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The Inhibition of RNF20 Causes Inflammation in the Model of Myocardial Infarction through the NLRP3 Inflammsaome Activation

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KEYWORDS Degradation. Inflammsaome. Myocardial Infarction. Ring Finger Protein 20. Vitamin D Receptor

ABSTRACT This study aimed to examine the function and its possible molecular biological mechanisms of Ring Finger Protein 20 (RNF20) in the model of myocardial infarction (MI). Patients with MI infarction were collected and analysed. C57BL6J mice or H9c2 cell were induced using the left anterior descending coronary artery or hydrogen peroxide and Lipopolysaccharides. The serum mRNA expression of RNF20 levels in patients with MI or in the mice with MI was down regulated. RNF20 gene reduced inflammation *in vitro* model of MI. Knock-out of RNF20 causes inflammation and MI in the mice model. RNF20 suppressed NLRP3 inflammasome by Vitamin D Receptor. RNF20 interacted with Vitamin D Receptor to reduce degradation of Vitamin D Receptor. Vitamin D Receptor interacted with NLRP3 protein. The inhibition of RNF20 causes inflammation in the model of MI through NLRP3 activation by Vitamin D Receptor, and might be a potential treatment target for the MI -related diseases.

INTRODUCTION

Epidemiological investigation has found that the death of patients caused by cardiovascular related diseases ranks first in the world. Among them, Myocardial Infarction (MI) caused by coronary artery stenosis caused by various factors is one of the pathogenic factors. MI is one of the serious types of Coronary Heart Disease (Dergilev et al. 2021). The incidence rate of MI increased year by year, and the mortality rate is high, which seriously threatens the health of the elderly (Marinkoviæ et al. 2020; Montone and La Vecchia 2021). Therefore, prevention or reduction of it is of positive significance.

At present, the main clinical treatment for acute myocardial infarction is intervention and bypass surgery, which effectively reduces the mortality of patients with myocardial infarction (Ganeshan et al. 2022). However, most patients with myocardial infarction will gradually develop chronic complications related to myocardial ischemia and necrosis, leading to ventricular remodeling, which not only increases the difficulty of postoperative rehabilitation, but also seriously interferes with the quality of life of patients (Iaremenko et al. 2022; Li et al. 2022b; Wienbergen et al. 2022).

After myocardial infarction, the number of cardiomyocyte apoptosis increased, a large number of cardiomyocytes were lost, myocardial collagen fibres increased, cardiac pathological remodelling and cardiac function decreased significantly, which are important reasons for the heart failure (Marinkoviæ et al. 2020). It has been found that ventricular remodeling may occur in elderly patients after myocardial infarction, which may be related to inflammatory factors secreted by myocardial cells after ischemia, which may also lead to apoptosis of myocardial cells in the injured area (Komal et al. 2022). Therefore, how to control the inflammatory response of myocardial cells in elderly patients after myocardial infarction will be one of the key and difficult points to control ventricular remodeling after myocardial infarction (Li et al. 2022a; Li et al. 2022b).

The importance of inflammatory mediators in cardiac pathophysiology has increased, but in the following three centuries, the role of inflammatory response in the heart failure after myocardial infarction has not been paid attention (Ong et al. 2018). There is a large amount of leukocyte infiltration in the myocardial infarc-

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tion area. In the next half-century, a number of animal experiments have proved that anti-inflammatory therapy targeting leukocyte mediated inflammatory response after solid infarction can effectively reduce myocardial injury (Prabhu and Frangogiannis 2016). The research on the role and mechanism of inflammatory response and anti-inflammatory therapy in the heart failure has become a hot spot in the field of heart failure (Thackeray et al. 2018) . Inflammatory response is one of the important pathological mechanisms in the process of ventricular remodeling caused by myocardial infarction (Zhang et al. 2022). After myocardial infarction, the endogenous immune system is activated to induce the release of a large number of inflammatory mediators, promote the migration and chemotaxis of inflammatory cells to the injured myocardial tissue, release a large number of inflammatory substances again, promote myocardial fibrosis, and finally form scar tissue to affect cardiac contractile function (Zhang et al. 2022) .

NLRP3 is involved in the pathophysiological process of MI, ischemia/reperfusion injury and cardiac remodeling after myocardial infarction, and affects the clinical outcome (Buckley and Libby 2019). Effective regulation of NLRP3 is helpful to prevent and treat cardiovascular diseases (Gao et al. 2019; Gao et al. 2021) Up regulation of NLRP3 expression in MI ischemic myocardium can promote caspase-1 and IL-1β And IL-18 expression increased, inducing myocarditis cascade. Inhibiting the expression of NLRP3 inflammatory bodies can reduce inflammation and improve cardiac dysfunction and remodeling in MI rats. Intervening in the abnormal expression of NLRP3 inflammatory bodies may become a new target or strategy for the prevention and treatment of MI.

Toll like receptor recognition on myocardial fibroblast membrane promotes intracellular NF-κB and NLRP3 expression; ATP dependent k+ efflux and a large amount of ROS produced during reperfusion occurred in the damaged myocardial fibroblasts, which further activated NLRP3 in the fibroblasts (Hua et al. 2022; Pu et al. 2022) .

Rnf20 can play the role of ubiquitin ligase alone (Jeon et al. 2020). Both RNF20 proteins contain a C-terminal ring finger domain to form a tight heterodimer (Liang et al. 2018) . It is the main E3 ligase responsible for the monoubiquitination of histone H2B lysine (k) 120 (h2bk120ub) in mammalian cell (Tarcic et al. 2017; Liang et al. 2021). The decreased expression of RNF20 and H2Bub in ox LDL stimulated macrophages suggests that RNF20 may modify the epigenetics of H2Bub modified genes and affect ox-LDL stimulated macrophage migration. The results of this study showed that after overexpression of RNF20, H2Bub expression increased and NF-êB and IL-6 expression were inhibited, and macrophage migration was reduced, suggesting that RNF20 could affect histone h2bub gene epigenetics, thus regulating macrophage migration and inflammatory factors. VanDusen et al. showed that RNF20/40 is the epigenetic regulator of cardiomyocyte maturation (VanDusen et al. 2021). Therefore, RNF20 might regulate the disease progression of the heart disease.

