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M1 macrophage-derived migrasomes exacerbate post-myocardial infarction injury via guanylate binding protein 5

Qingfu Zhang^{1†}, Aolin Du^{1†}, Zhichao Li^{1†}, Hui Gu², Wanqi Huang², Ying Li¹, Su Han^{1*}, Chuanhe Wang^{1*} and Zhijun Sun^{1*}

Abstract

Myocardial infarction (MI) is a complex pathological process characterized by vascular injury, myocardial necrosis, and dynamic immune interactions. Migrasomes are recently identified organelles generated during cell migration, serving as key mediators of intercellular communication. However, the contribution of migrasomes to immune-mediated myocardial injury remains largely unexplored. This study demonstrated an increase in migrasome production following MI. Migrasomes can be produced by macrophages, and M1 macrophage-derived migrasomes (M1-Migs) were particularly found to exacerbate myocardial tissue injury. Quantitative proteomic sequencing demonstrated increased levels of guanylate binding protein 5 (GBP5) within M1-Migs. Viral knockdown experiments demonstrated that M1-Migs mediate their deleterious effects predominantly via GBP5. Pathway enrichment analysis further indicated that GBP5 activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling, thereby promoting myocardial cell apoptosis. Analysis of clinical samples has also demonstrated a positive correlation between macrophage-derived migrasomes and MI. Notably, colchicine may mitigate post-infarction myocardial injury by suppressing migrasome production by M1 macrophages. Overall, these findings identify macrophage-derived migrasomes as key amplifiers of myocardial injury, providing potential therapeutic targets for MI and may provide additional evidence for the clinical application of colchicine.

Keywords Myocardial Infarction, Migrasome, Macrophage, Organelle, Apoptosis

Background

Acute myocardial infarction (AMI) remains a major cause of morbidity and mortality worldwide. Following AMI, severe circulatory disturbances induce severe myocardial hypoxia and necrosis with limited myocardial regeneration [1, 2]. The extent of cardiomyocyte proliferation remains inadequate to offset the loss of viable tissue caused by myocardial necrosis. Consequently, necrotic myocardium is replaced by fibrotic scar tissue, leading to adverse ventricular remodeling, progressive cardiac dysfunction, and ultimately heart failure [3]. While cardiomyocyte death induces inflammatory responses, macrophages, as primary immune cells, orchestrate

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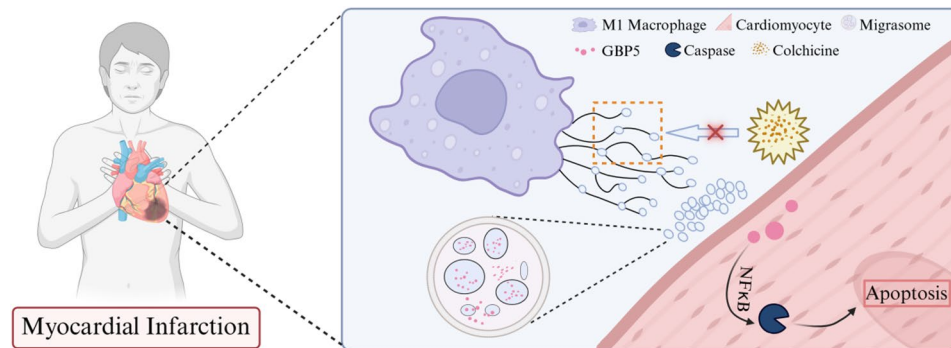
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Graphical abstract

inflammation progression and resolution, thereby influencing cardiac repair and remodeling [4–6]. M1 and M2 macrophages are the most well-characterized and predominant subsets of AMI cells, with M1 macrophages predominating during the acute phase. These cells produce high levels of pro-inflammatory cytokines (e.g., tumor necrosis factor- α , interleukin-1 β , and proteases), exhibiting phagocytic and proteolytic activity to remove cellular debris [7]. However, prolonged pro-inflammatory states accelerate extracellular matrix degradation and cell death, further expanding the infarct area and impairing post-infarction cardiac repair. Excessive M1 macrophage activation has been reported to accelerate cardiomyocyte death, inhibit myocardial regeneration, promote myocardial fibrosis, suppress neovascularization, and ultimately predispose to cardiac rupture [8, 9]. Therefore, elucidating the molecular pathways linking M1 macrophages to AMI may enable the development of novel therapeutic targets.

Migrasomes are recently identified organelles (500–3000 nm in size) that form during cell migration and are mainly composed of the tetraspanin (TSPAN4) protein family. They contain a variable number of vesicles and are tethered to their parent cells through contractile fibers. Migrasomes mediate intercellular communication, homeostasis maintenance, embryonic development, mitochondrial quality control, and various diseases by transporting diverse cargo, including nucleic acids, proteins, lipids, enzymes, and metabolites [10–12]. Macrophages, highly migratory immune cells, represent the principal source of migrasomes *in vivo* [13]. Evidence indicates that monocyte/macrophage-derived migrasomes facilitate vascular angiogenesis in chicken embryos via transporting angiogenic factors and C-X-C motif chemokine ligand 12 (CXCL12) [14]. Moreover, in cerebral amyloid angiopathy, macrophage-secreted migrasomes enriched in CD5 antigen-like (CD5L) act on endothelial cells, compromising the blood-brain barrier and accelerating disease progression; this underscores

the role of macrophage-derived migrasomes in various pathological processes [15]. Therefore, investigation of migrasomes may elucidate the pathological mechanisms of M1 macrophage-mediated AMI and identify potential therapeutic targets.

Colchicine, a well-established anti-inflammatory agent traditionally used to treat gout, has recently been repurposed as a promising anti-inflammatory treatment for cardiovascular disease (CVD), with expanding clinical applications and research in multiple CVDs [16]. Clinical evidence demonstrates that low-dose colchicine significantly decreases the incidence of major adverse cardiovascular events [17]. Pathophysiologically, colchicine modulates critical processes influencing AMI prognosis through its anti-inflammatory properties, including the acute inflammatory burst and the chronic inflammation-induced ventricular remodeling following infarction. Colchicine modulates pro-inflammatory cells, including M1 macrophages. Moreover, colchicine acts on the microtubule system, a critical cytoskeleton component required for cell migration and migrasome formation [18, 19]. Therefore, we aimed to elucidate the specific mechanisms by which colchicine exerts its therapeutic effects through a migrasome-centered approach to provide further evidence for its clinical application.

Transmission electron microscopy (TEM) demonstrated migrasome-like structures in post-infarction myocardial tissue, and quantitative analysis revealed a significant increase in migrasome production within the tissue following MI. Subsequent experiments confirmed that macrophages produce migrasomes and that M1-Migs aggravate myocardial injury. Proteomic profiling of M1-Migs and M0-Migs demonstrated significantly increased guanylate-binding protein 5 (GBP5) levels in M1-Migs, which was confirmed by viral-mediated GBP5 knockdown as the primary mediator of M1-Mig-induced myocardial injury. Pathway analysis of enriched proteins demonstrated that GBP5 promotes cardiomyocyte apoptosis through the activation of the nuclear factor- κ B

(NF- κ B) signaling pathway. Clinical sample analysis confirmed a positive association between macrophage-derived migrasomes and AMI. Notably, our observations imply that colchicine might mitigate post-infarction myocardial injury through suppressing migrasome production in M1 macrophages. Overall, this study demonstrated that M1 macrophages exacerbate myocardial injury via migrasome-mediated mechanisms, providing potential molecular targets for AMI therapy and experimental evidence supporting the clinical application of colchicine in AMI.

Results

Migrasomes derived from M1 macrophages are implicated in MI

TEM demonstrated migrasome-like structures in the interstitial spaces of myocardial tissues from AMI mouse models (Fig. 1A, blue arrow, yellow tint). Morphologically, these structures were roundish vesicles with a diameter of 0.5–3.0 μ m, containing variable numbers of small vesicles and damaged mitochondrial structures, consistent with the previously reported migrasome features [11, 20]. Notably, these migrasomes appeared to originate from macrophages migrating into the myocardial interstitium (Fig. 1A, III; red arrow, green tint). Therefore, this study hypothesized that macrophage-derived migrasomes may play a pivotal role in AMI. To further confirm this hypothesis, the expression of migrasome-related markers at different stages of MI was analyzed using western blotting. The results demonstrated an upregulated expression of migrasome-related genes following MI, including TSPAN4, EOGT, and PIGK [21]. Among these, TSPAN4 and PIGK expression peaked at day 3 post-infarction, while EOGT was significantly upregulated (Fig. 1B). To further confirm this, immunohistochemistry for TSPAN4 in myocardial tissues on day 3 post-infarction was conducted and demonstrated a significant increase in TSPAN4 expression (Figure S1A). Following MI, macrophages rapidly accumulated at the infarcted myocardium, with the M1-type macrophages predominating on day 3. On this basis, we further investigated the association between migrasomes and different cell types in infarcted myocardial tissues using immunofluorescence colocalization assays. The results demonstrated a prominent colocalization between TSPAN4, a specific marker of migrasomes, and iNOS, a marker of M1 macrophages. In contrast, the colocalization signals of TSPAN4 with α -SMA (a fibroblast marker) and CD11b (a neutrophil marker) were negligible (Figure S1B). Given that migrasomes are formed during cell migration and that macrophages are highly motile [13, 20], this study inferred that M1-Migs may play a pivotal role in the pathophysiology of MI.

Because M1 macrophages originate from M0 macrophages and migrasome cargo is determined by their parental cells, M0-derived migrasomes (M0-Migs) were used as controls to elucidate the role of M1-Migs through a comparative analysis [22]. To test this hypothesis, in vitro-cultured macrophages were stained with wheat germ agglutinin (WGA), a migrasome-specific fluorescein dye [23]. Numerous WGA-positive migrasomes were observed in both M0 and M1 macrophages (Fig. 1C, white arrow). Scanning electron microscopy (SEM) results revealed the presence of migrasomes tethered to the cell body through retraction fibers (Fig. 1D). In parallel, we performed WGA immunofluorescence staining and SEM analysis on M2 macrophages for comparative purposes (Figure S1C and S1D). Subsequently, migrasomes were isolated from in vitro-cultured cells through ultracentrifugation and gradient centrifugation (Fig. 1E). Western blot and other detection techniques were used to confirm that the isolated vesicles were migrasomes. Western blot confirmed the expression of migrasome markers in the isolated vesicles, including TSPAN4, Integrin α 5, EOGT, and PIGK. To distinguish the isolated vesicles from exosomes (another type of extracellular vesicle), exosome-specific markers (CD9, CD81, Alix, and TSG101) were examined and were undetectable (Fig. 1F) [24], indicating high purity of the purified migrasomes. Nanoparticle tracking analysis was conducted to determine the size of the in vitro-isolated migrasomes and their stability (Fig. 1G). Morphologically, TEM demonstrated that the purified vesicles exhibited typical migrasome structures, including mature migrasomes separated from contraction filaments and immature migrasomes tethered to contraction filaments (Fig. 1H). Super-resolution microscopy demonstrated single WGA-stained migrasome structures (Fig. 1I). Overall, these results demonstrated that migrasome expression was significantly upregulated in vivo under MI conditions and that abundant migrasomes were produced by M1 macrophages during the acute phase, potentially exerting a profound influence on disease progression.

M1-Migs exacerbate post-myocardial infarction injury

Given the dominance of M1 macrophages and our data in Fig. 1B demonstrating that migrasome expression reaches its peak in this critical stage, we hypothesize that M1-Migs play a pivotal role in MI progression [25, 26]. To confirm this hypothesis, we conducted a multilevel experimental verification. At the animal level, in vivo, M0-Migs or M1-Migs derived from RAW264.7 macrophages were intramyocardially injected into the infarct zone of MI mice subjected to standardized left anterior descending artery (LAD) ligation across groups (Fig. 2A and S2A). The survival rate of mice treated with M1-Migs was lower than that of the M0-Migs-treated or MI groups

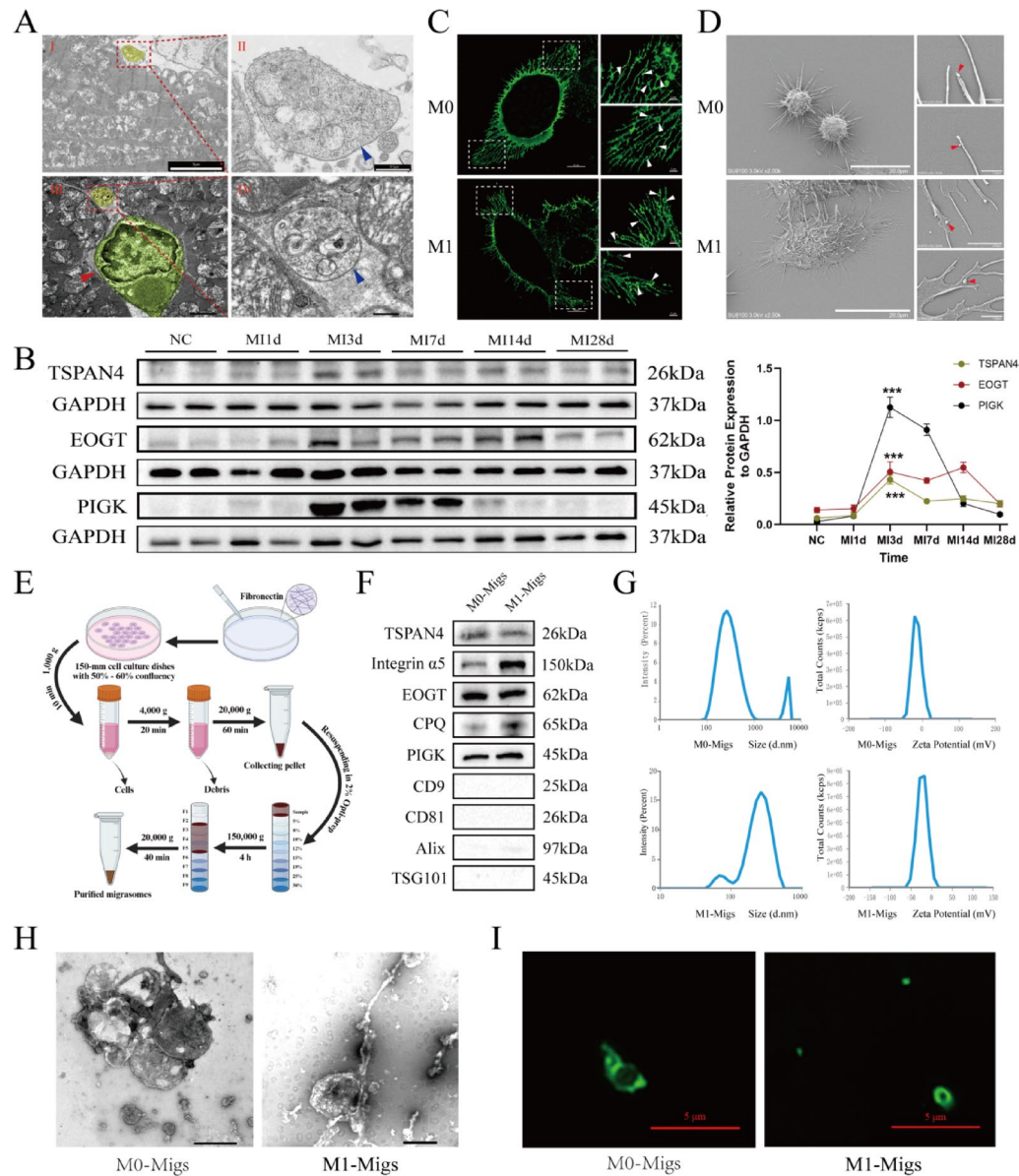


Fig. 1 Migrasomes detected in MI animal models and in vitro macrophages. **(A)** TEM images of myocardial tissue samples from MI mice. Blue arrows and yellow regions indicate migrasomes, while red arrows and green regions indicate macrophages. Data are representative of three biologically independent experiments. **(B)** Western blot analysis of migrasome-related marker proteins in myocardial tissues of MI mice at different time points, and the quantitative results are presented as the relative expression of proteins to GAPDH. Quantification and trend of protein expression are demonstrated in the line graph ($n=6$ mice/group). **(C)** Immunostaining images of wheat germ agglutinin (WGA)-labeled M0 and M1 macrophages, with magnified images of migrasomes on the right ($n=3$). White arrows indicate migrasomes. Scale bars: low magnification = 10 μm ; high magnification = 2 μm . **(D)** SEM images of M0 and M1 macrophages ($n=3$). Red arrows indicate migrasomes. Scale bars: low magnification = 20 μm ; high magnification = 2 μm –1 μm . **(E)** Schematic illustration of the migrasome purification procedure (created with BioRender.com). **(F)** Western blot assessment of migrasome marker expression in purified migrasomes. Data are representative of three biologically independent experiments. **(G)** Diameter and Zeta potential of M0-Migs and M1-Migs. Data are representative of three biologically independent experiments. Lines in the graph indicate the mean value. **(H)** Representative TEM images of purified M0 and M1 macrophage-derived migrasomes through negative staining ($n=3$). Scale bars = 500 nm. **(I)** Super-resolution microscopy images of WGA-stained purified migrasomes ($n=3$). Scale bars = 10 μm . Results are described as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. One-way ANOVA or two-way ANOVA was applied for multivariate analysis, and unpaired t-tests were applied for comparisons between two groups

(Fig. 2B). Twenty-eight days following MI, echocardiography demonstrated that M0-Migs treatment did not significantly influence cardiac function when assessed against MI alone. However, M1-Migs injection reduced

left ventricular ejection fraction (LVEF) and fractional shortening (LVFS) and increased left ventricular end-diastolic volume (LVEDV) more than did M0-Migs treatment or MI, leading to further deterioration of

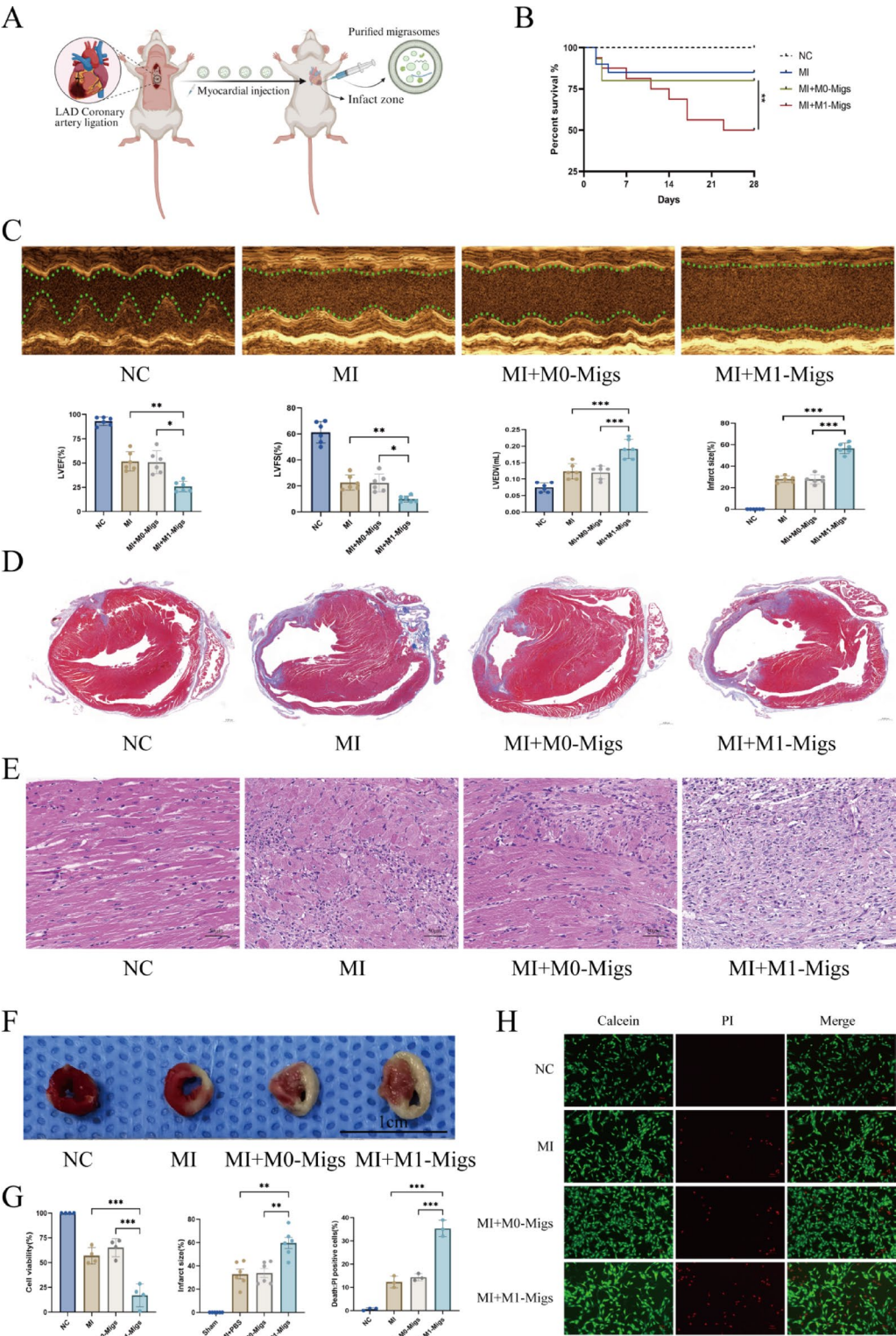


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Fig. 2 M1-Migs exacerbate cardiomyocyte and tissue injuries. **(A)** Schematic illustration of mouse model construction by ligating the left anterior descending (LAD) artery, followed by the intramyocardial injection of migrasomes into the infarct zone (created with BioRender.com). **(B)** Comparison of the survival rates of MI mice after intramyocardial injection of M0-Migs or M1-Migs (normal control (NC) group $n=6$, MI group $n=20$, M0-Migs group $n=15$, M1-Migs group $n=16$). **(C)** Echocardiography analysis 28 days post-infarction. Comparison of left ventricular ejection fraction (LVEF), fractional shortening (LVFS), and end-diastolic volume (LVEDV) among groups ($n=6$). **(D)** Masson's trichrome staining of cardiac tissue sections for infarct area comparison. Scale bars=0.500 mm ($n=6$). **(E)** Hematoxylin and eosin (HE) staining to detect myocardial tissue injury in each group. Scale bars=50 μ m ($n=6$). **(F)** Triphenyltetrazolium chloride (TTC) staining demonstrating a significantly larger MI area in the M1-Migs group than that in the M0-Migs and MI groups. Scale bars=1 cm ($n=6$). **(G)** Cell counting kit-8 (CCK-8) assay for viability of HL-1 cells under different treatments. The stimulation concentration of migrasomes was 100 μ g/ml ($n=4$). **(H)** Calcein/propidium iodide (PI) cell viability and toxicity assay for HL-1 cells under different treatments. Scale bars=100 μ m ($n=3$). Results are described as mean $\pm$ SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. One-way or two-way ANOVA was applied for multivariate analysis, and unpaired t-tests were applied for comparison between the two groups

left ventricular systolic function (Fig. 2C). Masson's trichrome staining of the tissue sections demonstrated that M1-Migs treatment enlarged the infarct area in mice (Fig. 2D). Hematoxylin and eosin (HE) staining demonstrated that M1-Migs injection promoted inflammatory cell infiltration, aggravated the disarray of muscle fibers, and resulted in severe myocardial injury (Fig. 2E). Triphenyl Tetrazolium Chloride (TTC) staining of cardiac tissues confirmed these results, with significantly larger infarct areas in M1-Migs-treated mice than in the MI and M0-Migs groups (Fig. 2F). TEM observations of tissue samples from different treatment groups demonstrated more pronounced swelling of cardiomyocytes in the M1-Migs group, blurred sarcomere bands, clear structural fractures of myocardial fibers, and misaligned mitochondria with severe ultrastructural damage, including outer membrane lysis, disappearance of the cristae space, fractures, and vacuolar changes (Figure S2B). At the cellular level, an in vitro cell model mimicking MI was established by treating mouse HL-1 cardiomyocytes with 200 μ M hydrogen peroxide (H_2O_2) [27]. Subsequently, cardiomyocytes were co-cultured with M0-Migs or M1-Migs. Cell counting kit-8 (CCK-8) assays demonstrated that M1-Migs significantly inhibited cardiomyocyte viability more than did M0-Migs at equivalent concentrations (Fig. 2G). Moreover, a dose-dependent aggravation of cardiomyocyte injury was observed with increasing M1-Migs concentrations (Figure S2C). Calcein/propidium iodide (PI) viability and toxicity assays confirmed that the results were consistent with those of CCK-8, demonstrating that M1-Migs significantly promoted cardiomyocyte mortality (Fig. 2H). Overall, the results from both animal and cellular experiments demonstrate that M1-Migs exacerbate myocardial injury more than M0-Migs following MI.

GBP5 is packed in M1-Migs

For further investigation of the molecular mechanism by which M1-Migs exacerbate myocardial tissue injury, quantitative proteomic analysis of M0-Migs and M1-Migs was conducted (Fig. 3A and Figure S3A). Volcano plots and heat maps demonstrated significant differences in protein content between M0-Migs and

M1-Migs (Fig. 3B and C). Given that previous experiments confirmed the deleterious effects of M1-Migs on cardiomyocytes, proteins associated with cellular injury were prioritized in the enrichment results. M1-Migs were enriched in the guanine nucleotide-binding protein (GBP) family. As interferon-induced guanosine triphosphatases (GTPases), GBPs have been reported to contribute to cellular injury processes through multiple mechanisms. Among these, GBP5 demonstrated the most pronounced content differences in the sequencing results [28, 29]. Studies have demonstrated that GBP5 is a key factor that exacerbates myocardial injury and that downregulation of GBP5 expression mitigates myocardial injury [30]. Thus, GBP5 may serve as a potential therapeutic target for ischemic heart disease and as a biomarker for injury severity evaluation. Therefore, this study hypothesized that M1-Migs mainly exacerbate cardiomyocyte injury through enriching GBP5 expression.

To confirm this hypothesis, we first validated the quantitative proteomic results. Western blot analysis of the isolated M0-Migs and M1-Migs demonstrated that M1-Migs expressed higher levels of GBP5 than did M0-Migs at the same total protein amount (Fig. 3D). Notably, myocardial tissues from MI mice not only demonstrated higher expression of migrasome-related proteins but also demonstrated higher GBP5 protein expression than did those from normal mice (Fig. 3E), indicating that GBP5 protein expression increases after MI in mice and that the increased GBP5 is likely derived mainly from M1-Migs. Moreover, immunofluorescence localization experiments using fluorescent labeling of migrasomes and GBP5 demonstrated that M1-Migs were rich in GBP5. The process by which M1 macrophages transferred GBP5 to migrasomes via retraction fibers and subsequent release of GBP5 from migrasomes into the extracellular environment was observed (Fig. 3F). To confirm the role of GBP5 in the injurious effects of M1-Migs, GBP5 expression in M1 macrophages was knocked down (Figure S3B), and the migrasomes derived from M1 macrophages with low GBP5 expression (M1-Migs (GBP5-KD)) were collected. Notably, immunofluorescence staining of M0 macrophages demonstrated that GBP5 was barely detectable in M0-Migs (Figure S3C).

The effects of M1-Migs and M1-Migs (GBP5-KD) were compared at both animal and cellular levels. Following intramyocardial injection of M1-Migs and M1-Migs (GBP5-KD) into MI mice, their effects on myocardial tissues were compared. Echocardiography results demonstrated that the M1-Migs (GBP5-KD) group exhibited significantly higher LVEF and LVFS and lower LVEDV than did the M1-Migs group, indicating that the knockdown of GBP5 substantially suppressed the inhibitory effect of M1-Migs on cardiac function (Fig. 3G). Masson's trichrome staining of myocardial tissue sections demonstrated a smaller infarct area in the M1-Migs (GBP5-KD) group than in the M1-Migs group (Fig. 3H), consistent with the TTC staining results (Fig. 3I), suggesting that GBP5 knockdown alleviated the myocardial infarct size. Furthermore, HE staining of tissue sections demonstrated that following GBP5 knockdown, inflammatory cell infiltration was significantly decreased, muscle fibers were better aligned, and structural damage was alleviated (Fig. 3J). At the cellular level, *in vitro* HL-1 cells mimicking MI were co-cultured with M1-Migs or M1-Migs (GBP5-KD). Calcein/PI viability and toxicity assays demonstrated lower cardiomyocyte mortality in the M1-Migs (GBP5-KD) group than in the M1-Migs (Fig. 3K), and CCK8 assays demonstrated higher cardiomyocyte viability in the M1-Migs (GBP5-KD) group than in M1-Migs (Fig. 3L), indicating that GBP5 knockdown inhibited the deleterious effects of M1-Migs. Overall, these data indicate that M1-Migs induce myocardial injury mainly through GBP5. Therefore, decreasing GBP5 expression in M1 macrophages can attenuate myocardial injury after MI to a certain extent.

M1-Migs exacerbate injury through promoting cardiomyocyte apoptosis in AMI

The specific mechanism by which M1-Migs promote cardiomyocyte injury via GBP5 was further investigated. Gene Ontology (GO) analysis of differentially expressed proteins between M1-Migs and M0-Migs revealed multiple apoptosis-related pathways among the enriched proteins (Fig. 4A), indicating that M1-Migs exacerbate myocardial injury after MI, primarily through activating the apoptotic pathway. GBP5 is closely associated with apoptosis; previous studies have demonstrated that GBP5 can induce hepatocyte apoptosis and aggravate liver injury and inflammation [31]. Accordingly, it was hypothesized that M1-Migs induce myocardial injury mainly through promoting apoptosis via GBP5. Subsequently, we conducted validation at both the cellular and animal levels. MI mice were intramyocardially injected with different sources of migrasomes, including M0-Migs, M1-Migs, and M1-Migs (GBP5-KD). Western blot results demonstrated increased expression of apoptotic proteins in the myocardial tissues of the M1-Migs

group, including elevated ratios of Bcl-2-associated X protein (BAX)/B-cell lymphoma 2 (BCL-2) and cleaved caspase3/caspase3, indicating more severe apoptosis in myocardial tissues (Fig. 4B). However, GBP5 knockdown significantly decreased the apoptotic effect on M1-Migs. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of tissue sections from different groups demonstrated the highest apoptosis rate in the M1-Migs group, which was mitigated by GBP5 knockdown (Fig. 4C). At the cellular level, we co-cultured HL-1 cells under *in vitro* MI-mimicking conditions with migrasomes from different sources and collected cellular proteins for western blot analysis of apoptotic protein expression. The results were consistent with those observed at the tissue level (Fig. 4D). Flow cytometry analysis of cells under different treatments demonstrated the highest apoptosis rate in HL-1 cells co-cultured with M1-Migs, whereas GBP5 knockdown decreased the apoptosis rate (Fig. 4E). TUNEL staining analysis of cells under different treatments also confirmed histological consistency (Fig. 4F). These data demonstrate that M1-Migs exacerbate myocardial tissue injury primarily by promoting cardiomyocyte apoptosis through GBP5.

The specific molecular pathways underlying apoptosis were also investigated. Previous studies have demonstrated that GBP5 expression is abnormally increased in damaged livers, and GBP5 induces hepatocyte apoptosis through activating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway [31]. Therefore, it was hypothesized that GBP5 also induces cardiomyocyte apoptosis through the NF- κ B pathway during myocardial injury. Western blot detection of NF- κ B pathway-related proteins in tissue and cell samples demonstrated significantly upregulated P-P65/P65 and inhibitor of kappa B alpha (I κ B α) expression in cardiomyocytes of the M1-Migs treatment group, while GBP5 knockdown significantly inhibited this effect (Fig. 4G-H), indicating the importance of GBP5 for M1-Migs in the activation of the NF- κ B pathway. Overall, these results demonstrate that M1-Migs exacerbate myocardial injury after MI primarily through activating the NF- κ B pathway through GBP5 to promote cardiomyocyte apoptosis.

Increased expression of migrasome-related proteins in monocytes in patients with MI

To investigate the association between macrophage-derived migrasomes and myocardial injury in patients with AMI, the findings were validated using clinical patient samples. Analysis of the GSE172270 dataset from the GEO database, based on the expression profile of key genes involved in migrasome formation, demonstrated that macrophage lineage cells in the serum of patients with AMI exhibited significantly higher expression of

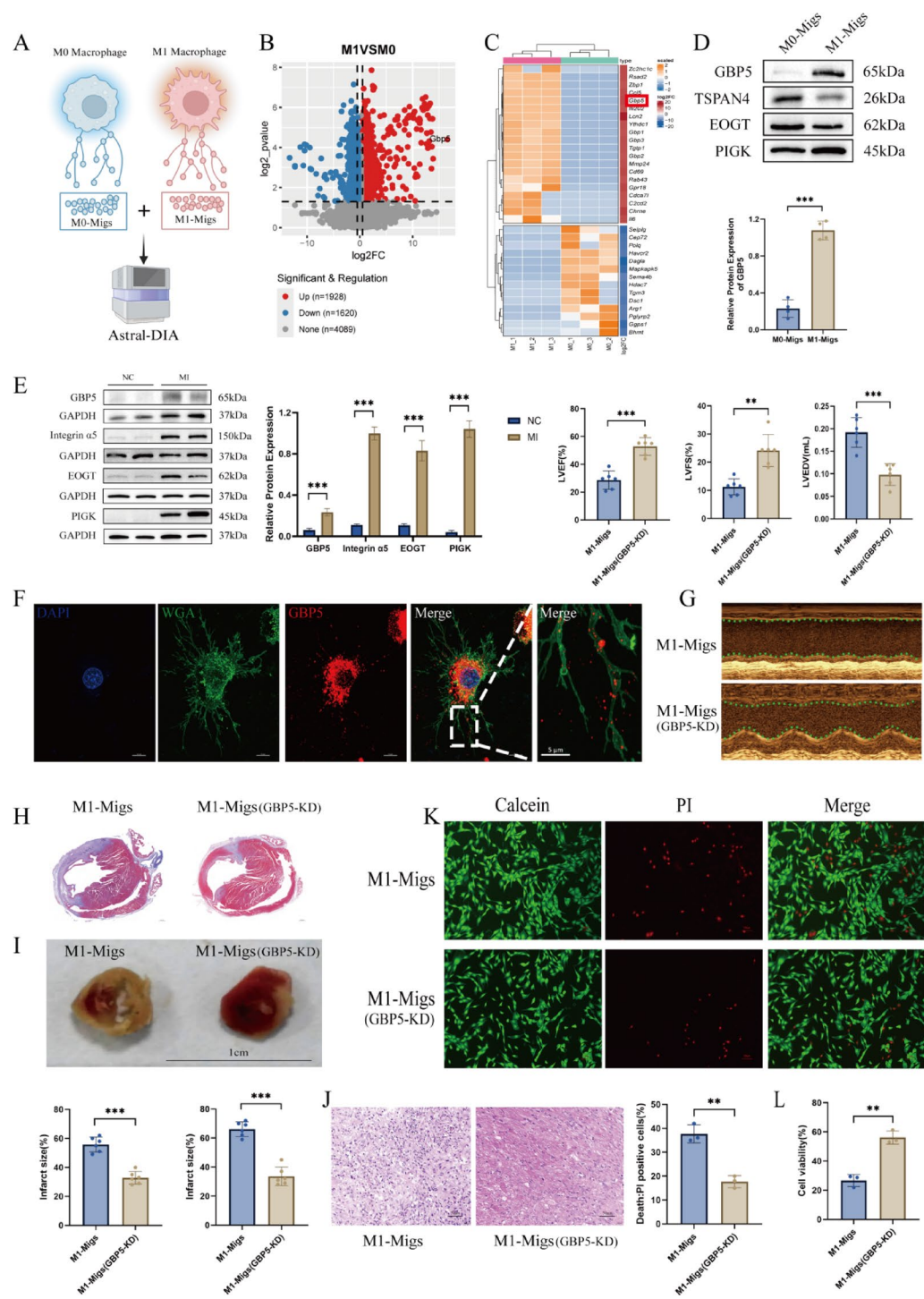


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TSPAN4 and carboxypeptidase Q (CPQ) than that of healthy controls (Fig. 5A). Both genes are critical for migrasome biogenesis. These findings provide preliminary evidence that macrophages produce more migrasomes in patients with AMI. To further validate these results, we analyzed the GSE145154 dataset by isolating immune cells (CD45⁺ cells) from the peripheral blood of patients

with heart disease and subjecting them to single-cell sequencing. First, quality control was applied to the raw sequencing data, and low-quality data were filtered out to ensure the accuracy of subsequent analyses (Figure S4A). The harmony algorithm was used to integrate single-cell sequencing data. Following batch-effect correction, the harmony-corrected principal components were used to

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Fig. 3 M1-Migs exert injurious effects primarily through guanylate binding protein-5 (GBP5). **(A)** Flowchart of quantitative proteomic sequencing (Astral-DIA) for M0-Migs and M1-Migs ($n=3$). **(B)** Quantitative volcano plot demonstrating differentially expressed proteins between M0-Migs and M1-Migs, with GBP5 ranked fifth among upregulated proteins in M1-Migs/M0-Migs. **(C)** Heat map of differentially expressed proteins between M0-Migs and M1-Migs, with GBP5 highlighted with red rectangles. **(D)** Western blot analysis comparing the protein profiles of M0-Migs and M1-Migs with the same amount of total protein ($n=4$). **(E)** Western blot comparison of GBP5 and migrasome-related protein expression between normal controls (NC) and MI groups. The quantitative results are presented as bar graphs ($n=6$). **(F)** Immunofluorescence localization of GBP5 in M1-Migs. Scale bars = 10 μm (low magnification) and 5 μm (high magnification). Data are representative of three biologically independent experiments. **(G)** Echocardiographic comparison of left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), and left ventricular end-diastolic volume (LVEDV) between groups ($n=6$). **(H)** Masson's trichrome staining for infarct area comparison. Scale bars = 0.500 mm ($n=6$). **(I)** Triphenyltetrazolium chloride (TTC) staining demonstrating a significantly larger infarct area in the M1-Migs group than in the M1-Migs guanylate binding protein 5 knockdown (GBP5-KD) group. Scale bars = 1 cm ($n=6$). **(J)** Hematoxylin and eosin (HE) staining to detect myocardial tissue injury in both groups. Scale bars = 50 μm ($n=6$). **(K)** Calcein/propidium iodide (PI) viability assay for HL-1 cell mortality under two treatments. Scale bars = 100 μm ($n=3$). **(L)** Cell Counting Kit-8 (CCK8) assay to assess HL-1 cell viability under two treatments ($n=3$). Results were shown as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. One-way or two-way ANOVA variance was applied for multivariate analysis. Unpaired t-tests were applied to compare two groups

construct unified uniform manifold approximation and projection (UMAP) and t-distributed stochastic neighbor embedding (t-SNE) embedding spaces (Fig. 5B). Following data integration, marker genes relevant to cell-type identification, such as the monocyte-associated genes protein tyrosine phosphatase receptor type C (PTPRC) and CD14, were identified. Bubble plots were generated with cell subsets on the horizontal axis and gene names on the vertical axis. Bubble size represented the proportion of cells expressing each gene, and color intensity indicated the average expression level. This approach yielded clear and intuitive bubble plots that represent the expression distribution of key genes across different cell subsets (Fig. 5C). Subsequently, nonlinear dimensionality reduction algorithms (t-SNE and UMAP) were applied to project high-dimensional data into a two-dimensional space to obtain distinct cell subsets, which were then annotated (e.g., monocytes, B cells, and T cells) (Fig. 5D). These steps ultimately resulted in the single-cell data analysis. Bubble plots revealed that monocytes exhibited increased expression of migrasome-related genes (TSPAN4, TSPAN9, phosphatidylinositol glycan anchor biosynthesis class K (PIGK), and CPQ) during myocardial injury (Fig. 5E). These data indicate that serum monocytes produce more migrasomes during myocardial injury.

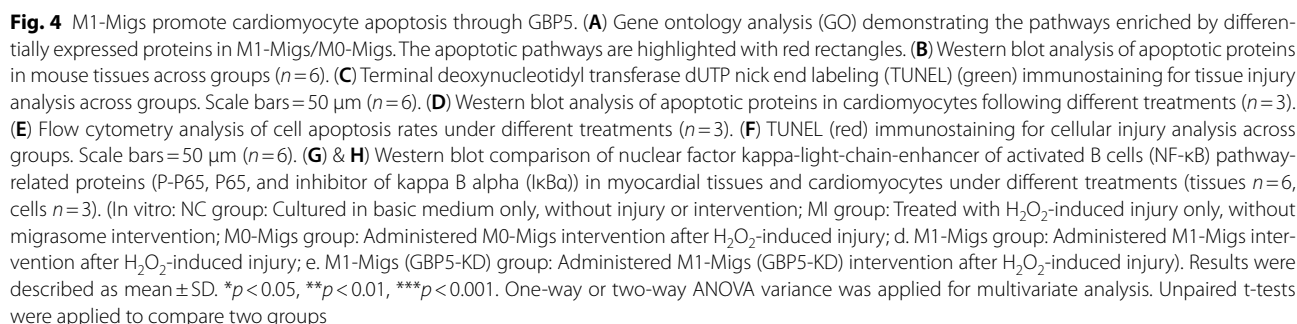
To confirm this finding, plasma from patients with AMI and healthy controls was collected, and migrasomes were isolated. Immunofluorescence staining with WGA followed by microscopic imaging demonstrated significantly higher migrasome abundance in the plasma of patients with AMI than in healthy controls (Fig. 5F). These findings indicate that macrophage lineages are responsible for excessive migrasome production in patients with AMI. Plasma monocytes were isolated by magnetic bead sorting (Figure S4B). Flow cytometry analysis demonstrated that monocytes from patients with AMI expressed higher levels of TSPAN4, Integrin $\alpha 5$, EOGT, and PIGK than did those from normal controls (Fig. 5G). Overall, clinical patient samples further

confirmed that the number of monocyte/macrophage-derived migrasomes was significantly increased in MI.

Colchicine alleviates post-myocardial infarction injury by inhibiting the formation of migrasomes

M1 macrophages were confirmed to exacerbate myocardial injury through production of migrasomes. Therefore, it is hypothesized that inhibition of migrasome biogenesis could mitigate the deleterious effects of M1 macrophages. A review of the literature and clinical evidence demonstrated that colchicine, beyond its established role in suppressing pro-inflammatory responses in M1 macrophages, acts as a classical microtubule inhibitor through binding to the β -subunit of tubulin to form a colchicine-tubulin complex [18, 19, 32]. This complex inhibits tubulin polymerization into microtubules and promotes the depolymerization of existing microtubules, resulting in intracellular microtubule network disruption. Given that microtubules are essential components of the cytoskeleton and play a critical role in cell migration, colchicine-induced disruption of their structure and function profoundly affects cell motility. Given the intimate association between migrasome biogenesis and migration, colchicine likely suppresses migrasome biogenesis through inhibiting cell migration. Clinical evidence demonstrates that low-dose colchicine significantly decreases major adverse cardiovascular events in patients with acute coronary syndrome and stable coronary artery disease. Its recent Food and Drug Administration (FDA) approval as the first anti-inflammatory drug for coronary heart disease highlights its therapeutic potential in cardiovascular medicine [16]. These findings warrant further investigation into the role of colchicine in the inhibition of macrophage migration and alleviation of myocardial injury following infarction.

The inhibitory effect of colchicine on cell migration was validated. Macrophages pretreated with 1 μM colchicine for 24 h were subsequently seeded into the upper chamber of Transwell inserts and incubated for another 48 h. Quantitative analysis revealed a greater decrease in migrated cell numbers in the colchicine-treated



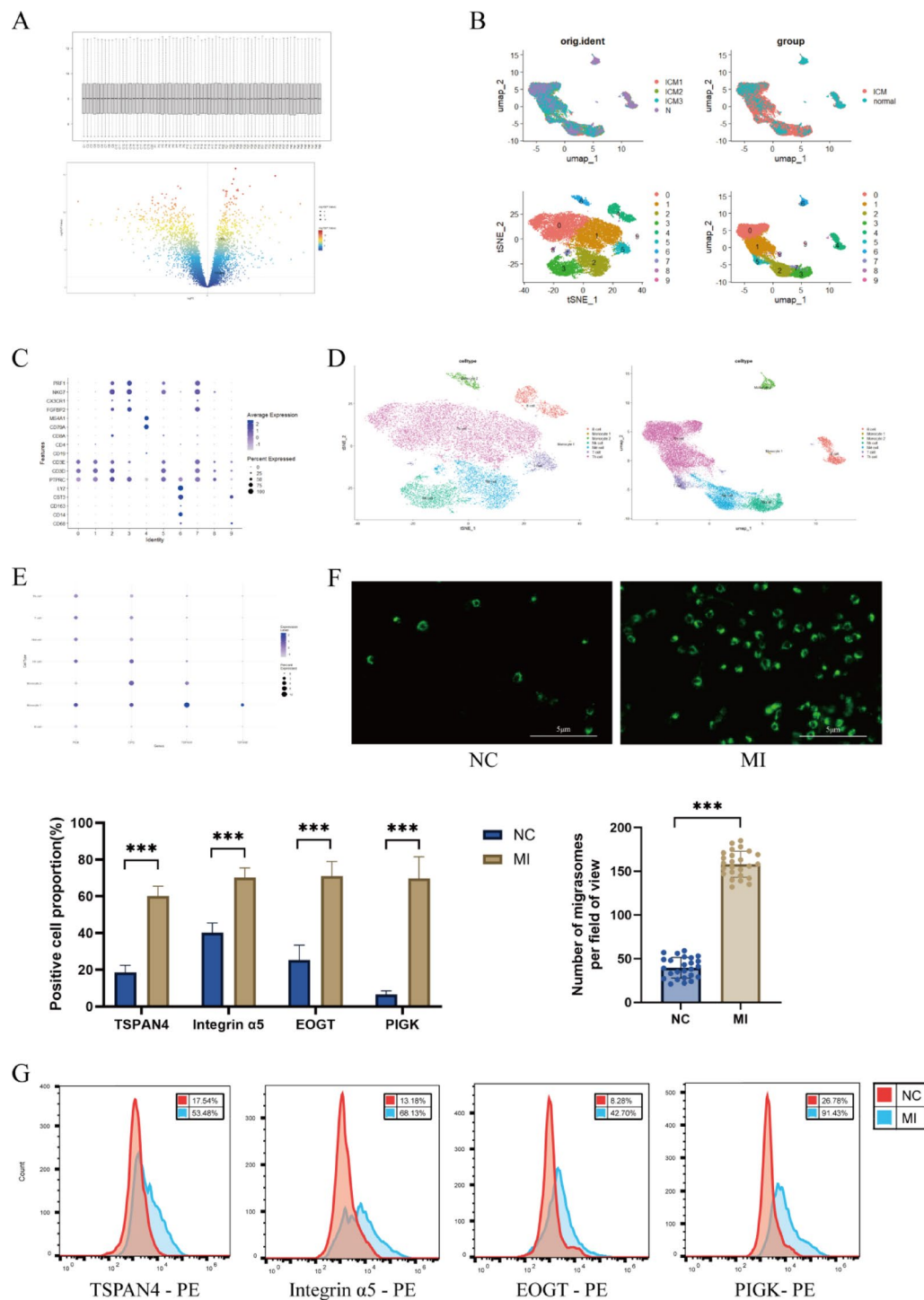


Fig. 5 Correlative analysis of macrophage-derived migrasomes and MI in clinical samples. **(A)** Box plots and volcano plots comparing macrophages in serum of patients with acute myocardial infarction and healthy controls, with tetraspanin 4 (TSPAN4) and carboxypeptidase Q (CPQ) annotated. **(B)** Uniform manifold approximation and projection (UMAP) and t-distributed stochastic neighbor embedding (t-SNE) plots integrating single-cell sequencing data. **(C)** Bubble plots demonstrating expression distribution of key genes across different cell subsets. **(D)** UMAP and t-SNE plots for annotation of distinct cell subsets. **(E)** Bubble plots demonstrate expression levels of migrasome-related genes in different cell subsets. Bubble size demonstrates the percentage of cells expressing each gene, and color indicates expression intensity. **(F)** Confocal microscopy images of wheat germ agglutinin (WGA)-labeled migrasomes from different plasma samples. Scale bars = 5 μ m ($n=20$). **(G)** Flow cytometry analysis of migrasome-associated markers (TSPAN4, Integrin α 5, EGF domain-specific O-linked N-acetylglucosamine transferase (EOGT), phosphatidylinositol glycan anchor biosynthesis class K (PIGK)) in monocytes ($n=12$). Results were described as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. A one-way ANOVA test or two-way ANOVA test was applied for multivariate analysis. Unpaired t-tests were applied for the comparison of two groups

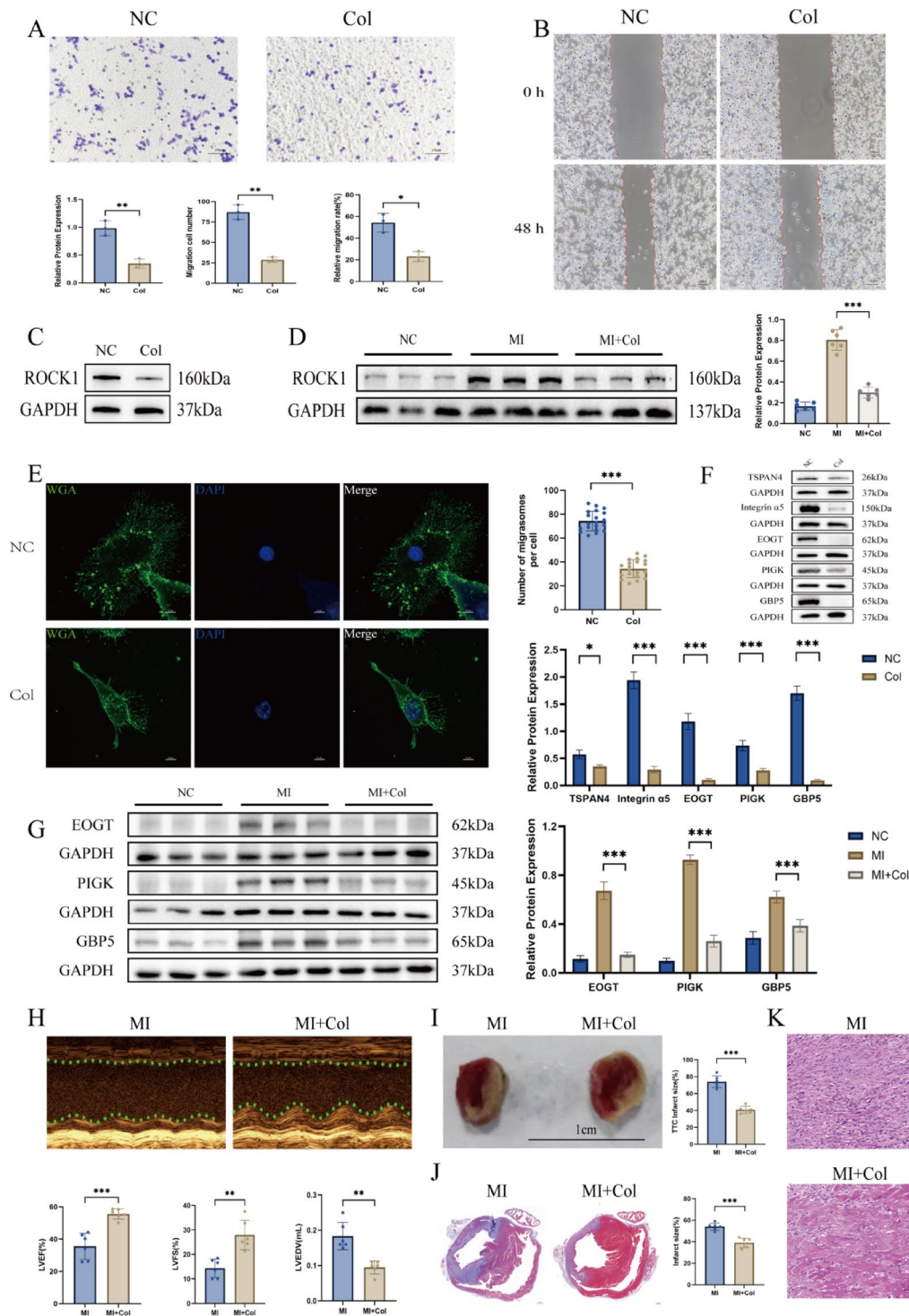


Fig. 6 (See legend on next page.)

macrophage group than in the controls (Fig. 6A and Figure S5A). Consistent with these results, wound-healing assays further demonstrated a decreased migration area in colchicine-treated macrophages (Figs. 6B and S5B), confirming the inhibitory effect of colchicine on macrophage migration. To explore the underlying cytoskeletal

mechanism, rho-associated coiled-coil containing protein kinase 1 (ROCK1) expression was assessed, a downstream effector kinase of RhoA-GTPase that enhances actin-myosin contractility through myosin light chain phosphorylation to drive cytoskeletal reorganization and focal adhesion dynamics for cell migration [33–35].

(See figure on previous page.)

Fig. 6 Colchicine ameliorates myocardial injury by inhibiting migrasomes. **(A)** Transwell assay for migration of M1 macrophages treated with colchicine. Scale bars = 100 μ m ($n=3$). **(B)** Wound healing assay for migration area of M1 macrophages following colchicine treatment. Scale bars = 100 μ m ($n=3$). **(C)** Western blot analysis of ROCK1 expression in M1 macrophages treated with colchicine ($n=3$). **(D)** Western blot analysis of ROCK1 expression in cardiac tissues following colchicine treatment ($n=6$). **(E)** Confocal microscopy quantification of migrasomes in M1 macrophages after 4',6-diamidino-2-phenylindole (DAPI)/wheat germ agglutinin (WGA) staining. Scale bars = 5 μ m ($n=20$). **(F)** Western blot analysis of migrasome-associated proteins (Tetraspanin 4 (TSPAN4), Integrin α 5, EGF domain-specific O-linked N-acetylglucosamine transferase (EOGT), phosphatidylinositol glycan anchor biosynthesis class K (PIGK), and guanylate-binding protein 5 (GBP5)) in M1 macrophages treated with colchicine ($n=3$). **(G)** Western blot analysis of migrasome-associated proteins (EOGT, PIGK) and GBP5 in cardiac tissues of colchicine-treated mice ($n=6$). **(H)** Echocardiographic comparison of left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), and left ventricular end-diastolic volume (LVEDV) between the control and colchicine groups ($n=6$). **(I)** Triphenyltetrazolium chloride (TTC) staining showing decreased myocardial infarct size after colchicine treatment. Scale bars = 1 cm ($n=6$). **(J)** Masson's trichrome staining for infarct size comparison between groups. Scale bars = 0.500 mm ($n=6$). **(K)** Hematoxylin and eosin staining for the evaluation of myocardial tissue injury between groups. Scale bars = 50 μ m ($n=6$). Results were described as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. A one-way ANOVA test or two-way ANOVA test was applied for multivariate analysis. Unpaired t-tests were applied for the comparison of two groups

Western blot analysis revealed decreased ROCK1 expression in colchicine-treated macrophages (Fig. 6C). Intraperitoneal injection of 2 mg/kg colchicine into MI mice similarly decreased ROCK1 expression in cardiac tissues (Fig. 6D), indicating that colchicine modulates this migrasome-associated marker. Immunofluorescence labeling with WGA further confirmed that colchicine treatment directly decreased migrasome production during macrophage migration (Figs. 6E and S5C). Western blot analysis demonstrated decreased expression of migrasome-associated proteins in colchicine-treated macrophages (Fig. 6F), consistent with the tissue-level findings (Fig. 6G). Notably, colchicine concomitantly suppressed GBP5 expression alongside the inhibition of migrasome formation, indicating that M1-Migs are likely the primary source of GBP5.

The current findings point to the possibility that colchicine mitigates post-infarction myocardial injury via the inhibition of migrasome formation. Echocardiographic assessment of the colchicine-treated infarcted mice demonstrated a significant improvement in cardiac function (Fig. 6H). Masson's trichrome and TTC staining of the cardiac sections demonstrated decreased infarct size (Figs. 6I–J), whereas HE staining demonstrated decreased inflammatory cell infiltration and improved tissue architecture (Fig. 6K). Overall, these results suggest that colchicine may ameliorate post-infarction myocardial injury by inhibiting M1 macrophage migration, providing supportive preclinical evidence for its potential clinical application.

Discussion

This study demonstrated the critical role of migrasomes in the macrophage-mediated progression of MI. M1 macrophages exacerbate myocardial injury after MI through generating migrasomes (M1-Migs), containing GBP5. M1-Migs promote myocardial cell apoptosis through GBP5-mediated activation of the NF- κ B pathway. Moreover, colchicine was found to alleviate myocardial injury through inhibiting migrasome biogenesis.

MI is a life-threatening disease that requires an in-depth exploration of its pathological mechanisms to

identify novel therapeutic targets and improve outcomes. It occurs when coronary blood flow is abruptly interrupted, resulting in ischemic necrosis of cardiomyocytes [36]. The immune system dynamically orchestrates all stages of MI, from early inflammation to late tissue repair; however, dysregulated immune activation or functional imbalance significantly worsens infarcted myocardial prognosis. Although inflammation initially contributes to the degradation of necrotic cells and restoration of tissue homeostasis, prolonged inflammation following tissue necrosis increases the risk of adverse MI outcomes [37]. M1 macrophage, a principal pro-inflammatory immune cell in MI, expresses high levels of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6), as well as inducible nitric oxide synthase (iNOS), leading to the production of nitric oxide (NO) [5]. These mediators degrade the myocardial extracellular matrix, increase vascular permeability, and induce cardiomyocyte apoptosis. Persistent inflammation promotes myocardial remodeling, fibrosis, and dysfunction. Previous studies have demonstrated that inhibition of M1 macrophage polarization decreases MI size, underscoring M1 macrophages as a promising therapeutic target for MI [6, 8].

Using TEM, migrasome structures were identified in the infarcted myocardial tissues. Western blotting and immunohistochemical analyses confirmed increased migrasome production in the infarcted myocardium. Migrasomes are recently discovered organelles that mediate intercellular communication; some researchers classify them as extracellular vesicles because of their structural similarity to exosomes [20]. Previous studies have underscored the pivotal role of exosomes in mediating immune cell-myocardium crosstalk during MI. For instance, M1 macrophage-derived exosomes exacerbate cardiac dysfunction through inhibiting neovascularization or altering vascular repair after injury, emphasizing the need to delineate the mechanisms through which M1 macrophages influence MI [38]. Unlike the exosomes produced by most cells, migrasomes are formed by highly migratory cells and carry source-cell-specific signatures [20]. Structurally, migrasomes contain multiple

internal vesicles that encapsulate diverse cargoes, including nucleic acids, proteins, damaged mitochondria, and autophagosomes, with the proposed hypothesis that exosomes are derived from migrasomal vesicle release [11, 20, 39]. Accordingly, investigating migrasomes may provide new insights into the mechanisms by which M1 macrophages aggravate MI and facilitate the identification of novel therapeutic targets. Recent studies in CVDs have demonstrated that migrasomes play a critical role in low-intensity pulsed ultrasound (LIPUS)-mediated mitochondrial quality control and endothelial cell-induced M1 macrophage polarization in atherosclerosis [40, 41]. This study demonstrated that M1-Migs exacerbate myocardial injury, thereby identifying M1-Migs as a potential therapeutic target to mitigate adverse cardiovascular events.

Proteomic analysis of M1-Migs demonstrated abundant GBP5, and pathway enrichment analysis indicated its association with apoptosis. It was confirmed that M1-Migs activate the NF- κ B pathway through GBP5 to promote cardiomyocyte apoptosis and exacerbate myocardial injury. GBP5 is a conserved GTP-binding protein that orchestrates the host defense mechanisms, inflammation, and cell death. It was first reported to mediate hepatocyte apoptosis in liver injury and promote vascular smooth muscle cell proliferation and foam cell formation during atherosclerosis [31]. Recent studies have demonstrated that GBP5 aggravates cardiac ischemic injury through amplifying myocardial inflammation [30]. This study demonstrated that GBP5 is a key mediator of myocardial injury, with migrasomes functioning as the major transport vehicle for GBP5, indicating that inhibiting migrasome biogenesis may represent a novel strategy for targeting GBP5.

Given the detrimental effects of M1-Migs in MI, colchicine, a well-established anti-inflammatory drug recently approved by the FDA for the anti-inflammatory therapy of coronary artery disease, was investigated [16, 17]. Colchicine acts by inhibiting tubulin polymerization, thereby disrupting cytoskeletal stability and cell migration, which are critical for cell migration [18]. In this study, colchicine was found to suppress Rho-associated coiled-coil containing protein kinase 1 (ROCK1), a key regulator of cell migration and migrasome formation [33–35]. Western blotting and immunofluorescence analyses confirmed that colchicine inhibited M1 macrophage migration, thereby mitigating post-MI injury. These findings provide mechanistic and translational evidence supporting the clinical applications of colchicine in cardiovascular diseases.

Our study has some limitations. First, migrasome isolation using ultracentrifugation and density gradient methods may yield preparations contaminated by cell debris and other vesicles. It is also important to note that

trace residual vesicles or soluble proteins from FBS could theoretically exert a weak interference on the quantitative analysis or functional validation of migrasomes. This underscores the need for improved purification techniques. For future research, we propose the following optimization directions: we plan to use commercially available EV-depleted FBS to further enhance the efficiency of serum pretreatment, and combine density gradient centrifugation with immunomagnetic separation (e.g., TSPAN4 antibody-conjugated magnetic bead sorting) to achieve high-purity enrichment of migrasomes. This integrated approach is expected to fundamentally address the potential risk of serum-derived contamination. Second, current migrasome research is confronted with a technical bottleneck characterized by ambiguous boundaries between the qualitative and quantitative analysis of biomarkers. Traditional biomarkers, typified by TSPAN4 involved in this study, can verify the basic molecular characteristics of migrasomes. However, their expression is susceptible to interference from multiple factors including cell polarization and microenvironmental signals, which renders them unfit to serve as the absolute standard for evaluating migrasome yield, purity and functional activity. In tissue sample detection, the signals of these biomarkers are more vulnerable to the impacts of factors such as complex cellular origins, thus only providing directional clues related to migrasome-associated biological events. This highlights the urgency of developing specific quantitative biomarkers for migrasomes. An ideal biomarker should possess high specificity and stability, and be capable of independently reflecting the quantity and functional status of migrasomes. Future research needs to combine multi-omics and high-resolution imaging technologies to establish an integrated detection system that incorporates both qualitative and quantitative analyses. Breaking through this technical bottleneck will enable more precise elucidation of the regulatory mechanisms of migrasomes in the pathophysiological processes of diseases such as myocardial infarction, thereby promoting the transition of migrasome research from phenotypic description to mechanistic interpretation. Third, while our study preliminarily confirmed the association between migrasome generation inhibition and alleviation of GBP5-mediated myocardial injury via colchicine intervention, this experimental strategy has inherent limitations, primarily due to insufficient effect decoupling caused by colchicine's non-specificity. Existing evidence confirms that colchicine suppresses not only migrasome biogenesis but also macrophage inflammatory activation and tissue infiltration independently [32]. This raises the possibility that the observed myocardial injury alleviation may partially result from its off-target anti-inflammatory effects rather than solely through inhibiting migrasome-mediated GBP5 transport. Furthermore, in

line with extracellular vesicle research principles, non-specific inhibitor data require validation with more specific approaches to ensure rigor. Colchicine's inability to distinguish between its effects on migrasome generation and other cellular functions hinders full confirmation of the core conclusion that GBP5 exerts its function in a migrasome-dependent transport manner.

Conclusion

This study demonstrates increased migrasome production in the setting of MI. Functionally, M1-Migs exacerbated post-infarction myocardial injury. Mechanistically, M1-Migs promoted cardiomyocyte apoptosis through GBP5-mediated activation of the NF- κ B pathway. Moreover, colchicine may mitigate myocardial injury by inhibiting migrasome biogenesis. These findings highlight migrasome biogenesis as a novel therapeutic target in MI and strengthen the evidence base for the clinical use of colchicine in cardiovascular disease. Future studies should develop precise therapies targeting M1-Migs to balance therapeutic benefits and risks.

Methods

Animal Experiments: eight-to-twelve-week-old wild type (WT) C57BL/6 J male mice were purchased from Medical Laboratory Animal Center of Shengjing Hospital Affiliated to China Medical University. (Liaoning, China). During the experiment, the mice were kept on a 12 h light/12 h dark cycle in pathogen-free conditions with free access to food and water at the institutional animal care facility. They were anesthetized with 2.0% isoflurane during MI model operation. After the study, all the mice were first anesthetized by allowing them to inhale 2.0% isoflurane and subjected to cervical dislocation. The AMI model: the mice were anesthetized with 2.0% isoflurane, ventilated with room air, and placed on a Small Animal Gas Anesthesia Machine (MIDMARK, USA) under direct vision. The third intercostal space was exposed, and the thoracic cavity was penetrated. The pericardium was dissected, and the left anterior descending (LAD) coronary artery was ligated using a 7–0 polyester suture. Mice in the NC-operated group underwent the same surgical procedure but without the LAD ligation. Migrasomes were administered via intramyocardial injection at a single dose of 10 mg/kg into the hearts of MI mice: specifically, three injection sites were targeted within the myocardial infarction (MI) area, with 10 μ L of migrasome suspension delivered per site (total injection volume: 30 μ L per animal). The injection was performed concurrently with MI induction to ensure immediate exposure of the ischemic myocardium to migrasomes. We labeled the purified migrasomes with PKH26 fluorescent dye (Sigma), and then injected the labeled migrasomes into the infarcted myocardial tissue. The tropism

of migrasomes toward the infarcted region was verified via fluorescence imaging. Colchicine (HY-16569, MCE) was intraperitoneally injected into MI mice at a dose of 2 mg/kg each time, once daily for one week after MI modeling.

Cell Lines Mouse macrophage cell line Raw 264.7 cell line and Mouse myocardial cell line HL-1 cell line were purchased from ATCC. All cell lines were tested for mycoplasma contamination prior to use, and the results confirmed they were contamination-free. All cell cultures were confirmed to be mycoplasma-free. RAW264.7 macrophages and HL-1 cardiomyocytes were cultured in Dulbecco's Modified Eagle Medium (DMEM, C11995500 BT, Gibco, USA) supplemented with 1% penicillin/streptomycin (P/S, 15140122, Gibco, USA) and 10% fetal bovine serum (FBS, 10091148, Gibco, USA). When macrophage confluency reached 60%–70%, cells were stimulated with 100 ng/ml lipopolysaccharide (LPS, L2880, Sigma) and 20 ng/ml interferon- γ (IFN- γ , 315-05-20, Peprotech) to promote polarization of M0 macrophages to M1 macrophages; separate aliquots of cells were treated with 20 ng/ml interleukin-4 (IL-4, 214-14-10, Peprotech) and 20 ng/ml interleukin-13 (IL-13, 210-13-10, Peprotech) under the same cell density conditions to drive M0 macrophage polarization toward the M2 phenotype. HL-1 cardiomyocytes were treated with 200 μ M hydrogen peroxide (H_2O_2) to establish an in vitro cell model mimicking MI. Migrasomes were used at a stimulation concentration of 100 μ g/ml.

Bulk and Single-Cell Sequencing: Bulk transcriptomic profiles (GSE172270) were used to assess human primary monocyte-derived macrophages between AMI patients and healthy volunteers. Single-cell RNA sequencing datasets (scRNA-seq) from peripheral blood in both normal and failed human heart (GSE145154) were obtained from the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/gds>).

Isolation of migrasomes from culture cells and plasma samples: 150-mm culture dishes were pre-coated with 0.1 μ g/ml fibronectin (BD 354008), and the coating solution was discarded before adding complete medium for macrophage culture. To prevent contamination from extracellular vesicles, soluble proteins, and potential migrasome-like structures in FBS, the following serum handling procedures were implemented prior to migrasome collection: For cell culture systems dedicated to migrasome harvesting, serum deprivation was initiated 24 h before sample collection by replacing the regular serum-containing medium with serum-free basal medium. For experimental groups with high serum dependency, maintaining cell viability is critical for normal migrasome biogenesis in these groups. The medium for these groups was prepared using vesicle-depleted FBS pretreated by

a two-step ultracentrifugation protocol. The first step involved centrifugation at $100,000 \times g$ for 12 h, and the pellet was discarded to remove most large-sized vesicles. The second step was centrifugation at $150,000 \times g$ for 18 h to further eliminate residual small-sized exosomes and soluble protein complexes. After aspirating the culture supernatant, cells were digested with 0.25% trypsin, and the resulting cell digestion mixture was collected into 50-mL centrifuge tubes for subsequent migrasome isolation. Centrifugation was performed at 4°C : cells and large debris were removed by centrifugation at $1000 \times g$ for 10 min followed by $4000 \times g$ for 20 min. Crude migrasomes were separated as pellets by ultracentrifugation at $20,000 \times g$ for 1 h, and then subjected to a density gradient (30% $\rightarrow$ 25% $\rightarrow$ 19% $\rightarrow$ 15% $\rightarrow$ 12% $\rightarrow$ 10% $\rightarrow$ 8% $\rightarrow$ 5% $\rightarrow$ 2%) prepared with 400 μL 1 $\times$ Extraction Buffer, 253 μL Optiprep (60%) (Sigma-Aldrich, LYSIS 1), and 137 μL 1 $\times$ Dilution Buffer. Except for the 800- μL crude migrasome fraction, each gradient layer was 500 μL . The gradient was centrifuged at $150,000 \times g$ for 4 h in an MLS-50 rotor (Beckman, Optima MAX-XP). Fractions 3 to 5 (F3–F5) were sequentially collected from top to bottom at a volume of 500 μL per fraction following centrifugation. To remove organic components, each fraction was mixed with an equal volume of PBS and centrifuged twice at $20,000 \times g$ for 40 min to collect pure migrasome pellets. Blood samples were collected in anticoagulant tubes using standard venipuncture protocols. Large debris were removed by centrifugation at $1000 \times g$ for 10 min followed by $4000 \times g$ for 20 min. Crude migrasomes were collected as pellets by centrifugation at $20,000 \times g$ for 30 min, washed with PBS, and re-centrifuged at $20,000 \times g$ for 20 min. The sample preparation method was compatible with Western blot and mass spectrometry analyses. The particle size and zeta potential of isolated migrasomes were measured using a Litesizer 500 particle analyzer (Anton Paar, Austria).

GBP5 Knockdown via Lentiviral Transduction RAW264.7 macrophages were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C with 5% CO_2 . To induce M1 polarization, cells were treated with 100 ng/mL lipopolysaccharide (LPS, L2880, Sigma) and 20 ng/mL interferon- γ (IFN- γ , 315-05-20, Peprotech) for 24 h. Lentiviral vectors encoding short hairpin RNA (shRNA) targeting mouse GBP5 and scrambled negative control shRNA were constructed using the pLKO.1-puro vector. Recombinant lentiviruses were produced by co-transfecting shRNA plasmids with packaging plasmids (psPAX2 and pMD2.G) into HEK293T cells via Lipofectamine 3000 (Invitrogen, USA). Viral supernatants were harvested at 48 h and 72 h post-transfection, filtered through 0.45 μm membranes, and concentrated by ultracentrifugation. M1 macro-

phages were seeded in 6-well plates at 2×10^5 cells/well and infected with lentivirus at a multiplicity of infection (MOI) of 10 in the presence of 5 $\mu\text{g/mL}$ polybrene (Sigma-Aldrich, USA). After 24 h of transduction, the medium was replaced with fresh complete medium. At 72 h post-infection, stable transfectants were selected with 2 $\mu\text{g/mL}$ puromycin for 48 h. Knockdown efficiency was validated by Western blot analysis to detect GBP5 protein expression levels. M1-polarized RAW264.7 cells without transduction and those transduced with sh-NC served as blank control and negative control, respectively.

Western blot analysis: to obtain protein samples, an appropriate amount of RIPA cell lysis buffer (Solarbio, Cat#R0020) was added to cell or migrasome samples, which were then lysed by sonication at 50 W (Scientz, China) for 30 s. Forty micrograms of protein from each sample were used for Western blot experiments. Western blotting was performed using standard SDS-polyacrylamide gel electrophoresis and ECL Western HRP substrate (SA00001-1, Proteintech). The following primary antibodies were used: rabbit anti-TSPAN4 (LS-C446997-100, Lifespan, 1:1000), rabbit anti-Integrin $\alpha 5$ (Abcam ab150361, 1:2000), rabbit anti-NDST1 (Proteintech 26203-1-AP, 1:1000), rabbit anti-PIGK (Proteintech 15151-1-AP, 1:1000), rabbit anti-EOGT (Abcam ab190693, 1:2000), rabbit anti-CPQ (Proteintech 16601-1-AP, 1:1000), rabbit anti-CD9 (Proteintech 20597-1-AP, 1:2000), rabbit anti-CD81 (Proteintech 27855-1-AP, 1:1000), rabbit anti-Alix (Proteintech 12422-1-AP, 1:5000), rabbit anti-TSG101 (Proteintech 28283-1-AP, 1:1000), rabbit anti-ROCK1 (CST 28999 S, 1:1000), rabbit anti-GBP5 (Abcam ab313390, 1:1000), rabbit anti-Caspase 3 (Proteintech 19677-1-AP, 1:1000), rabbit anti-BAX (Abmart AB_2910262, 1:1000), rabbit anti-BCL-2 (Abmart AB_2929011, 1:1000), rabbit anti-P65 (Proteintech 80979-1-RR, 1:1000), rabbit anti-P-P65 (Proteintech 82335-1-RR, 1:1000), rabbit anti-I κ B α (Abmart AB_2937048, 1:1000), rabbit anti-iNOS (Abcam ab15323, 1:1000), rabbit anti-CD11b (Abcam ab184308, 1:1000), and mouse anti-GAPDH (Proteintech 60004-1-Ig, 1:1000).

Immunofluorescence Staining macrophages were seeded onto glass slides at 40%–50% confluency for 24 h to observe migrasomes. After 24 h, cells were fixed with 4% paraformaldehyde for 15 min, washed with PBS, and stained with 1 $\mu\text{g/mL}$ WGA (W11261, Invitrogen, USA) for 15 min. Migrasomes were then visualized using a confocal microscope (Lsm900, Zeiss). Purified migrasomes were observed under a super-resolution microscope (N-SIM + N-STORM, Nikon). Fixed macrophages were incubated overnight at 4°C with anti-GBP5 antibody, followed by labeling with fluorescent secondary antibody (HA720228F, HUABIO). After co-staining with WGA and

DAPI, the localization of GBP5 in M1-Migs was observed under the confocal microscope.

Transmission Electron Microscopy (TEM) before sample collection, a petri dish containing TEM fixative (G1102, Servicebio) was pre-prepared. Tissues were cut into small blocks ($\leq 1 \text{ mm}^3$) with a scalpel directly in the fixative within 1–3 min after ex vivo isolation, and immediately immersed in the petri dish. The samples were fixed with 1% osmium tetroxide prepared in 0.1 M phosphate buffer (PB, pH 7.4), dehydrated at room temperature, infiltrated and embedded with acetone, polymerized, positioned, ultrathin-sectioned, stained with 2% uranyl acetate in saturated ethanol (protected from light), and 2.6% lead citrate (protected from CO_2). Microstructural observation of tissues was performed using a transmission electron microscope (HT7800/HT7700, Hitachi). For isolated migrasomes, membrane structures were visualized by TEM after negative staining with 3% phosphotungstic acid.

Scanning Electron Microscopy (SEM) macrophages were cultured on 20-mm cell slides pre-coated with 10 $\mu\text{g}/\text{ml}$ fibronectin for 24 h, fixed with 4% paraformaldehyde for 1 h, followed by post-fixation with 1% osmium tetroxide for 1 h. Dehydration was performed using a gradient of ethanol concentrations (30% $\rightarrow$ 50% $\rightarrow$ 70% $\rightarrow$ 80% $\rightarrow$ 90% $\rightarrow$ 95% $\rightarrow$ 100%). The cell slides were attached to sample holders with conductive adhesive and sputter-coated in an ion sputtering device.

TUNEL Staining tissue sections or cell slides were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100, followed by staining according to the instructions of the TUNEL kit (HY-K1078-50T, MCE). Apoptotic cells were observed and imaged under a fluorescence microscope for distribution and quantification.

Flow Cytometry Analysis: to evaluate differences in migrasome markers on the surface of monocytes in plasma between AMI patients and healthy individuals, monocytes were first isolated from plasma using magnetic bead sorting (8804-6837-74, Invitrogen). The purity of isolated monocytes was analyzed by flow cytometry after labeling with anti-human CD14 antibody (367108, Biolegend). Migrasome markers were labeled with different primary antibodies overnight, including rabbit anti-TSPAN4 (LS-C446997-100, Lifespan, 1:200), rabbit anti-Integrin $\alpha 5$ (Abcam ab150361, 1:200), rabbit anti-PIGK (Proteintech 15151-1-AP, 1:100), and rabbit anti-EOGT (Abcam ab190693, 1:200). Cells were then labeled with anti-rabbit secondary antibody (SA00008-2, Proteintech) for flow cytometry analysis using a FACS flow cytometer (BD Biosciences, San Jose, CA). For determining cardiomyocyte apoptosis rates, cells under

different treatments were labeled with a cell apoptosis detection kit (HY-K1073-50T, MCE) and analyzed by flow cytometry.

Transwell Assay to investigate the effect of colchicine on macrophage migration, colchicine-treated macrophages were seeded into the upper chamber of Transwell inserts (BL 910 A, Biosharp). After 48 h of incubation, the inserts were fixed with 4% paraformaldehyde for 20 min, followed by staining with 0.1% crystal violet (G1063, Solarbio) for 15 min. Cells in the upper chamber were then removed with cotton swabs. Migrated cells were observed and counted under a microscope in at least three randomly selected fields of view. The number of migrated cells was quantified using ImageJ software, and migration differences were analyzed with GraphPad Prism 10.

Wound Healing Assay macrophages were seeded into 6-well plates and treated with colchicine. When cell confluency reached 80%–90%, uniform wounds were created by scraping the cell monolayer vertically with a sterile 200 μL pipette tip. Detached cells in the wounded area were removed by gentle washing with PBS for 2–3 times. Images of the wounded regions were captured under a microscope at 0 h and 48 h, respectively. The width of the wounds at each time point was measured using ImageJ software to calculate the wound healing rate. