 479 wall at numerous stages during reperfusion to confirm the 480 role of Bnip3 pathway in PCSK9 cardioprotection, while 481 PCSK9 siRNA was transfected into cells before intrinsic 482 hypoxia-reoxygenation. As expected, in the presence of 483 Bnip3-siRNA, Bnip3 and Beclin-1 protein expressions 484 were repressed, P62 was boosted, and the upregulated 485 effect of PCSK9 on autophagy was significantly reduced. 486 These findings show that the key protective mechanism 487 of PCSK9 against MIRI is autophagy inhibition via the 488 Bnip3 pathway. 489

Inflammasome activation plays a vital role in host defense. 490 Simultaneously, autophagy is naturally linked to the adaptive 491 immune system and strongly linked to inflammation. Zhang 492 et al. discovered that knocking down lincRNA-Cox2 acti-493 vated caspase-1, resulting in decreased IL-1 production and 494 increased autophagy [28]. Furthermore, Wang et al. discov-495 ered that neuregulin-1 could reduce reactive oxygen species 496 formation by inhibiting NADPH oxidase 4 and inhibiting 497 NLRP3/caspase-1 pathway in MIRI to minimize oxidative 498 damage and inflammation [29]. A recent study disclosed that 499 normocholesterolemic subjects with lower plasma PCSK9 500 and higher white adipose tissue surface expression of LDLR 501 and CD36 had higher NLRP3 inflammasome activation [30]. 502 To test whether PCSK9 can control inflammatory factors 503 and hence worsen MIRI, PCSK9 knockdown was followed 504 by NLRP3 and IL-1 downregulation, as confirmed by WB 505 and Q-PCR. The above data display that PCSK9 knock-506 down inhibits autophagy and attenuates the inflammatory 507 response, thereby ameliorating MIRI. 508

PCSK9 is mainly secreted by the liver and can be released 509 into the blood. Circulating PCSK9 levels are associated 510 with LDL-c levels. LDL-c is readily oxidized by ROS, and 511 LOX-1 can be activated by autophagy, thereby regulating 512 infarct size [31]. Based on this information, we hypothe-513 size that MIRI can be controlled indirectly by modulating 514 autophagy and LDL-c levels. PCSK9 inhibitors are com-515 monly used in lipid-lowering therapy, although their use 516 in individuals with myocardial infarction is debatable. In 517 the Atherosclerosis Risk in Communities trial, deletion of 518 one copy of PCSK9 saved 88% of human cardiovascular 519 events [32]. This study provided experimental evidence for 520 reducing reperfusion injury in patients with acute myocar-521 dial infarction. A significant number of clinical trials remain 522

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required to validate the clinical use of PCSK9 inhibitors in

524 patients with myocardial infarction.

525 Conclusion

In ischemic/hypoxic circumstances, PCSK9 expression
was dramatically increased. PCSK9 knockdown alleviated
MIRI via Bnip3-mediated autophagic pathway and improved
inflammatory response, myocardial infarct size, and cardiac
function.

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Author Contributions GWH, XYL, and WL designed and performed experiments, analyzed and interpreted data, and prepared the manuscript. KZ, ZGD, XLX, MZL, CL, YQL and LX participated in the design of the study and performed the statistical analysis. HYZ and ZHL conceived of the study, and participated in its design and coordination and review of this manuscript. All authors read and approved the final manuscript.

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545Data AvailabilityAll data, models, or code generated or used during546the study are available from the corresponding author by request.

547 **Code Availability** Not applicable.

548 **Declarations**

549 Conflict of interest The authors declare no conflict of interest.

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560 **References**

- Kuhn, T. C., Knobel, J., Burkert-Rettenmaier, S., et al. (2020).
 Secretome analysis of cardiomyocytes identifies PCSK6 (proprotein convertase subtilisin/kexin type 6) as a novel player in cardiac remodeling after myocardial infarction. *Circulation*, 141(20), 1628–1644.
- Huang, Z.-Q., Xu, W., Wu, J.-L., et al. (2019). MicroRNA-374a
 protects against myocardial ischemia–reperfusion injury in mice
 by targeting the MAPK6 pathway. *Life Sciences, 232*, 116619.