Objective of the Study

This paper aimed to investigate the function of ring finger protein 20 (RNF20) in the model of myocardial infarction (MI) and its possible molecular biological mechanisms, in order to find the potential treatment target for the MI-related diseased patients.

MATERIAL AND METHODS

Patients Clinical Experiments

Informed consent of this study was subscribed by selected personnel. Patients with MI (n = 12) and normal volunteers (n = 12) were collected and analysed from the Ningbo Hangzhou Bay Hospital. The protocols for the experiments in this study were approved by University Research Committee of Ningbo Hangzhou Bay Hospital.

Animals, Experimental Model and Lentivirus Injection and Histopathologic Assay

All experimental procedures involving animals were approved by The Institutional Animal Care and Use Committee of Ningbo Hangzhou Bay Hospital. C57BL6J male mice (WT) and $RNF20^{-/-}$ mice were received ordinary feed and had free access to food and water for 1 week. All

mice were injected with 50 mg/kg pentobarbital sodium and fixed on the operating table. Mice was induced as literature (Maruyama et al. 2021) and the intraoperative electrocardiogram displayed the ST segment for the MI model.

The heart tissues were fixed with four percent paraformaldehyde for 24 hours. 5-ìm-thick sections were stained with hematoxylin-eosin (H&E) and were obtained using fluorescence microscopy (Nikon Eclipse TE2000-U, Japan).

Cell Culture and Transfection

H9c2 cell lines were obtained from Shanghai Cell Bank, and cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with ten percent foetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA) in five percent CO_2 and ninety-five percent air. H9c2 cell were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 48 hour of transfection, cell was induced with 250 μM hydrogen peroxide (H_2O_2) and 100 ng of LPS for 24 hours.

Quantitative Analysis of mRNAs and Gene Microarray Hybridization

Total RNA was extracted from the cells or tissues using the TRIzol reagent (Life Technologies). Quantitative RT-PCR was carried out using an ABI Prism 7900 Sequence detection system (Applied Biosystems, Canada) as literature (Maruyama et al. 2021). Primers for qPCR: RNF20: Forward, GGGTGTCTCTTCAACGGAGG; Reverse, CATCATCGGTGGCCTGTCTT. The relative expression levels of mRNAs were calculated by the $2^{-\Delta\Delta Ct}$ method.

Total RNA was labelled using cyanine-5-cTP and hybridised to the SurePrint and G3 Mouse Whole Genome GE 8x60 K Microarray G4852A platform. Images were quantified and feature extracted using Agilent Feature Extraction Software (A.10.7.3.1; Agilent Technologies, Inc.).

ELISA

IL-1β (H002), CK-MB (H197-1-1), CK (A032-1-1), IL-6 (H007-1-1), and TNF-α (H052-1) kits were used to measure correlation factors by ELISA kits (Nanjing Jiancheng Bioengineering Research Institute). INF-γ (PI511 and PI508) kits were used to measure correlation factors by ELISA kits (Beyotime).

Immunofluorescence and Western Blotting

Cells were fixed with four percent paraformaldehyde, and Immunofluorescence were executed as literature (Maruyama et al. 2021). Cells were treated with primary antibodies RNF20 (ab181032, 1:200, abcam), Vitamin D Receptor (ab109234, 1:200, abcam) at 4°C overnight. Cells were observed under a fluorescent illumination microscope (Olympus IX71, Tokyo, Japan).

Western Blotting were executed as literature (Pu et al. 2022). The membranes were blocked in five percent skim milk for 1 hour and incubated with primary antibodies against: RNF20 (ab181032, 1:1000, abcam), Vitamin D Receptor (Vitamin D R, ab109234, 1:1000, abcam), NLRP3 (ab263899, 1:1000, abcam) and β-Actin (ab8226, 1:5000, abcam) at 4°C overnight.

Co-Immunoprecipitation

Cell samples were lysed using lysed, samples were mixed with 4× SDS–PAGE sample buffer for 5 minutes after equal protein concentration. Then, samples were examined by Western blotting. Co-IP assays were performed as described previously (Zhang et al. 2021).

Statistical Analysis

Data are presented as means ± standard deviations using SPSS 18.0 (SPSS, Chicago, IL, USA) and Student's t-test or one-way analysis of variance (ANOVA) as appropriate. $p < 0.05$ was considered to represent statistical significance.

RESULTS

RNF20 Expression in the Model of MI

Firstly, in this experiment, the serum expression of RNF20 mRNA level in patients with MI (0.184±0.166) was down-regulated, as compared with normal healthy volunteers (0.724±0.398) ($P<0.01$, Fig. 1A). Additionally, the serum mRNA of RNF20 had a negative correlation with serum

