Prmt1 upregulated by Hdc deficiency aggravates acute myocardial infarction via NETosis

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Abstract Neutrophils are mobilized and recruited to the injured heart after myocardial infarction, and neutrophil count has been clinically implicated to be associated with coronary disease severity. Histidine decarboxylase (HDC) has been implicated in regulating reactive oxidative species (ROS) and the differentiation of myeloid cells. However, the effect of HDC on neutrophils after myocardial infarction remains unclear. Here, we found that neutrophils were disorderly recruited into the ischemic injured area of the myocardium of Hdc deficiency (Hdc\textsuperscript{−/−}) mice. Moreover, Hdc deficiency led to attenuated adhesion but enhanced migration and augmented ROS/neutrophil extracellular traps (NETs) production in neutrophils. Hdc\textsuperscript{−/−} mouse-derived NETs promoted cardiomyocyte death and cardiac fibroblast proliferation/migration. Furthermore, protein arginine methyltransferase 1 (PRMT1) was increased in Hdc\textsuperscript{−/−} mouse-derived neutrophils but decreased with exogenous histamine treatment. Its expression could be rescued by blocking histamine receptor 1 (H1R), inhibiting ATP synthesis or reducing SWItch/sucrose non fermentable (SWI/SNF) chromatin remodeling complex. Accordingly, histamine or MS023 treatment could decrease ROS and NETs \textit{ex vivo}, and ameliorated cardiac function and fibrosis, along with the reduced NETs in plasma \textit{in vivo}. Together, our findings unveil the role of HDC in NETosis by histamine–H1R–ATP–SWI/SNF–PRMT1–ROS signaling and provide new biomarkers and targets for identifying and tuning the detrimental immune state in cardiovascular disease.
1. Introduction

Myocardial infarction (MI) is one of the leading causes of death worldwide. After infarction, three sequential processes, inflammatory response, cardiomyocyte necrosis, and scar formation, determine the final ejection fraction and the survival rate of patients with MI. The inflammatory response, regulated by recruited immune cells, including neutrophils, monocytes/macrophages, and lymphocytes, profoundly influences heart remodeling.

Neutrophils, as sentinels of inflammation, first infiltrate the infarcted zone in great numbers after MI. Although neutrophils have a relatively short lifespan, they secrete inflammatory mediators, purge debris of apoptotic cardiomyocytes, and activate fibroblasts. A decade ago, neutrophils were found to form a net-like structure, containing double-stranded DNA, citrullination of histones and neutrophil granule protein, called neutrophil extracellular traps (NETs). Originally, NETs were regarded as the protection of our bodies from fungal and bacterial diseases. Recently, excessive NETs have been reported to be involved in the progression of other diseases, including atherosclerosis, lupus erythematosus, and coronavirus disease 2019 (COVID-19). NETs were also found in patients with MI/acute coronary syndromes, and the level of NETs in plasma was related to infarct size.

Histidine decarboxylase (HDC) catalyzes L-histidine to histamine and is involved in allergic responses, gastric acid secretion, immune modulation, and the development of hematopoietic stem cells. In the bone marrow of mice, over 50% of CD11b-Ly6G granulocytic cells highly express HDC, and HDC-expressing granulocytes derived histamine plays important roles in the differentiation of immune cells. Moreover, it has been reported that serum histamine concentrations significantly increased after MI, indicating the potential roles of HDC/histamine in MI. However, the role of HDC/histamine in the regulation of neutrophils after MI has not been fully clarified.

In this study, we report that HDC plays a key role in regulating the functions of neutrophils. Recruitment, oxidative burst, and NETosis were significantly altered in Hdc−/− mouse-derived neutrophils, which augmented the death of cardiomyocytes and the activation of fibroblasts. Blocking neutrophils to a certain extent could aid reduction of myocardial injury in Hdc−/− mice. Furthermore, by using transcriptomics and proteomics, we found that aggravated cardiac injury in Hdc−/− mice was due to excessive NETs through HDC–protein arginine methyltransferase 1 (PRMT1)–reactive oxygen species (ROS) pathway. The administration of exogenous histamine or the inhibitor of PRMT1 could ameliorate cardiac injury in Hdc−/− mice.

2. Methods and materials

2.1. Animals and MI model

Hdc knockout (Hdc−/−, BALB/c background) mice were generously provided by Professor Timothy C. Wang from Columbia University (New York, USA). The generation of Hdc−/− mice has been described in previous papers. The adopted Hdc−/− mice in the present work is the offspring of Hdc−/− homozygous parents, generating not enough littermates as controls. Thus male wild-type (WT, in BALB/c background) mice at 8 to 12-week-old purchased from CAVENS. LA (Changzhou, China) were used as controls, which were shown the same phenotypes as WT littermates of Hdc−/− mice, as previously described. All procedures in this study were approved by the Institutional Review and Ethics Board of Zhongshan Hospital of Fudan University (Shanghai, China). The myocardial infarction model was performed as described before. Briefly, male mice were anaesthetized with 3% isoflurane inhalation in a chamber. After exposing the hearts by left thoracotomy, a small incision was made at the fourth intercostal space to pop out the heart. The left main descending coronary artery (LCA) was permanently ligated at a site 3 mm from its origin. After the heart was placed back into the intrathoracic space, fast air evacuation and chest wall closure were performed. Histamine (Sigma, St. Louis, MO, USA) was injected with the dose of 4 mg/kg/day intraperitoneally (i.p.) beginning from 3 days before surgery and continuing until euthanasia.

2.2. Histopathological analysis

Hearts were embedded in optimal cutting temperature compound (O.C.T.; Sakura® Finetek Japan Co., Ltd., Tokyo, Japan) or in paraform (Leica Biosystems, Wetzler, Germany). 5 μm thick paraform sections were used for stained with haematoxylin and eosin (H&E) and Masson’s trichrome staining according to standard procedures.

Immunofluorescent staining was performed to detect neutrophil infiltration (neutrophil elastase and α-actinin, Abcam, Cambridge, UK) and NETs formation [anti-histone H3 citrulline R2/R8+R17, and myeloperoxidase (MPO), Abcam]. Co-staining was visualized by fluorescence microscope with 488- and 596-conjugated secondary antibodies (Abcam) mounted with fluoroshield mounting medium with 4′,6-diamidino-2-phenylindole (DAPI, Abcam).

2.3. Enzyme-linked immunosorbent assay (ELISA) assay

CK-MB (Jiancheng Bioengineering Institute, Nanjing, China) and MPO (R&D Systems, Minneapolis, MN, USA) were measured with the respective ELISA kits, according to the manufacturer’s instructions. Briefly, plasma samples were collected from mice in each group at the indicated time after MI, and centrifuged at 3000 × g for 10 min, after which the supernatant was collected. The plasma of 100–200 μL was added into the 96-well plates for the measurement of concentrations based on the absorption values.
2.4. Neutrophil isolation

Mice neutrophils were harvested and purified as previously described. In brief, bone marrow cells were gathered, added into Percoll gradient consisting of 52%, 65%, 78% Percoll layers (GE, Boston, MA, USA), and centrifuged at 2500 × g for 30 min at room temperature. The cells between 65% and 78% layers were harvested, and red blood cells were lysed with red blood cells lysis buffer (BD, New York, NY, USA).

2.5. Adult mouse cardiomyocyte and fibroblasts isolation

The isolation of adult mouse cardiomyocytes was performed as described previously. In brief, hearts of mice were incised, after injected with EDTA buffer from apical region of left ventricle. Then left ventricle was digested and disassociated to isolate cardiomyocytes and cardiac fibroblasts through gravity sedimentation. For hypoxia, the cardiomyocytes were placed in an anaerobic incubator filled with gas consisting of 1% O₂, 5% CO₂, and 94% N₂, at 37 °C for 24 h.

2.6. Adhesion assay

Isolated neutrophils were labeled with Calcein-AM (Thermo Fisher Scientific, Waltham, MA, USA) and plated on human umbilical vein endothelial cells (HUVEC) with the presence of 10 ng/mL tumor necrosis factor-α (TNF-α) as previously described. Then wells were washed with PBS. The number of adhering neutrophils was counted, and the percentage was calculated.

2.7. Transwell migration assay

Neutrophil Transwell migration assay was performed as described previously. Briefly, bone marrow derived neutrophils from each genotype were labeled with Calcein-AM, and seeded on human umbilical vein endothelial cells (HUVEC) at 5 × 10⁵ cells per well. For hypoxia, the cells were incubated in an anaerobic incubator filled with gas consisting of 1% O₂, 5% CO₂, and 94% N₂, at 37 °C for 24 h.

2.8. Quantification of ex vivo NETs formation and ROS

The 5 × 10⁵ cells per well were seeded onto coverslips coated with poly-L-lysine (Beyotime, Nantong, China), and treated with phorbol myristate acetate (PMA, 200 nmol/L, MedChemExpress) for 18 h. Neutrophils were stained with DAPI/Sytox green (Thermo Fisher Scientific). Percentage values of NETs were calculated as the area of Sytox green positive cells relative to DAPI positive cells. The parameter was set to filter the Sytox green positive cells with small area to ignore the dead points of neutrophil for more accuracy.

To measure the level of ROS, isolated neutrophils were treated with DCFH-DA (MCE) for 30 min. Then cells were reconstituted with serum-free RPMI 1640 medium and transferred to black at 96-fold intervals, with or without PMA (200 nmol/L) treatment. The plate was measured at an excitation wavelength of 488 nm and an emission length of 530 nm using an automated plate monochrome reader (Molecular Devices, Silicon Valley, CA, USA).

2.9. TdT-mediated dUTP nick-end labeling (TUNEL) assay

TUNEL assay using the One Step TUNEL Apoptosis Assay Kit (Beyotime) was performed in heart tissue sections or cultured cardiomyocytes following the manufacturer’s instructions. Briefly, cardiomyocytes were permeabilized with 0.05% Triton X-100 before labeling the segmented DNA of the apoptotic cells using TUNEL detection buffers. Then the samples were co-stained with anti-α-actinin primary antibody (CST, Danvers, MA, USA) followed by the Alexa Fluor-conjugated secondary antibodies (CST). Each staining in different groups was visualized with identical light exposure parameters under a fluorescence microscope (Leica Biosystems).

2.10. 5-Ethynyl-2-deoxyuridine (EdU) incorporation assay

EdU incorporation assay was analyzed by using BeyoClick™ EdU Cell Proliferation Kit (Beyotime) according to the manufacturer’s instructions. In brief, isolated fibroblasts were incubated in DMEM (Thermo Fisher Scientific) with 10 μmol/L EdU for 2 h at 37 °C/5% CO₂. After the incubation, the cells were washed with PBS to remove the DMEM and the free EdU probe. Then fibroblasts were fixed in 4% paraformaldehyde at room temperature and stained with vimentin primary antibody (CST) followed by the Alexa Fluor-conjugated secondary antibodies (CST).

2.11. Cell migration assay/wound healing

Fibroblasts labeled with Calcein-AM were seeded in 24-well plates and cultured until cell monolayers formed. Monolayers were wounded by manual scraping with a 10 μL micropipette tip. The cells were then incubated with NETs from neutrophils of each genotype pretreated with PMA for 4 h. Wound repair was analyzed through measuring the injured area recovered from the wounding borders.

2.12. DNA accessibility (DNase I sensitivity) assay

DNA accessibility assay were carried out as described previously. In brief, DNA from HL60 cells treated with histamine for 12 h was digested by DNase I (Takara, Kyoto, Japan) and accessed by quantitative real-time PCR (qRT-PCR).

2.13. ELISA for MPO—DNA complexes

Capture ELISA was used to measure the MPO/NA complexes as described. Briefly, capture antibody for myeloperoxidase (R&D Systems) was diluted and added into an ELISA plate overnight. After blocking, diluted plasma samples were added to the plate overnight. The supernatant was discarded, and diluted PicoGreen (Thermo Fisher Scientific) with 1 × Tris-EDTA (TE) buffer was added to the plate. Fluorescence of the samples was measured using an automated plate monochrome reader (Molecular Devices).

2.14. Measurement of DNA concentrations

DNA concentrations were measured using the Quant-iT PicoGreen dsDNA assay kit (Thermo Fisher Scientific) following the manufacturer’s instructions. In brief, plasma was mixed with the PicoGreen dye, added to the microplate wells, and then incubated
in the dark for 10 min. The florescence of the samples was measured using an automated plate monochrome reader (Molecular Devices)\textsuperscript{15}.

### 2.15. Statistical analysis

Data are shown as mean ± standard error of mean (SEM) of at least three independent experiments for each cellular experimental group and at least five independent experiments for each animal group. The size and count of NETs or cells were accessed by Python using OpenCV (https://github.com/Winston-00/NET-analysis). We evaluated the data with Student’s t-test for two-group comparisons, and one or two way analysis of variance for multiple comparisons. The value of \( P < 0.05 \) was considered statistically significant.

### 3. Results

#### 3.1. Transcriptomic profiling of Hdc\textsuperscript{-/-} mice with deteriorative outcomes after MI

To elucidate the role of histamine deficiency in myocardial infarction, we compared the severity of myocardial infarction injury between Hdc\textsuperscript{-/-} and WT (Hdc\textsuperscript{+/+}) mice. As expected, the levels of CK-MB increased significantly on Day 1 after MI in both Hdc\textsuperscript{-/-} and Hdc\textsuperscript{+/+} mice (Fig. 1A). However, the level of CK-MB in the plasma of Hdc\textsuperscript{-/-} mice was much higher than that in Hdc\textsuperscript{+/+} mice (Fig. 1A), indicating that Hdc deficiency aggravated cardiomyocyte death. Furthermore, cardiac function on Day 7 after MI surgery was measured by echocardiography. The results demonstrated that the ejection fraction (EF) and fractional shortening (FS) (Fig. 1B and C, and Supporting Information Fig. S1A for the representative images) in Hdc\textsuperscript{-/-} mice were significantly lower than those in Hdc\textsuperscript{+/+} mice. These data indicated that Hdc deficiency was associated with poor prognosis after MI.