- Liu, W., Chen, C., Gu, X., et al. (2021). AM1241 alleviates myocardial ischemia–reperfusion injury in rats by enhancing Pink1/ Parkin-mediated autophagy. *Life Sciences*, 272, 119228.
 571
- 4. Elgebaly, S. A., Poston, R., Todd, R., et al. (2019). Cyclocreatine protects against ischemic injury and enhances cardiac recovery during early reperfusion. *Expert Review of Cardiovascular Therapy*, *17*(9), 683–697.
- Sulaiman, D., Li, J., Devarajan, A., et al. (2019). Paraoxonase 2 protects against acute myocardial ischemia–reperfusion injury by modulating mitochondrial function and oxidative stress via the PI3K/Akt/GSK-3β RISK pathway. *Journal of Molecular and Cellular Cardiology, 129*, 154–64.
- Zheng, Y., Shi, B., Ma, M., et al. (2019). The novel relationship between Sirt3 and autophagy in myocardial ischemia–reperfusion. *Journal of Cellular Physiology*, 234(5), 5488–5495.
- Abate, N., Sallam, H. S., Rizzo, M., et al. (2014). Resistin: An inflammatory cytokine. Role in cardiovascular diseases, diabetes and the metabolic syndrome. *Current Pharmaceutical Design*, 20(31), 4961–9.
- Horton, J. D., Cohen, J. C., & Hobbs, H. H. (2007). Molecular biology of PCSK9: Its role in LDL metabolism. *Trends in Biochemical Sciences*, 32(2), 71–77.
- Gu, H.-M., & Zhang, D.-W. (2015). Hypercholesterolemia, low density lipoprotein receptor and proprotein convertase subtilisin/ kexin-type 9. *Journal of Biomedical Research*, 29(5), 356–361.
- 10. Poncelas, M., Inserte, J., Vilardosa, Ú., et al. (2015). Obesity induced by high fat diet attenuates postinfarct myocardial remodeling and dysfunction in adult B6D2F1 mice. *Journal of Molecular and Cellular Cardiology*, *84*, 154–61.
- 11. Inserte, J., Aluja, D., Barba, I., et al. (2019). High-fat diet improves tolerance to myocardial ischemia by delaying normalization of intracellular PH at reperfusion. *Journal of Molecular and Cellular Cardiology*, *133*, 164–73.
- Andreadou, I., Schulz, R., Badimon, L., et al. (2020). Hyperlipidaemia and cardioprotection: Animal models for translational studies. *British Journal of Pharmacology*, 177(23), 5287–5311.
- Ding, Z., Wang, X., Liu, S., et al. (2018). PCSK9 expression in the ischaemic heart and its relationship to infarct size, cardiac function, and development of autophagy. *Cardiovascular Research*, *114*(13), 1738–1751.
- Momtazi-Borojeni, A. A., Sabouri-Rad, S., Gotto, A. M., et al. (2019). PCSK9 and inflammation: A review of experimental and clinical evidence. *European Heart Journal Cardiovascular Pharmacotherapy*, 5(4), 237–245.
- Palee, S., Mcsweeney, C. M., Maneechote, C., et al. (2019). PCSK9 inhibitor improves cardiac function and reduces infarct size in rats with ischaemia/reperfusion injury: Benefits beyond lipid-lowering effects. *Journal of Cellular and Molecular Medicine*, 23(11), 7310–7319.
- Wiviott, S. D., Giugliano, R. P., Morrow, D. A., et al. (2020). Effect of evolocumab on type and size of subsequent myocardial infarction: A prespecified analysis of the FOURIER randomized clinical trial. *JAMA Cardiology*, 5(7), 787–793.
- Schwartz, G. G., Steg, P. G., Szarek, M., et al. (2018). Alirocumab and cardiovascular outcomes after acute coronary syndrome. *The New England Journal of Medicine*, 379(22), 2097–2107.
- Shankar, P., Manjunath, N., & Lieberman, J. (2005). The prospect of silencing disease using RNA interference. *JAMA*, 293(11), 1367–1373.
- Zhou, H., Mo, L., Huang, N., et al. (2022). 3-Iodothyronamine inhibits apoptosis induced by myocardial ischemia reperfusion via the Akt/FoxO1 signaling pathway. *Annals of Translational Medicine*, 10(4), 168.
- Medicine, 10(4), 168.
 20. Rossello, X., Hall, A. R., Bell, R. M., et al. (2016). Characterization of the Langendorff perfused isolated mouse heart model of global ischemia–reperfusion injury: Impact of ischemia and 634

- reperfusion length on infarct size and LDH release. *Journal of Cardiovascular Pharmacology and Therapeutics*, 21(3), 286–295.
- Crucet, M., Wüst, S. J. A., Spielmann, P., et al. (2013). Hypoxia
 enhances lipid uptake in macrophages: Role of the scavenger receptors Lox1, SRA, and CD36. *Atherosclerosis*, 229(1), 110–117.
- 22. Ning, K., Jiang, L., Hu, T., et al. (2020). ATP-sensitive potassium
 channels mediate the cardioprotective effect of panax notoginseng saponins against myocardial ischaemia–reperfusion injury
 and inflammatory reaction. *BioMed Research International*, 2020,
 3039184.
- Li, L., Li, X., Zhang, Z., et al. (2020). Protective mechanism and clinical application of hydrogen in myocardial ischemia–reperfusion injury. *Pakistan Journal of Biological Sciences*, 23(2), 103–112.
- Wang, J., Toan, S., & Zhou, H. (2020). New insights into the role
 of mitochondria in cardiac microvascular ischemia/reperfusion
 injury. *Angiogenesis*, 23(3), 299–314.
- Liu, L., Jin, X., Hu, C.-F., et al. (2017). Exosomes derived from mesenchymal stem cells rescue myocardial ischaemia/reperfusion injury by inducing cardiomyocyte autophagy via AMPK and Akt pathways. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology, 43*(1), 52–68.
- 26. Yu, S.-Y., Dong, B., Fang, Z.-F., et al. (2018). Knockdown of lncRNA AK139328 alleviates myocardial ischaemia/reperfusion injury in diabetic mice via modulating miR-204-3p and inhibiting autophagy. *Journal of Cellular and Molecular Medicine*, 22(10), 4886–4898.
- Yu, W., Xu, M., Zhang, T., et al. (2019). Mst1 promotes cardiac
 ischemia–reperfusion injury by inhibiting the ERK-CREB pathway and repressing FUNDC1-mediated mitophagy. *The Journal of Physiological Sciences*, 69(1), 113–127.
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Journal : Large 12012

- Xue, Z., Zhang, Z., Liu, H., et al. (2019). lincRNA-Cox2 regulates NLRP3 inflammasome and autophagy mediated neuroinflammation. *Cell Death and Differentiation*, 26(1), 130–145.
- Wang, F., Wang, H., Liu, X., et al. (2021). Neuregulin-1 alleviate oxidative stress and mitigate inflammation by suppressing NOX4 and NLRP3/caspase-1 in myocardial ischaemia–reperfusion injury. *Journal of Cellular and Molecular Medicine*, 25(3), 1783–1795.
- 30. Cyr, Y., Lamantia, V., Bissonnette, S., et al. (2021). Lower plasma PCSK9 in normocholesterolemic subjects is associated with upregulated adipose tissue surface-expression of LDLR and CD36 and NLRP3 inflammasome. *Physiological Reports*, 9(3), e14721.
- Li, D., Williams, V., Liu, L., et al. (2003). Expression of lectinlike oxidized low-density lipoprotein receptors during ischemia– reperfusion and its role in determination of apoptosis and left ventricular dysfunction. *Journal of the American College of Cardiology*, 41(6), 1048–1055.
- Cohen, J. C., Boerwinkle, E., Mosley, T. H., et al. (2006). Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *The New England Journal of Medicine*, 354(12), 1264–1272.

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26(1), 130–145.
(2021). Neuregulin-
flammation by suppl
ocardial ischaemia-re
Molecular Medicine,
et al. (2021). Lower
ubjects is associate
pression of LDLR and
gical Reports, 9(3), e
2003). Expression of
receptors during isch
ation of apoptosis a
e American College o
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sley, T. H., et al. (
DL, and protection
eland Iournal of Me

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