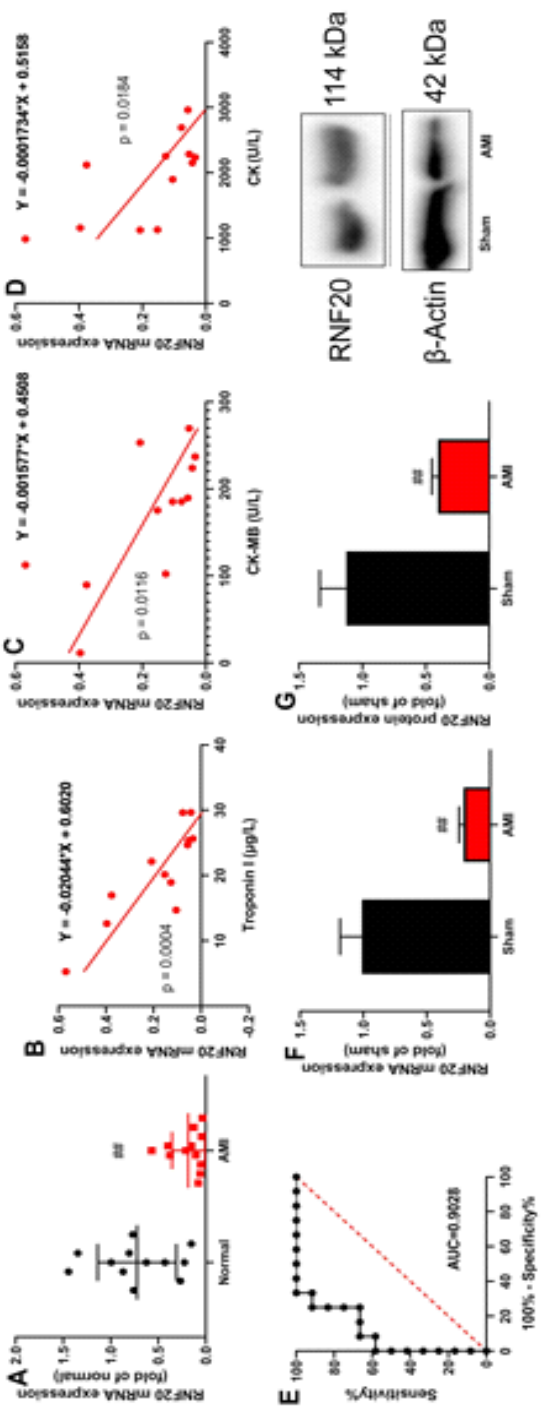

Fig. 1. RNF20 expression in the model of MI
(A) RNF20 mRNA expression, (B) the serum mRNA of Jmjd3 was negative correlation with serum troponin I, (C) CK-MB or (D) CK levels, (E) ROC in patients with MI; (F) RNF20 mRNA expression and (G) protein expression in the mice with MI.
##p<0.01 compared with normal volunteer or sham group

troponin I (20.483±6.948), CK-MB (169.535±73.358) or CK (1917.146±635.299) levels in patients with MI and the receiver operating characteristic (ROC) curve (AUC=0.9028) was constructed to assess diagnostic value of RNF20 level (all P<0.01, Fig. 1B-1E). Among the mice with MI, RNF20 protein (1.130± 0.208 versus 0.406±0.047) and mRNA expression (1.014±0.172 versus 0.210±0.036) in lung tissue was reduced (all P<0.01, Fig.1F-1G). These results showed that RNF20 might be participating in the disease progression of MI.

RNF20 Gene Reduced Inflammation *In Vitro* Model of MI

The researchers investigated the anti-inflammation effects of RNF20 *in vitro* model of MI. The RNF20 mRNA expression was increased in RNF20 over-expression group (1.071±0.374 versus 6.038±1.068) and RNF20 mRNA expression (1.024±0.211 versus 0.689±0.235 versus 0.213±0.079 versus 0.135±0.021) was down-regulated in si-RNF20 group (P<0.01, Fig. 2A). RNF20 decreased IL-1β (470±85.81 versus 155±52.08), IL-6 (158±5.14 versus 36.67±28.39), INF-γ (625±128.32 versus 205 ±115.76) and TNF-α (56.66±9.39 versus 20.33±3.30) levels *in vitro* model of MI (Figs. 2B-2E). Si-RNF20 promoted IL-1β (292.5±74.25 versus 1315±269.47), IL-6 (68.67±14.70 versus 152.33±32.17), INF-γ (471.67 ±187.85 versus 1083.33±288.02) and TNF-α (47.67± 6.94 versus 136.667±15.15) levels *in vitro* model of MI (all P<0.01, Figs. 2F-2I).

Knock Out of RNF20 Causes Inflammation and MI in the Mice Model

The researchers next further examined the function of RNF20 in the mice model of myocardial infarction. Knock out of RNF20 ($RNF20^{-/-}$) promoted CK level (1480±368.51 versus 4060±398.25) and LDH activity level (8900±803.12 versus 13916. 66667±758.65), increased myocardial infarction, reduced the left ventricular ejection fraction (55.43± 3.49 versus 29.02±1.37), the left ventricular fractional shortening (39.61±1.33 versus 11.02±1.06) and the left ventricular stroke volume (51.32±2.51 versus 21.77±1.54), enhanced the left ventricular internal diameter (5.02±0.10 versus 9.01±0.51), and heightened IL-1β (377.5±36.91 versus 1242.5±60.42), IL-6 (134.67±14.73 versus 407.3333333±49.38), INF-γ (52.67±5.56 versus 180±4.32) and TNF-α (491.67± 131.99 versus 1178.333333±190.15) levels, compared with WT group (all P<0.01, Fig. 3). So, the researchers thought RNF20 reduced inflammation to present myocardial infarction in the model.

RNF20 Suppressed NLRP3 Inflammasome by Vitamin D Receptor

To understand the mechanism of RNF20 in the model of MI, microarray analysis was performed to screen the gene expression (Fig. 4A). $RNF20^{-/-}$ suppressed Vitamin D Receptor (Vitamin D R) protein (1.139±0.136 versus 0.228±0.093) expression and induced NLRP3 protein expression level (1.131±0.26 versus 12.678±0.329) in the heart tissue (all P<0.01, Fig. 4B).

RNF20 Interacted with Vitamin D Receptor to Reduce Degradation of Vitamin D Receptor

In vitro model, over-expression of RNF20 induced Vitamin D R mRNA expression (1.098±0.427 versus 1.198±0.755 versus 4.422±1.856 versus 14.804±2.047) and down-regulation of RNF20 reduced vitamin D R mRNA (1.086±0.390 versus 0.718± 0.307 versus 0.450±0.089 versus 0.350±0.095) (all P <0.01, Figs. 5A-5B). Immunofluorescence showed that over-expression of RNF20 induced RNF20 and Vitamin D R mRNA expression *in vitro* model (P <0.01, Fig. 5C). Over-expression of RNF20 reduced Vitamin D R ubiquitination and down-regulation of RNF20 increased Vitamin D R ubiquitination *in vitro* model (P<0.01, Fig. 5D).

Vitamin D Receptor Interacted NLRP3 Protein

Next, the researchers confirmed the association between NLRP3 and Vitamin D R proteins by Co-IP (Fig. 6A). Over-expression of RNF20 induced RNF20 (1.173±0.133 versus 2.887±0.303) and Vitamin D R protein expressions (1.076±0.085 versus 2.512±0.442), and suppressed NLRP3 protein expression (1.037±0.057 versus 0.337±0.068) in vitro model of myocardial infarction (all P<0.01, Fig. 6B-6D). Down-regulation of RNF20 reduced RNF20 (1.200±0.152 versus 0.350±0.019) and Vitamin D R protein expressions (1.170±0.230 versus 0.350±0.045), and induced NLRP3 protein expression (1.153±0.108 versus 2.724±0.364) in vitro model of myocardial infarction (all P<0.01, Fig. 6E-6G). These results indicated that the inhibition of RNF20 causes inflammation in the

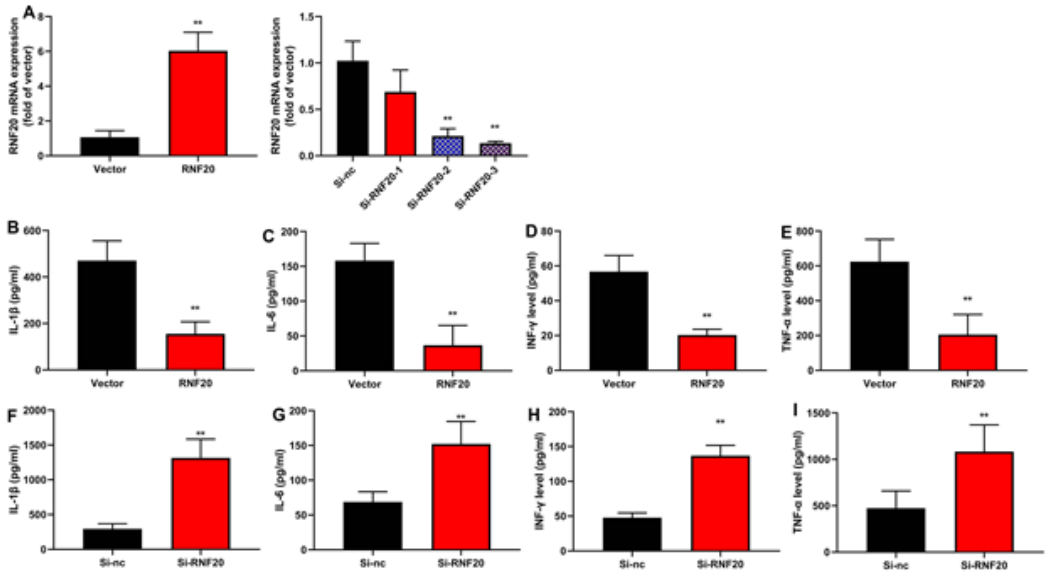

Fig. 2. RNF20 gene reduced inflammation *in vitro* model of MI
(A) RNF20 mRNA expression, (B) IL-1β, (C) IL-6, (D) INF-γ and (E) TNF-α levels by RNF20; (F) IL-1β, (G) IL-6, (H) INF-γ and (I) TNF-α levels by si-RNF20
****p<0.01 compared with negative group**

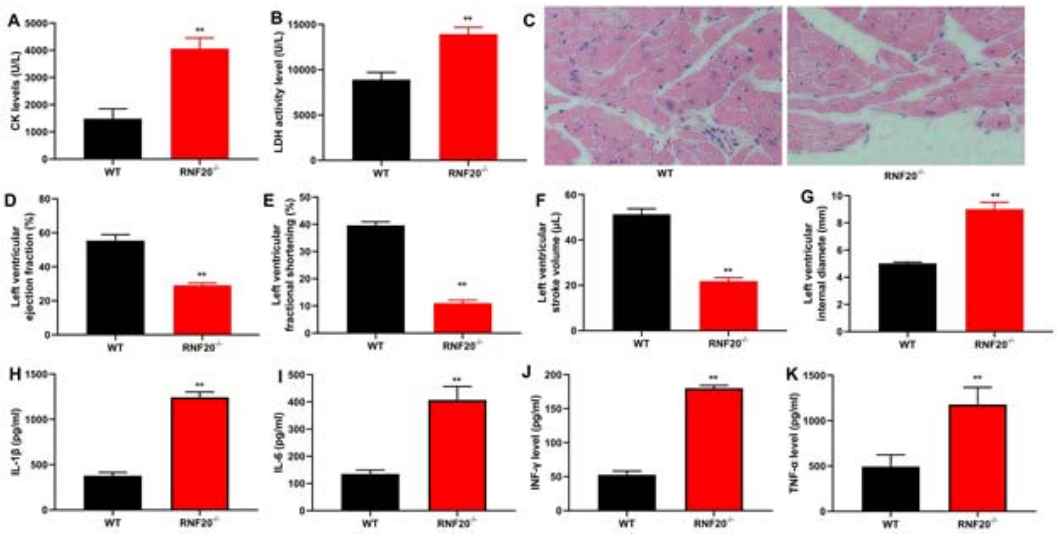

Fig 3. Knock out of RNF20 causes inflammation and MI in the mice model
(A) CK activity level, (B) LDH activity level, (C) myocardial infarction, (D) the left ventricular ejection fraction, (E) the left ventricular fractional shortening, (F) the left ventricular stroke volume, (G) the left ventricular internal diameter, (H) IL-1β, (I) IL-6, (J) INF-γ and (K) TNF-α levels
****p<0.01 compared with WT group**

model of myocardial infarction through the suppression of NLRP3 activation by the induction of degradation of Vitamin D Receptor.

Inhibition of Vitamin D Receptor Reversed the Effects of RNF20 in Vitro Model of Myocardial Infarction

To understand the mechanism of RNF20 in vitro model of myocardial infarction, over-expression of Vitamin D R induced Vitamin D R protein expression (1.124±0.097 versus 0.289±0.059 versus 0.734±0.072), and suppressed NLRP3 protein expression (0.986±0.110 versus 2.469±0.117 versus 1.615±0.144) in vitro model of myocardial infarction by si-RNF20, compared with si-RNF20 group (all $P<0.01$, Figs. 7A-7B). Down-regulation of Vitamin D R suppressed Vitamin D R protein expression (1.286±0.213 versus 2.901±0.214 versus 1.429±0.153), and induced NLRP3 protein expression (1.099±0.157 versus 0.266±0.123 versus 0.802±0.128) in vitro model of myocardial infarction by over-expression of RNF20, compared with over-expression of RNF20 group (all $P<0.01$, Figs. 7C-7D). Over-expression of Vitamin D R reduced IL-1β (410±53.50 versus 1280±61.95 versus 772.5±76.73), IL-6 (142.667±35.11 versus 376.67± 6.80 versus 207.33±32.43), INF-γ (58.33±6.18 versus 192.67±28.17 versus 106.67±17.52) and TNF-α (548.33±127.63 versus 1275±278.57 versus 605± 240.56) levels in vitro model of myocardial infarction by si-RNF20, compared with si-RNF20 group (all $P<0.01$, Figs. 7E-7H). Down-regulation of Vitamin D R increased IL-1β (610±70.98 versus 200± 24.75 versus 307.5±24.49), IL-6 (197.33±31.26 versus 47.33±13.60 versus 110.67±16.36), INF-γ (75.67± 15.92 versus 22±2.94 versus 54.67±7.41) and TNF-α (686.67±185.53 versus 155±72.57 versus 395± 72.57) levels in vitro model of myocardial infarction by over-expression of RNF20, compared with over-expression of RNF20 group (all $P<0.01$, Figs. 7I-7L).

DISCUSSION

With the accelerating aging of the population in China, the incidence rate of myocardial infarction in elderly patients is also gradually increasing, and the heart function of the elderly is worse than that of the young. Under the same degree of coronary embolism, the situation of elderly patients is even worse. Ventricular remodelling after myocardial infarction is an important reason for the development of heart failure (Jacobsen et al. 2021). To effectively prevent the occurrence and development of ventricular remodelling has become a research hotspot in the prevention and treatment of the heart failure in recent years (Montalto et al. 2021). At present, although the treatment methods such as revascularisation and antagonising the abnormal activation of neuroendocrine have achieved good results, the 5-year mortality of patients with the heart failure is still close to fifty percent (VanDusen et al. 2021) .

The researchers report for the first time that the serum expression of RNF20 mRNA level in patients with MI (0.184±0.166) was down-regulated. RNF20 protein (1.130±0.208 versus 0.406± 0.047) and mRNA expression (1.014±0.172 versus 0.210±0.036) in lung tissue was reduced in the mice with MI.

VanDusen et al. showed that RNF20/40 is epigenetic regulators of cardiomyocyte maturation (VanDusen et al. 2021), suggesting that RNF20 might be a potential anti-inflammatory factor for MI.

Inflammatory response plays an important role in this process (Wang et al. 2018). Appropriate inflammatory reaction can reduce the infarct size, promote the formation of scar in the infarct area, maintain the stability of the environment around the infarct area, and contribute to the recovery of ischemic myocardium (Takahashi 2019b). Excessive inflammatory reaction causes cardiomyocyte apoptosis, hypertrophy and fibrosis of myocardial tissue in non-infarct area, resulting in pathological remodelling of ischemia related tissues and myocardial dysfunction (Toldo and Abbate 2018).

Notably, the researchers found that knock out of RNF20 ($RNF20^{-/-}$) promoted CK level (1480±368.51 versus 4060±398.25) and LDH activity level (8900±803.12 versus 13916.66667± 758.65), increased MI, reduced the left ventricular ejection fraction (55.43±3.49 versus 29.02±1.37), the left ventricular fractional shortening (39.61±1.33 versus 11.02±1.06) and the left ventricular stroke volume (51.32±2.51 versus 21.77±1.54), enhanced the left ventricular internal diameter (5.02±0.10 versus 9.01±0.51), and heightened IL-1β (377.5±36.91 versus 1242.5±60.42), IL-6 (134.67±14.73 versus 407.3333333±49.38), INF-γ (52.67±5.56 versus

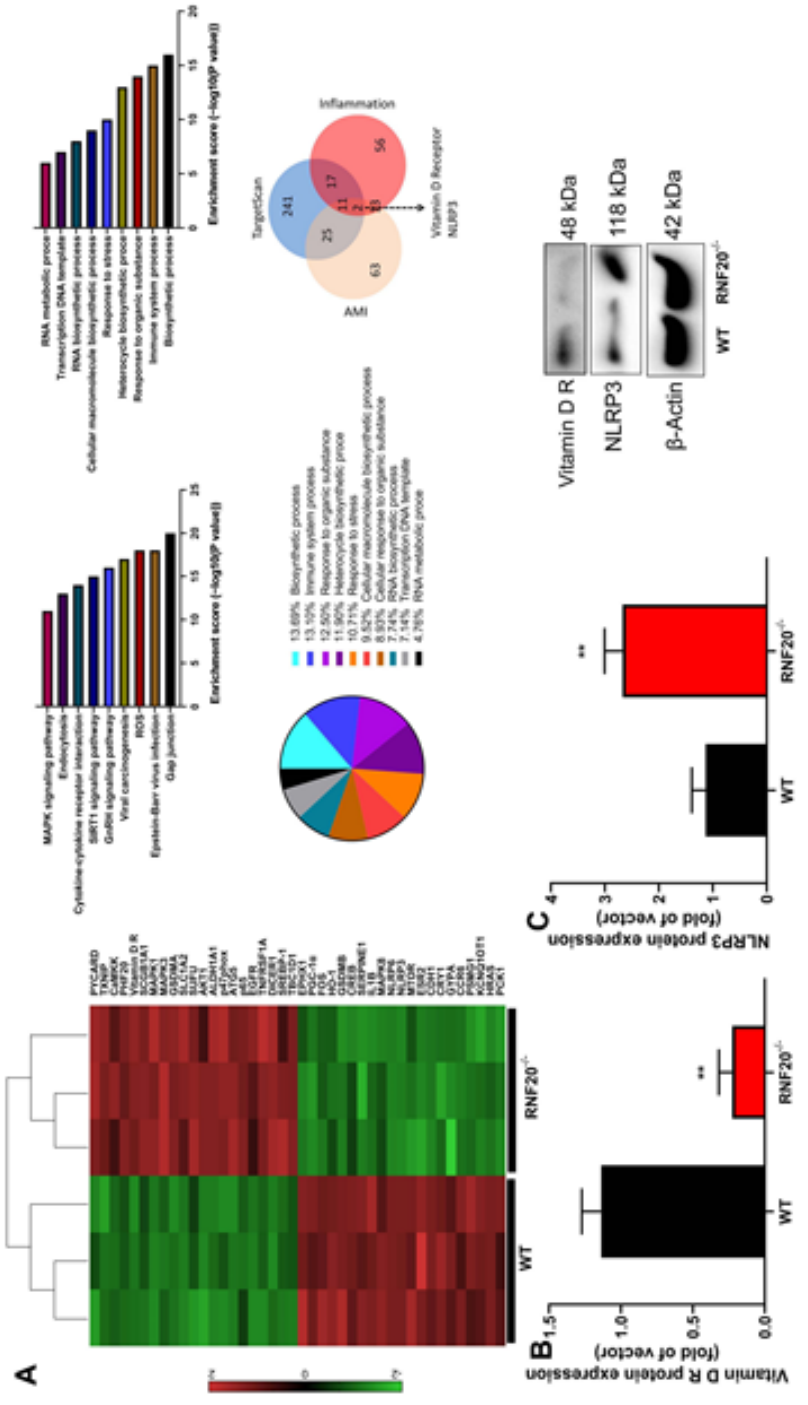

Fig. 4. RNF20 suppressed NLRP3 inflammasome by Vitamin D Receptor
(A) heat map and analysis results, (B, C)Vitamin D Receptor protein expression.
****p<0.01 compared with WT group**

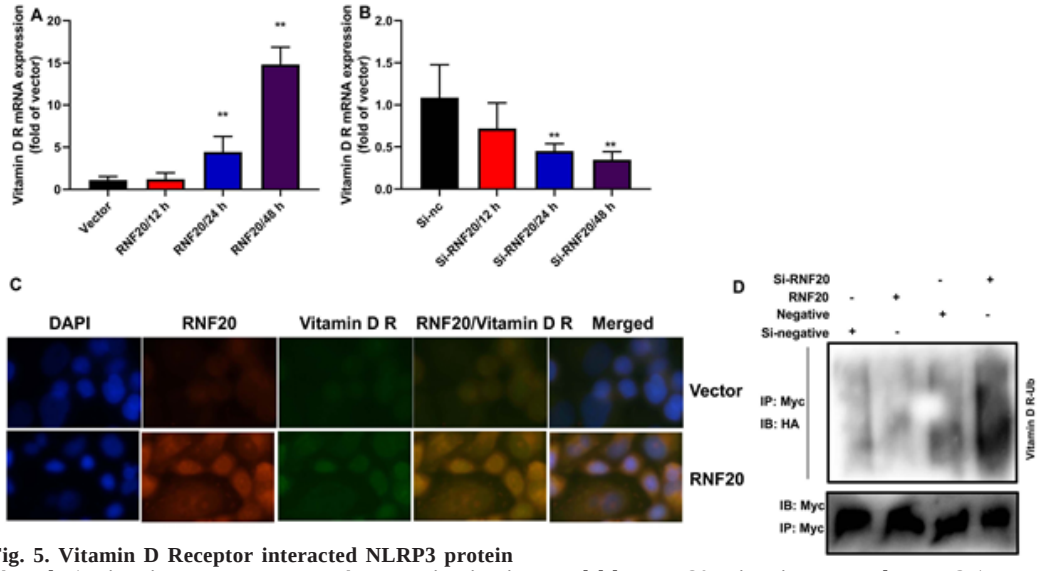

Fig. 5. Vitamin D Receptor interacted NLRP3 protein
(A and B) Vitamin D Receptor mRNA expression in vitro model by RNF20, Vitamin D R and NLRP3 (Immunofluorescence, C), (D)Vitamin D Ubiquitination
****p<0.01 compared with negative group**

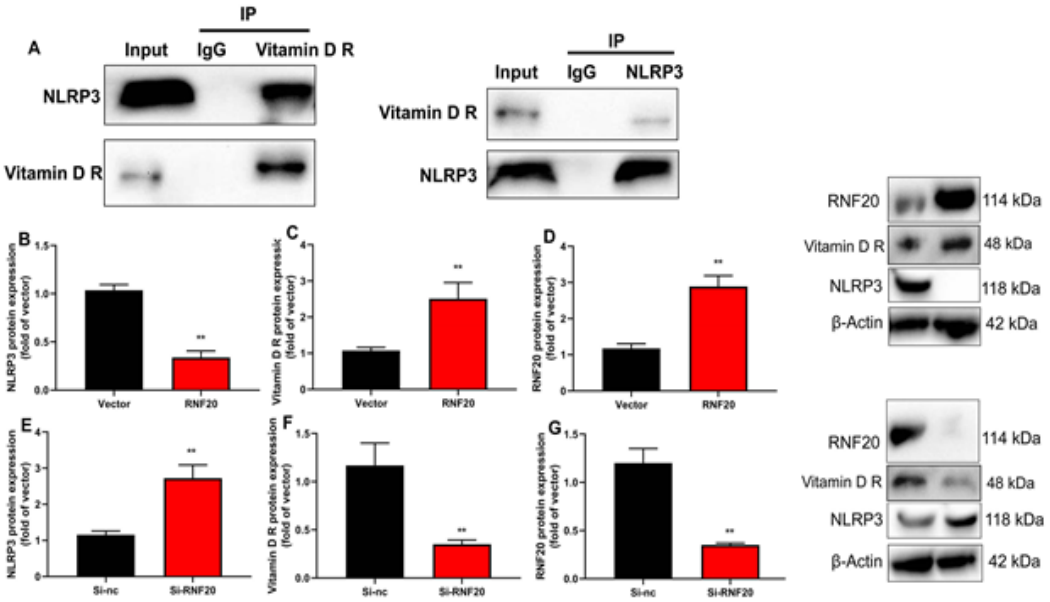

Fig. 6. Vitamin D Receptor interacted NLRP3 protein
(A) NLRP3 and Vitamin D R proteins by Co-IP (A) , (B, C and D) RNF20, Vitamin D R and NLRP3 protein expressions in vitro model of MI by RNF20, (E, F and G)
****p<0.01 compared with negative group**

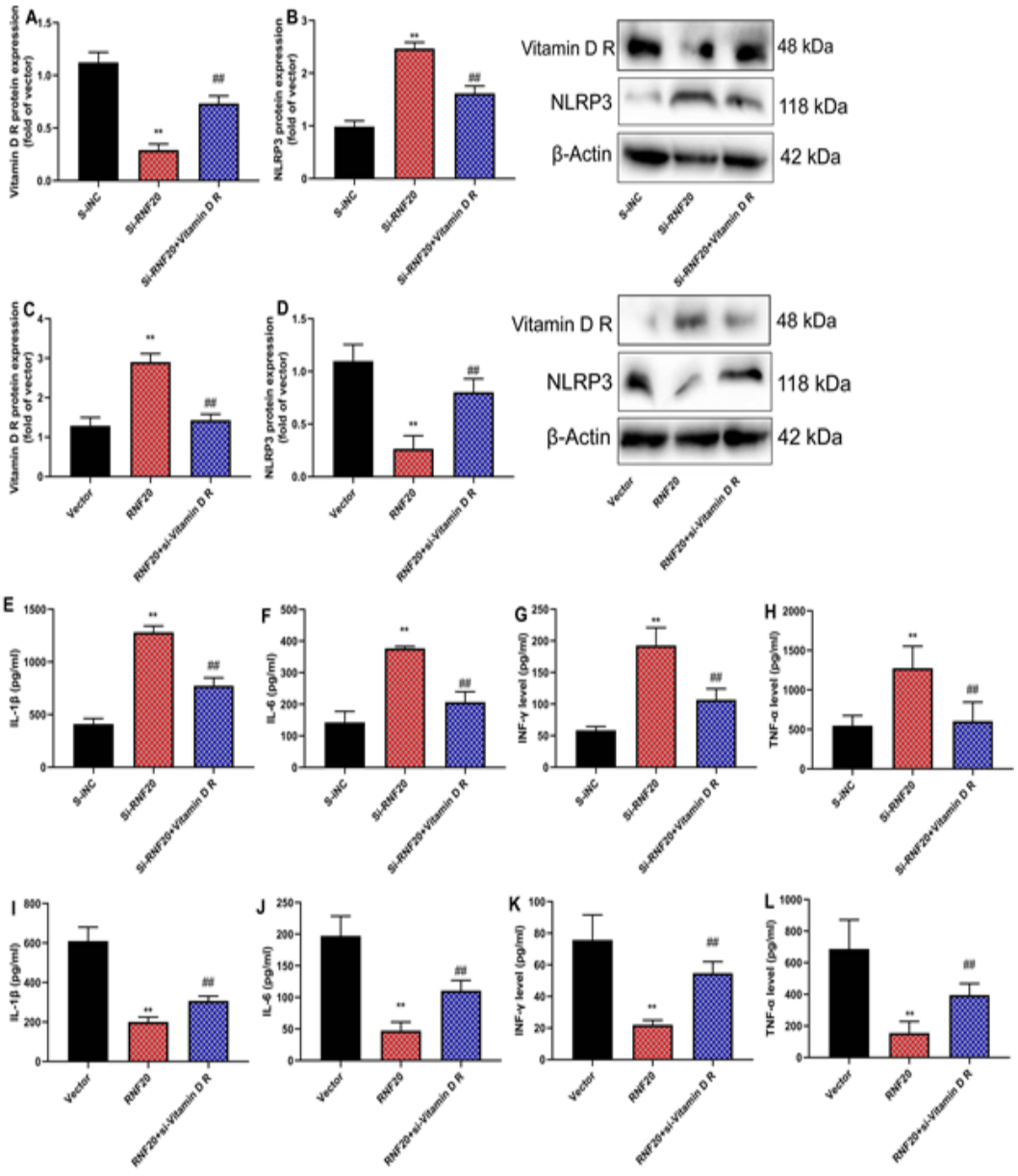

Fig. 7. The inhibition of Vitamin D Receptor reversed the effects of RNF20 in vitro model of MI
(A and B) Vitamin D R and NLRP3 protein expression in vitro model of RNF20 and down-regulation of Vitamin D R, (C and D) Vitamin D R and NLRP3 protein expression in vitro model of si-RNF20 and Vitamin D R, (E) IL-1β, (F) IL-6, (G) INF-γ and (H) TNF-α levels in vitro model of RNF20 and si-Vitamin D R, (I) IL-1β, (J) IL-6, (K) INF-γ and (L) TNF-α levels in vitro model of si-RNF20 and Vitamin D R.
**p<0.01 compared with negative group, $^{\#\#}$p<0.01 compared with RNF20 group or si-RNF20 group

180±4.32) and TNF-α (491.67±131.99 versus 1178.333333±190.15) levels in the mice model of MI. Over-expression of RNF20 decreased IL-1β (470± 85.81 versus 155±52.08), IL-6 (158±5.14 versus 36.67± 28.39), INF-γ (625±128.32 versus 205±115.76) and TNF-α (56.66±9.39 versus 20.33±3.30) levels *in vitro* model of MI. Down-regulation of RNF20 promoted IL-1β (292.5±74.25 versus 1315±269.47), IL-6 (68.67±14.70 versus 152.33±32.17), INF-γ (471.67± 187.85 versus 1083.33±288.02) and TNF-α (47.67± 6.94 versus 136.667±15.15) levels *in vitro* model of MI.

Tarcic et al. reported that RNF20 modulates inflammation and inflammation-associated cancer in the mice (Tarcic et al. 2016). These data suggested that RNF20 mitigates inflammasome-related inflammation of MI.

Studies have shown that MI induces aseptic inflammatory response, ATP dependent K + outflow of damaged myocardial fibroblasts and ROS produced during reperfusion further activate NLRP3 in fibroblasts (Mauro et al. 2019; Takahashi 2019a; Silvis et al. 2021). By stimulating IL-1β release and collect bone marrow-derived macrophages and neutrophils, leukocytes of bone marrow origin can recognise damps and activate intracellular NLRP3 to amplify the inflammatory response (Takahashi 2019a).

This study suggested that over-expression of RNF20 reduced Vitamin D R ubiquitination and down-regulation of RNF20 increased Vitamin D R ubiquitination *in vitro* model. The researchers confirmed the association between NLRP3 and Vitamin D R proteins by Co-IP. Over-expression of RNF20 induced RNF20 (1.173± 0.133 versus 2.887±0.303) and Vitamin D R protein expressions (1.076±0.085 versus 2.512± 0.442), and suppressed NLRP3 protein expression (1.037± 0.057 versus 0.337±0.068) in vitro model of myocardial infarction. Down-regulation of RNF20 reduced RNF20 (1.200±0.152 versus 0.350± 0.019) and Vitamin D R protein expressions (1.170±0.230 versus 0.350±0.045), and induced NLRP3 protein expression (1.153±0.108 versus 2.724± 0.364) in vitro model of MI.

Kosinsky et al. reveal that RNF20 regulate vitamin D receptor-dependent signalling in inflammatory bowel disease (Kosinsky et al. 2021). Collectively, the researchers demonstrate that RNF20 induced Vitamin D Receptor to suppress NLRP3 inflammasome of myocardial infarction, suggesting that RNF20 treatment is a possible strategy for the treatment of NLRP3-related inflammatory diseases in MI.

CONCLUSION

In conclusion, these studies demonstrate that the inhibition of RNF20 causes inflammation in the model of myocardial infarction through the suppression of NLRP3 activation by the induction of degradation of Vitamin D Receptor. In summary, the researchers reported that RNF20 might be a potential treatment target for myocardial infarction-related diseases. Based on this, RNF20 might provide one potential therapeutic utility of targeting these for the benefit of myocardial infarction.

RECOMMENDATIONS

In order to prevent and treat MI -related diseases, more research of the function of ring finger protein 20 (RNF20) in the model of myocardial infarction (MI) should be actively recommended to prevent and treat complications.

ABBREVIATIONS LISTS

RNF20: ring finger protein 20
MI: myocardial infarction

ACKNOWLEDGEMENTS

Not applicable.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AVAILABILITY OF DATA AND MATERIALS

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

AUTHORS' CONTRIBUTIONS

All authors read and approved the final manuscript. NKJ, YJL and LH are the guarantor

of integrity of the entire study, contributed to the study concepts, manuscript preparation and manuscript editing and review. NKJ and JNH contributed to the data acquisition, the data analysis and statistical analysis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethics committee of the Ningbo Hangzhou Bay Hospital.

PATIENT CONSENT FOR PUBLICATION

All patients were informed and signed the informed consent voluntarily.

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