To investigate the detailed mechanisms underlying the deteriorated cardiac functions in Hdc\textsuperscript{-/-} mice after MI, RNA-sequencing was performed to dissect the transcriptomic profile of hearts from Hdc\textsuperscript{+/+} and Hdc\textsuperscript{-/-} mice on Days 0, 1 and 7 after MI (Fig. 1D). A total of 259 genes and 273 genes were identified as differentially expressed genes (DEGs, \( P < 0.05, \text{FC} > 1.5 \)) in the hearts of Hdc\textsuperscript{+/+} and Hdc\textsuperscript{-/-} mice on Days 1 and 7 after MI. Among them, 92 genes were consistently abnormally expressed both on Day 1 and on Day 7 after MI (Fig. 1E).

To identify the specific biological process (BP) and networks involved in the progression of MI, we performed gene ontology (GO) enrichment analysis with the Day 1 and Day 7 DEGs, respectively. The top 15 GO terms were enriched based on the DEGs and their changes are shown in the time course. In the GO analysis of BP, more than 50% of the top terms were related to the function of immune cells, including leukocyte chemotaxis, leukocyte migration, and regulation of the inflammatory response (Fig. 1F). The DEGs with leukocyte migration/chemotaxis and regulation of the inflammatory response were diminished at the 7th day of MI, while genes with T cell activation were augmented. In the GO analysis of cellular component (CC), DEGs were related to cell vesicles, including lysosomes, lytic vacuoles, and secretory granules (Fig. 1G). In the GO analysis of molecular function (MF), lists of genes were generated in the categories of amide binding, ATPase activity, coenzyme binding, and cytokine receptor binding (Fig. 1H). In general, the DEGs were mainly involved in immune cell function, including chemotaxis/migration, secretory granule production, and other inflammatory responses. These results suggested that immune processes likely play a vital role in the pathological progression of MI and subsequent heart remodeling.

#### 3.2. Infiltration of immune cells especially neutrophils was shifted in Hdc\textsuperscript{-/-} mice

The contribution of immunological phenomena to cardiac diseases is of particular clinical relevance\textsuperscript{24}. Great numbers of immunocytes successively infiltrate the infarcted zone after MI, influencing the subsequent process of myocardial healing. The results of H&E staining confirmed that there were many inflammatory cells infiltrating into the infarcted zone (Fig. 2A). Therefore, we estimated the pattern of the infiltrated immunocytes by employing the MCP counter algorithm. As shown in Fig. 2B, the infiltration of immunocytes, including neutrophils, monocytes, dendritic cells (DCs), B cells, and T cells, in the infarcted hearts, was changed after Hdc deletion. As Hdc is expressed in myeloid cells, especially in Ly6G\textsuperscript{+} granulocytes, we further focused on exploring the consequence of Hdc deficiency in neutrophils responding to ischemia injury. As flow cytometry data (Fig. 2C and Supporting Information Fig. S2A for the gating strategy) revealed, the number of infiltrated neutrophils in the infarcted zone peaked on Day 1 and greatly dropped on Days 3 and 7 after MI. However, there were still many more neutrophils in the cardiac infarcted zone of Hdc\textsuperscript{-/-} mice than in Hdc\textsuperscript{+/+} mice on Days 3 and 7 after MI. The immunostaining result was consistent with the flow cytometry data (Fig. 2D and E).

To figure out whether the increased neutrophils contributed to the aggravated cardiac injury of Hdc\textsuperscript{-/-} mice, the anti-Ly6G antibody was applied to neutralize neutrophils after myocardial infarction. The EF and FS in Hdc\textsuperscript{-/-} mice were improved by the administration of anti-Ly6G antibody but not IgG (Fig. 2F). Similarly, compared with IgG, anti-Ly6G antibody reduced the cardiac fibrosis of Hdc\textsuperscript{-/-} mice (Fig. 2G). These findings suggested that Hdc deficiency-aggravated myocardial injury after MI may be due to the effect of Hdc deficiency mainly on the regulation of neutrophils.

#### 3.3. Hdc deficiency dysregulates the characteristic cellular activities of neutrophils

In a previous study, Hdc deficiency was proposed to promote phagocyte infiltration in infectious inflammation\textsuperscript{24}. Nevertheless, little is known about its role in sterile inflammation. We first investigated the effect of Hdc deficiency on the adhesion and migration of neutrophils. Compared with Hdc\textsuperscript{+/+} neutrophils, Hdc\textsuperscript{-/-} neutrophils showed significantly decreased adhesion to HUVECs after stimulation with TNF-\alpha (Fig. 3A). However, Hdc\textsuperscript{-/-} neutrophils have a higher migration ability with the attraction of IL-8 through Transwells (Fig. 3B). These data indicated that Hdc deficiency led to disordered neutrophil priming in a sterile inflammatory environment.

The response of neutrophils to stimulation relies on the production of ROS and the formation of NETs under both infectious and sterile inflammatory conditions. Therefore, we investigated whether Hdc affected the generation of ROS in neutrophils by flow cytometry. Regardless of PMA treatment, Hdc\textsuperscript{-/-} neutrophils produced significantly higher levels of ROS than Hdc\textsuperscript{+/+} neutrophils did (Fig. 3C). Accumulating studies have reported
that excessive ROS promote the formation of NETs, which is a unique way of exacerbating peripheral cell death\(^\text{26}\). Therefore, we assessed the areas of NETs formation under PMA treatment. Bone marrow-derived neutrophils from \(\text{Hdc}^{-/-}\) mice, after treatment with PMA in vitro, were shown to have a larger area of Sytox green-positive cells that represented NETs (Fig. 3D and E).

Furthermore, although there was no significant difference in the number of neutrophils infiltrating into the infarcted zone on Day 1 after MI in \(\text{Hdc}^{+/+}\) and \(\text{Hdc}^{-/-}\) mice as shown in Fig. 2, the co-staining of neutrophil elastase and citrullinated histone H3 revealed that there were much more NETs in the cardiac infarcted zone of \(\text{Hdc}^{-/-}\) mice even on Day 1 after MI, compared with \(\text{Hdc}^{+/+}\) mice (Fig. 3F and G). These results indicated that \(\text{Hdc}\) played an essential role in regulating the characteristic cellular activities of neutrophils, which may be involved in the more severe pathological progression of MI in \(\text{Hdc}^{-/-}\) mice.

### 3.4. \(\text{Hdc}\) deficiency in neutrophils promotes cardiomyocyte death and cardiac fibroblast proliferation/migration through NETosis

\(\text{Hdc}\) deficiency in neutrophils affected the cellular activities of neutrophils, as observed above. Next, we investigated the effects of neutrophils on cardiomyocytes. Neutrophils isolated from the bone marrow of \(\text{Hdc}^{+/+}\) or \(\text{Hdc}^{-/-}\) mice were co-cultured with cardiac fibroblasts in vitro, were shown to have a larger area of Sytox green-positive cells that represented NETs (Fig. 3D and E).

The number of DEGs and the overlap genes consistently differentially expressed on Days 1 and 7 after MI were shown in Venn diagram (E). \(n = 3\) per group. (F–H) gene ontology (GO) analysis of DEGs between \(\text{Hdc}^{-/-}\) and \(\text{Hdc}^{+/+}\) mice. The top 15 GO terms of biological process (BP, F), cellular component (CC, G) and molecular function (MF, H) at the indicated time points were shown.
and cardiomyocyte death. These findings suggest that Hdc deletion in neutrophils promoted cardiomyocyte death through NETosis. Studies have reported that excessive NETs can induce fibrosis, which participates in multiple pathological processes. Thus, we explored whether Hdc deficiency altered the effect of neutrophils on cardiac fibroblasts. Cardiac fibroblasts were co-cultured with Hdc+/+ or Hdc−/− mouse-derived NETs. An EdU incorporation assay was employed to determine the proliferation of cardiac fibroblasts. The results revealed that Hdc−/− neutrophils boosted the proliferation of fibroblasts to a higher degree than Hdc+/+ neutrophils did (Fig. 4C). However, when treated with DNase I simultaneously, the ability of NETs to promote the proliferation of fibroblasts was diminished to similar levels in both groups (Fig. 4C). Next, we performed wound healing assays to investigate the influence of Hdc+/+ and Hdc−/− NETs on the migration of fibroblasts. The migration of fibroblasts co-cultured with Hdc−/− NETs was faster than that of fibroblasts co-cultured with Hdc+/+ NETs (Fig. 4D). Similarly, DNase I treatment decreased the migration ability of both groups to the same degree (Fig. 4D). All these results demonstrated that Hdc deficiency in neutrophils exerted its pro-effect on cardiomyocyte death and fibroblast proliferation and migration by NETosis.

3.5. PRMT1 is negatively regulated by histamine in myeloid cells

To unveil the mechanism through which Hdc regulates NETosis, we first sorted CD11b positive cells from the bone marrow of Hdc+/+ or Hdc−/− mice for microarray analysis and then screened out the DEGs regulated by Hdc deficiency or histamine, through combining our data and published myeloid cell transcriptome data from other research groups. Differential gene analysis revealed that there were 3096 DEGs between the Hdc+/+ and Hdc−/− groups, and 1551 DEGs between groups with or without histamine treatment (Fig. 5A). Additionally, according to a previous study, there were 2628 DEGs between the Hdc−/− mice injected with anti-Ly6G antibody or its isotype after infarction. Among them, 64 DEGs were overlapped in the three groups (Fig. 5A). Their relative expression levels were shown in the heatmaps (Fig. 5B). Among the 64 DEGs, we noted that the expression level of Prmt1 was downregulated in the histamine group and Hdc−GFPHigh group but augmented in the Hdc−/− group (Fig. 5C).

PRMT1 is a protein arginine methyltransferase that has been reported to methylate a variety of protein substrates, including...
those involved in gene transcription, DNA damage repair, signal transduction, and protein translocation

To verify the alteration of \( \text{Prmt1} \) level in myeloid cells, we firstly detected its expression in neutrophils. Data showed that the level of \( \text{Prmt1} \) was transcriptionally and translationally increased in \( \text{Hdc}^{-/-} \) and \( \text{Hdc}^{+/+} \) mouse-derived neutrophils (Fig. 5D and Supporting Information Fig. S3A).

To test whether the expression of PRMT1 is regulated by histamine in granule cell like HL60 cells, the concentration gradient and time course assay were performed on HL60 cells. Our data show that the expression of PRMT1 was decreased responding to the treatment of 10 and 100 \( \mu \text{mol/L} \) histamine (Fig. 5E). And it began to decrease at 6 h after histamine treatment, kept for more than 72 h (Fig. 5F and Fig. S3B).

Next, we investigated the expression of other members of PRMT family, with the treatment of histamine. As shown in Fig. S3C, only PRMT1, with the greatest \( C_t \) value, has been downregulated among PRMT1–6, after the treatment with histamine.

To verify whether the enhanced ROS generation and NETosis caused by \( \text{Hdc} \) deficiency in neutrophils were mediated through PRMT1, we inhibited the activity of PRMT1 using MS023. MS023 is the specific type I inhibitor of PRMTs, which had been reported to be applied in treating acute myeloid leukemia (AML) in a relatively low dose. To minimize its side effects, we had chosen the optimal concentration of MS023 on PRMT1 according to the reported C50 of MS023 on PRMT1. The data showed that the increased production of ROS and the augmented formation of citH3-positive NETs in \( \text{Hdc}^{-/-} \) and \( \text{Hdc}^{+/+} \) mouse-derived neutrophils were reduced after 24 h of treatment with MS023 or histamine (Fig. 5G and H).

3.6. Histamine repressed the transcription of PRMT1 by restricting the DNA accessibility of the transcriptional start site (TSS) region of PRMT1

There are four known histamine receptors (H1R, H2R, H3R, and H4R) expressed in myeloid cells, and we wondered which of them would be responsible for the repression of histamine on PRMT1 in neutrophils. Among the inhibitors of four histamine receptors, only the H1R antagonist pyrilamine rescued the decrease of PRMT1 induced by histamine treatment in HL60 cells (Fig. 6A), suggesting that the regulation of PRMT1 by histamine was dependent on H1R.

Next, we explored the mechanism by which histamine inhibited the expression of \( \text{Prmt1} \). Since \( \text{Prmt1} \) was decreased at the mRNA level, suggesting the regulation of its expression at the transcriptional level, we attempted to identify the key transcription factors of \( \text{Prmt1} \). Signal transducer and activator of
transcription 1 (STAT1) and CCAAT enhancer binding protein beta (CEBPB) have been reported to bind to the promoter region of PRMT1 in fibroblasts. However, knocking down STAT1 or CEBPB in HL60 cells did not abolish the repressive effect of histamine on the level of PRMT1 mRNA in HL60 cells (Fig. 6B), which indicated that there was a unique transcriptional mechanism different from those in known cell types during the process of histamine repressing the transcription of PRMT1.

To further characterize the pathway by which histamine regulates PRMT1 expression, DNA accessibility was assessed by DNase digestion assay. The data revealed that TSS of PRMT1 became DNase I resistant after histamine treatment (Fig. 6C and D).

Since the ATP-dependent SWI/SNF complex contributes to chromatin remodeling, we tested whether it was involved in the regulation of PRMT1. Silencing the catalytic subunits of the SWI/SNF complex, Brahma (BRM) and Brahma related gene 1 (BRG1), abolished the repressive effect of histamine on the expression of PRMT1 (Fig. 6E). Similarly, with oligomycin treatment, which inhibited ATP production, the repression of histamine on the mRNA level of PRMT1 was rescued (Fig. 6F). In general, these findings revealed that HDC/histamine down-regulated the transcription of PRMT1 by restricting the DNA accessibility of PRMT1 in an ATP-dependent SWI/SNF-dependent manner.

3.7. Asymmetric arginine dimethylation profiling of PRMT1 targets are regulated by histamine

To further identify the downstream proteins methylated by PRMT1, the proteins with asymmetric dimethylated arginine (ADMA) were harvested by co-immunoprecipitation (co-IP) from the lysate of cells with or without histamine treatment. Coo- massie blue staining showed that there was a significant decrease in the level of proteins with ADMA responding to histamine treatment (Fig. 7A and Supporting Information Fig. S4A).

Then, we performed mass spectrometry to identify the profile of the proteins with ADMA before and after histamine treatment. There were 1488 proteins detected in the group without histamine treatment and 1392 proteins detected in the group with histamine treatment. Among them, 339 unique proteins existed in the former, while 243 proteins uniquely existed in the latter (Fig. 7B and Table 1).
Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the differentially expressed proteins revealed that proteins with ADMA regulated by histamine were mainly enriched in the cellular process of endocytosis, RNA processing, DNA replication, and amino acid degradation (Fig. 7C). GO analysis indicated that proteins with ADMA regulated by histamine were enriched for autophagy and Golgi vesicle transport (Fig. 7D). Moreover, these proteins were enriched in mitochondrial, nuclear, and cell cortex regions of the cell (Fig. 7E), and their functions were focused on GTP binding, phosphatidylinositol phosphate binding, and nucleoside binding (Fig. 7F).

Furthermore, we noticed that 42 members of the Ras family were enriched in the proteins pulled down by the ADMA antibody (Table 1), among which 15 members were reported to be involved in ROS production. Significantly, 7 out of the 42 members, especially Rap1, the key component of NADPH oxidase, were specifically enriched in the untreated group but disappeared in the histamine-treated group. The AMDA modification of Rap1 was significantly reduced in histamine treated group (Fig. S4B), suggesting Rap1 was likely the downstream target of HDC–PRMT1 signal in regulating the ROS generation and NETs formation.
The above findings implied that Hdc deficiency was deleterious to heart function after MI, possibly through upregulating PRMT1, leading to promoted ROS generation and excessive NETosis. Thus, to evaluate the role of PRMT1 in cardiac function after MI, possibly through upregulating PRMT1, early phase contribute to the diversity of clinical pathological immune responses of patients to the ischemic injury during the acute myocardial infarction. This study revealed that Hdc/histamine regulated neutrophil mobilization and limited additional reactive oxidative species and NET generation after myocardial infarction by the H1R-SWI/SNF–PRMT1 pathway (Fig. 9). Our findings identify a new mechanism for neutrophil dependent inflammation and provide a rationale for neutrophils as a therapeutic target for acute myocardial infarction.

3.8. Inhibiting the activity of PRMT1 can rescue the aggravation of myocardial injury caused by Hdc deficiency after MI

The expression of Hdc in the bone marrow has been shown to decrease across the lifespan, implicating its immunological role in aging related diseases (Supporting Information Fig. S6). Previous data have shown that Hdc/histamine participates in regulating the progression of acute heart injury and heart failure, but the underlying mechanisms have not been fully unveiled. Here, we illustrated the transcriptional profile of the injured hearts of mice on Days 0, 1, and 7 after ligation of the left anterior descending coronary artery. Time course analysis of GO showed that immunological phenomena was closely related to the progression of MI and that the immune state in the heart changed along with the development of myocardial remodeling. Immune recruitment analysis indicated that immunocytes, neutrophils especially,
infiltrated infarcted hearts within a different time frame in $Hdc^{-/-}$ mice, compared with $Hdc^{+/+}$ mice. $Hdc$ deficiency greatly increased the counts of neutrophils in the myocardium on Days 3 and 7 after MI.

Although $Hdc$ was globally knockout in our experiments, our previous data had suggested that the expression of HDC in myocardium is extremely low to be detected (data not shown) and there were no abnormalities in the cardiac structure and function of $Hdc^{-/-}$ mice under normal conditions. Moreover, neutralizing Ly6G$^+$ neutrophils ameliorated the prognosis of MI in $Hdc^{-/-}$ mice in the present work, which suggested the contributing role of neutrophils in the aggravated cardiac function loss in $Hdc^{-/-}$ mice with MI.

Neutrophils are vital for clearing pathogens or debris in acute inflammation. However, in the context of MI, abnormal neutrophils are detrimental to cardiac function. Clinically, the neutrophil-to-lymphocyte ratio has been employed to predict the survival of patients with STEMI and the improvement of coronary artery disease events is found to be correlated with neutrophil reduction. Surprisingly, the adhesion of $Hdc^{-/-}$ neutrophils to endothelial cells was decreased. Histamine has been reported to activate endothelial cells by promoting the expression of adhesion related genes, but the role of histamine in the adhesion of neutrophils is still unknown. In $Hdc^{-/-}$ mice, with the increased migration but the decreased neutrophil adhesion, why the infiltrated neutrophils were significantly increased on Days 3 and 7, but not on Day 1 after injury is the next scientific question we are eager to answer. Moreover, it will be interesting to analyze the effect of $Hdc$ on neutrophils at the earlier stage such as 1, 3, 6, or 12 h post MI, as the adhesion of neutrophils affected the recruitment at the start of inflammation.

Oxidative burst is the way of neutrophils that eliminate pathogens or respond to inflammatory stimuli. Histamine is thought to inhibit ROS driven by opsonized zymosan particles or fMLP in a PKC-independent way. Our data showed that deletion of $Hdc$ led to neutrophils in a hyperoxidative state not only under the treatment of PMA, an agonist of PKC, but also in the baseline state.

NETosis is a unique mechanism of cell death in neutrophils driven by ROS with various stimulations. It has been reported that NETs lead to the death of cells by histones. In addition, NETs have recently been reported to promote fibrosis cell activation/proliferation by neutrophil elastase (NE) dependent
matrix remodeling. Herein, in vivo experiments revealed that excessive NETs accompanied with augmented cardiac injury after MI in Hdc<sup>−/−</sup> mice. Our data showed that cardiomyocyte death and fibroblast proliferation/migration were aggravated by Hdc<sup>−/−</sup>-NETs. These findings indicate that aggregated neutrophils, which migrate into the heart, may promote cell death of the myocardium, expand the injured zone, and pave the way for fibroblasts rather than purging cell debris or reducing inflammation<sup>30</sup>. To clarify the underlying mechanisms through which Hdc histamine regulated ROS generation and the formation of NETs, we analyzed the DEGs regulated by Hdc, by combining our transcriptomic data and the published transcriptomic data of myeloid cells in the GEO database. After screening, we found that the expression of PRMT1 was tightly repressed by HDC/histamine at the mRNA and protein levels, while rescued by the antagonist of H1R. PRMT1 is an important enzyme responsible for asymmetric arginine dimethylation. It has been reported that there is a negative correlation between histamine and methylation in the serum of patients with behavioral disorders<sup>61</sup>. In the current study, histamine treatment, which repressed the transcriptional level of PRMT1, reduced the level of proteins with ADMA modification, which is consistent with the phenomenon observed in behavioral disorders patients<sup>61</sup>. Moreover, inhibiting the catalytic activity of PRMT1 by MS023 could limit the excessive ROS generation and NETosis, and improve the cardiac function of Hdc<sup>−/−</sup>-mice post MI. Our results and the work of Pyun et al.<sup>69</sup> suggested that PRMT1 likely possess different functions in different cells and different diseases models.

STAT1 and CEBPB have been reported to be responsible for the regulation of PRMT1 transcription in fibroblasts<sup>3,34,62</sup>. However, in our work, the data showed that neither transcription factor participated in the regulation of histamine on PRMT1 expression. This may be due to the different kinds of cell types. Instead, histamine repressed PRMT1 transcription by restricting DNA accessibility to its TSS region through ATP-dependent chromatin remodeling. Chromatin remodeling occurs in the process of cell differentiation<sup>44</sup>, while PRMT1 has been reported to participate in the maintenance of short-term hematopoietic stem cells, which implies that PRMT1 may participate in histamine-induced myeloid cell maturation. This suggested that ATP analogues or inhibitors of enzymes of chromatin remodeling are the potential targets to modulate PRMT1 expression and NETosis. Beside that histamine repressed the transcription of PRMT1 through restricting the DNA accessibility to the TSS region of PRMT1, our results of co-IP revealed that PRMT1 and HDC could interact with each other slightly, which was not affected by histamine or MS023 treatment as shown in Fig. S6B and C. The phenomenon aroused an interesting question that besides for the catalytic activity of arginine methylation, would PRMT1 act on HDC in a novel way as a negative feedback regulation of histamine inhibiting PRMT1 transcription? Or may PRMT1 and HDC form a complex with other proteins to function in cells? These questions need more efforts to figure out in the future work.

Type I PRMTs, including PRMT1–4, PRMT6, and PRMT8, promote ADMA, reducing the positive charge and hydrophilicity of proteins<sup>31</sup>. Many published studies certified that this type of modification involved in multiple cell activities, including gene transcription, cell signaling, mRNA translation, DNA damage signaling, protein trafficking, protein stability, and pre-mRNA splicing<sup>32,64–66</sup>. Previous study implicated that the deficiency of Ddah1, which is the key enzyme for ADMA degradation, raised ROS levels in cardiomyocytes. Moreover, it has also been reported that ADMA promotes ROS generation in macrophages<sup>67</sup> and MPO release in polymorphonuclear neutrophils in the context of cardiovascular disease<sup>68</sup>. In our work, the levels of ADMA were shown to be positively related to the levels of NETs in a mouse model of MI, implying that the ADMA playing a role in regulating ROS generation and the formation of NETs.

Although Pyun et al.<sup>69</sup> reported that mice null for cardiac PRMT1 could exhibit dilated cardiomyopathy after 2 months, there is no other work exploring the role of PRMT1 during acute MI up to now. Our work revealed that the expression of PRMT1 in neutrophils would be increased but showed no significant change in myocardium after Hdc deletion. Inhibiting PRMT1 could limit the excessive ROS generation and NETosis in Hdc-deficient neutrophils, improving the cardiac function of Hdc<sup>−/−</sup>-mice post MI. Similarly, directly reducing neutrophils could also attenuate cardiac injury post MI in Hdc<sup>−/−</sup>-mice. Based on these, we consider that PRMT1 mainly in neutrophils played a potential role in regulating the aggravated cardiac injury post MI in Hdc<sup>−/−</sup> mice. Our results and the work of Pyun et al.<sup>69</sup> suggested that PRMT1 likely possess different functions in different cells and different diseases models.

To determine the downstream factor of PRMT1 in regulating the formation of NETs, the ADMA modified profiling of proteins were determined by mass spectrometry in our work. GTP binding and GTPase activity were specifically enriched in the histamine

| Table 1 | ADMA proteins of the RAS family related to ROS. |
|------------------------|-----------------------------------------------|
| Group                  | ADMA protein<sup>a</sup> | ROS   |
| Only in Con            | RAP1B<sup>36</sup> | ↑   |
| Both in Con and HA     | RAP1A<sup>37</sup> | ↑   |
|                        | G3BP1<sup>18</sup> | ↑   |
|                        | IQGAP1<sup>39</sup> | ↑   |
|                        | RAC1<sup>36</sup> | ↑   |
|                        | RAC2<sup>40</sup> | ↑   |
|                        | RAB10<sup>41</sup> | ↑   |
|                        | RAB27A<sup>42</sup> | ↑   |
|                        | RAB35<sup>43</sup> | ↑   |
|                        | ARF6<sup>44</sup> | ↑   |
|                        | CDC42<sup>45</sup> | ↑   |
|                        | RASA3<sup>46</sup> | ↓   |
|                        | RAB31<sup>47</sup> | ↓   |
|                        | ARF4<sup>44</sup> | ↓   |
|                        | RASSF2, IQGAP2, RAB11A, RAB14, RAB1A, RAB1B, RAB21, RAB2A, RAB32, RAB5A, RAB5C, RAB6B, RAB7A, RAB8A, ARF5, ARF3, RAL | ↓ |
| Only in HA             | NRAS<sup>39</sup> | ↓   |
|                        | AK3, RAB8B, RAB8B | ↓   |

<sup>a</sup>The ADMA proteins of RAS family are divided into 3 groups: only in control (Con), both in Con and histamine (HA) groups, and only in HA group. Their roles in ROS production are indicated as arrows. ↑ represents the gene promotes the production of ROS; ↓ presents the gene decreases the production of ROS.
untreated group. In neutrophils, NADPH oxidase is the main complex responsible for ROS generation. It contains catalytic gp91phox (also known as NOX2), regulatory components, p22phox, p40phox, p47phox, p67phox, and Rac/Rap1. Rac/Rap1, belongs to the Ras family, many members of which are involved in the regulation of ROS (Table 1). The hydrophilicity of Rap1 would be reduced by its ADMA modification, which may facilitate its localization to the NADPH-oxidase complex on the membrane of mitochondria. The reduction of the ADMA modification of Rap1 caused by histamine suggested the downstream role of Rap1 in HDC–PRMT1 signaling. Taken together, our findings strongly suggested that the activity of NADPH oxidase could be regulated by PRMT1 to promote ROS production and NET formation, indicating that NADPH oxidase or Rap1 could
also be the potential target for the immune modulation for the cardiovascular disease.

5. Conclusions

Our study demonstrates that the lack of Hdc promotes NETosis by the H1R–SWI/SNF–PRMT1–ROS pathway, and hence leads to aggravated cardiomyocyte death and fibroblast activation. Targeting any one of them may provide insights into new treatments for myocardial infarction and consequent cardiac remodeling. These findings improve our understanding of the significant role of neutrophil and NET formation in cardiovascular disease and provide new biomarkers and pharmaceutical targets for identifying and tuning the detrimental immune state in cardiovascular disease.

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Author contributions

Zhiwei Zhang, Suling Ding, Zhe Wang, Xiangdong Yang, and Junbo Ge were involved in the study design, data analysis, and manuscript preparation. Zhiwei Zhang, Suling Ding, Zhe Wang, Zheliang Zhou, Xiaowei Zhu, Weiwei Zhang, and Zhe Wang performed the experiments. All authors reviewed the manuscript.

Conflicts of interests

The authors declare no competing conflict of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2021.10.016.

References

1. Ding S, Abudupataer M, Zhou Z, Chen J, Li H, Xu L, et al. Histamine deficiency aggravates cardiac injury through miR-206/216b-Atg13 axis-mediated autophagic-depadendent apoptosis. Cell Death Dis 2018;9:694.
2. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. Lancet 2006;367:356–67.
3. Frantz S, Bauersachs J, Ertl G. Post-infarct remodelling: contribution of wound healing and inflammation. Cardiovasc Res 2009;81:474–81.
4. Silvestre-Roig C, Braster Q, Ortega-Gomez A, Soehnlein O. Neutrophils as regulators of cardiovascular inflammation. Nat Rev Cardiol 2020;17:327–40.
5. Leoni G, Soehnlein O. (Re) solving repair after myocardial infarction. Front Pharmacol 2018;9:1342.
6. Curaj A, Schumacher D, Rusu M, Staudt M, Li X, Simsekylilmaz S, et al. Neutrophils modulate fibroblast function and promote healing and scar formation after murine myocardial infarction. Int J Mol Sci 2020;21:3685.
7. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303:1532–5.
8. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science 2015;349:316–20.
9. Yu Y, Su K. Neutrophil extracellular traps and systemic lupus erythematosus. J Clin Cell Immunol 2013;4:139.
27. Martinod K, Witsch T, Erpenbeck L, Savchenko A, Hayashi H, Cherpokova D, et al. Peptidylarginine deiminase 4 promotes age-related organ fibrosis. J Exp Med 2017;214:439–58.

28. Hofbauer TM, Mangold A, Scherz T, Seidl V, Panzenböck A, Ondracek AS, et al. Neutrophil extracellular traps and fibrocytes in ST-segment elevation myocardial infarction. Basic Res Cardiol 2019;114:33.

29. Chrysanthopoulou A, Mitroulis I, Apostolidou E, Aralaki S, Mikroulis D, Konstantinidis T, et al. Neutrophil extracellular traps promote differentiation and function of fibroblasts. J Pathol 2014;233:294–307.

30. Kifrin R, Grauers Wiktoria H, Nilsson MS, Aurelius J, Aydin E, Lenox B, et al. Anti-leukemic properties of histamine in monocytic leukemia: the role of NOX2. Front Oncol 2018;8:218.

31. Blanc RS, Richard S. Arginine metabolism: the coming of age. Mol Cell 2017;65:8–24.

32. He X, Zhu Y, Lin YC, Li M, Du J, Dong H, et al. PRMT1-mediated FLT3 arginine metabolism promotes maintenance of FLT3-ITD+ acute myeloid leukemia. Blood 2019;134:548–60.

33. Sun Q, Liu L, Mandal J, Molino A, Stolz D, Tam M, et al. PDGF-BB induces PRMT1 expression through ERK1/2 dependent STAT1 activation and regulates remodeling in primary human lung fibroblasts. Cell Signal 2016;28:307–15.

34. Sun Q, Fang L, Tang X, Lu S, Tam M, Stolz D, et al. TGF-β-regulated mitochondria mass through the SMAD2/3→C/EBPβ→PRMT1 signal pathway in primary human lung fibroblasts. J Immunol 2019;202:37–47.

35. Ji L, Zhao X, Zhang B, Kang L, Song W, Zhao B, et al. Slc6a8-mediated creatine uptake and accumulation reprogram macrophage polarization via regulating cytokine responses. Immunity 2019;51:272–84.e277.

36. Vara DS, Campanella M, Canobbio I, Dunn WB, Pizzorno G, Hirano M, et al. Autocrine amplification of integrin αIIβ3 activation and platelet adhesive responses by deoxyribose-1-phosphate. Thromb Haemostasis 2013;109:1108–19.

37. Li Y, Kim JG, Kim HJ, Moon MY, Lee JY, Kim J, et al. Small GTPases activation during the phagocytosis of IgG-opsonized zymosan in macrophages. Free Radic Biol Med 2012;52:1796–805.

38. Cho E, Than TT, Kim SH, Park ER, Kim MY, Lee KH, et al. G3BP1 deletion increases radiosensitisation by inducing oxidative stress in response to DNA damage. Anticancer Res 2019;39:6087–95.

39. Mo CF, Li J, Yang SX, Guo HJ, Liu Y, Luo XY, et al. Iqgap1 promotes anoikis resistance and metastasis through Rac1-dependent ROS accumulation and cell cycle defects in macrophages. J Immunol 2016;206:1154–63.

40. Raad H, Mouawad H, Hassan El-Sebany M, Arabi-Derkawi B, Boussett A, et al. The protein kinase A negatively regulates reactive oxygen species production by phosphorylating gp91phox/NOX2 in macrophages. J Exp Med 2011;208:689–99.

41. Wauters F, Cornelissen T, Imberechts D, Martin S, Koentjoro B, et al. Increased reactive oxygen species and cell cycle defects contribute to anemia in the RASA3 mutant mouse model. J Exp Med 2018;209:689–99.

10. Middleton EA, He XY, Denorme F, Campbell RA, Ng D, Salvatore SP, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood 2020;136:1169–79.

11. Liu J, Yang D, Wang X, Zhu Z, Wang T, Ma A, et al. Neutrophil extracellular traps and dsDNA predict outcomes among patients with ST-elevation myocardial infarction. Sci Rep 2019;9:11599.

12. Mangold A, Alia S, Scherz T, Hofbauer T, Jakowisch J, Panzenböck A, et al. Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. Circ Res 2015;116:1182–92.

13. Chen X, Deng H, Churchill MJ, Luscher LL, Du X, Chu TH, et al. Bone marrow myeloid cells regulate myeloid-biased hematopoietic stem cells via a histamine-dependent feedback loop. Cell Stem Cell 2017;21:747–76.e747.

14. Yang XD, Ai W, Asfaha S, Bhagat G, Friedman RA, Fin G, et al. Histamine deficiency promotes inflammation-associated carcinogenesis through reduced myeloid maturation and accumulation of CD11b+ Ly6G+ immature myeloid cells. Nat Med 2011;17:87–95.

15. Ding S, Abudupataer M, Zhou Z, Chen J, Li H, Xu L, et al. Histamine deficiency aggravates cardiac injury through miR-206/146b→Atg13 axis-mediated autophagic-dependent apoptosis. Cell Death Dis 2018;9:694.

16. Ohtsu H, Tanaka S, Terui T, Hori Y, Makabe-Kobayashi Y, Pejler G, et al. Mice lacking histidine decarboxylase exhibit abnormal mast cells. FEBS Lett 2001;502:53–6.

17. Chen J, Hong T, Ding S, Deng L, Abudupataer M, Zhang W, et al. Aggravated myocardial infarction-induced cardiac remodeling and heart failure in histamine-deficient mice. Sci Rep 2017;7:44007.

18. Gao E, Lei YH, Shang X, Huang ZM, Zuo L, Boucher M, et al. A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. Circ Res 2010;107:1445–53.

19. Zhou Z, Zhang S, Ding S, Abudupataer M, Zhang Z, Zhu X, et al. Excessive neutrophil extracellular trap formation aggravates acute myocardial infarction injury in apolipoprotein E deficiency mice via the ROS-dependent pathway. Oxid Med Cell Longev 2019;2019:1209307.

20. Ackers-Johnson M, Li PY, Holmes AP, O’Brien SM, Pavlovic D, Foo RS. A simplified, Langendorff-free method for concomitant isolation of viable cardiac myocytes and nonmyocytes from the adult mouse heart. Circ Res 2016;119:909–20.

21. Kessenbrock K, Fröhlich L, Stix M, Lümmermann T, Pfister H, Baiter M, et al. Proteinase 3 and neutrophil elastase enhance inflammation in mice by inactivating antiinflammatory prostaglandin. J Clin Invest 2008;118:2438–47.

22. Kukulski F, Yebdri FB, Lecka J, Kauffenstein G, Lévesque SA, Martin-Satué M, et al. Extracellular ATP and P2 receptors are required for IL-8 to induce neutrophil migration. Cytokine 2009;46:166–70.

23. Rezazadeh S, Yang D, Tombline G, Simon M, Regan SP, Seluanov A, et al. SIRT6 promotes transcription of a subset of NRF2 targets by mono-ADP-ribosylating BAFl70. Nucleic Acids Res 2019;47:7914–28.

24. Shi Y, Shi H, Niemann DC, Hu Q, Yang L, Liu T, et al. Lactic acid accumulation during exhaustive exercise impairs release of neutrophil extracellular traps in mice. Front Physiol 2019;10:709.

25. Arslan F, de Kleijn DP, Timmers L, Doevendans PA, Pasterkamp G, Bridging innate immunity and myocardial ischemia/reperfusion injury: the search for therapeutic targets. Curr Pharmaceut Des 2008;14:1205–16.

26. Hori Y, Nihei Y, Kurokawa Y, Kuramasa A, Makabe-Kobayashi Y, Terui T, et al. Accelerated clearance of Escherichia coli in experimental peritonitis of histamine-deficient mice. J Immunol 2002;169:1978–83.

27. Martinod K, Witsch T, Epenbeck L, Savchenko A, Hayashi H, Cherpokova D, et al. Peptidyldarginine deiminase 4 promotes age-related organ fibrosis. J Exp Med 2017;214:439–58.
50. Cavender MA, Milford-Beland S, Roe MT, Peterson ED, Weintraub WS, Ye F, Chai W, Xie M, Yang M, Yu Y, Cao L, et al. HMGB1 regulates Mayadas TN, Tsokos GC, Tsuboi N. Mechanisms of immune retinamide-induced cell death. Cancer Lett 2009;276:53–60.

51. Zhang KQ, Chu XD. GANT61 plays antitumor effects by inducing HDC-PRMT1 regulates NETosis post MI 1855.

52. Schaum N, Lehallier B, Hahn O, Pa’lovics R, Hosseinzadeh S, Lee SE, Vasicek O, Lojek A, Jancinova V, Nosal R, Ciz M. Role of histamine.

53. Deng L, Hong T, Lin J, Ding S, Huang Z, Chen J, et al. Histamine deficiency exacerbates myocardial injury in acute myocardial infarction through impaired macrophage infiltration and increased cardiomyocyte apoptosis. Sci Rep 2015;5:13131.

54. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1 receptor expression in NK cells with CD56dim and CD56bright phenotype: regulation by histamine and reactive oxygen species. Br J Haematol 2006;132:7–9.

55. Romero AI, Thore’n FB, Brune M, Hellstrand K. NKp46 and NKG2D complex-mediated neutrophil recruitment and tissue injury. Cell Biol Int 2012;36:1868.

56. Vasicek O, Lojek A, Jancinova V, Nosal R, Ciz M. Role of histamine receptors in the effects of histamine on the production of reactive oxygen species by whole blood phagocytes. Life Sci 2014;100:67–72.

57. Zhang Y, Jian W, He L, Wu J. Externalized histone H4: a novel target that orchestrates chronic inflammation by inducing lytic cell death. Acta Biochim Biophys Sin (Shanghai) 2020;52:336–8.

58. Saffarzadeh M, Juennemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. PLoS One 2012;7:e32366.

59. Albrengues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science 2018;361:eaao4227.

60. Schaier C, Janko C, Munoz LE, Zhao Y, Kienhöfer D, Frey B, et al. Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med 2014;20:511–7.

61. Walsh WJ, Glab LB, Haakenson ML. Reduced violent behavior following biochemical therapy. Physiol Behav 2004;82:835–9.

62. Jin A, Tang X, Zhai W, Li Y, Sun Q, Liu L, et al. TSLP-induced collagen type-I synthesis through STAT3 and PRMT1 is sensitive to calcitriol in human lung fibroblasts. Biochim Biophys Acta Mol Cell Res 2021;1868;119083.

63. Piazza F, Semenzato G. Molecular therapeutic approaches to acute myeloid leukemia: targeting aberrant chromatin dynamics and signal transduction. Expert Rev Anticancer Ther 2004;4:387–400.

64. Hartel NG, Chew B, Qin J, Xu J, Graham NA. Deep protein methylation profiling by combined chemical and immunoaffinity approaches reveals novel PRMT1 targets. Mol Cell Proteomics 2019;18:2149–64.

65. Liu LM, Sun WZ, Fan XZ, Xu YL, Cheng MB, Zhang Y. Methylation of C/EBPα by PRMT1 inhibits its tumor-suppressive function in breast cancer. Cancer Res 2019;79:2865–77.

66. Jeong MH, Jeong HJ, Ahn BY, Pyun JH, Kwon I, Cho H, et al. PRMT1 suppresses ATF4-mediated endoplasmic reticulum response in cardiomyocytes. Cell Death Dis 2019;10:903.

67. Chen CH, Zhao JF, Hsu CP, Kou YR, Lu TM, Lee TS. The detrimental effect of asymmetric dimethylarginine on cholesterol efflux of macrophage foam cells: role of the NOX/ROS signaling. Free Radical Biol Med 2019;143:354–65.

68. von Leitner EC, Klinke A, Atzler D, Slocum JL, Lund N, Kielstein JT, et al. Pathogenic cycle between the endogenous nitric oxide synthase inhibitor asymmetrical dimethylarginine and the leukocyte-derived hemoprotein myeloperoxidase. Circulation 2011;124:2735–45.

69. Pyun JH, Kim HJ, Jeong MH, Ahn BY, Vuong TA, Lee DI, et al. Cardiac specific PRMT1 ablation causes heart failure through CaMKII dysregulation. Nat Commun 2018;9:5107.

70. Panday A, Sahoo MK, Osorio D, Batra S. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cell Mol Immunol 2015;12:5–23.

71. Ferro E, Goitre L, Retta SF, Trabalzini L. The interplay between ROS and Ras GT Pases: physiological and pathological implications. J Signal Transduct 2012;2012:365769.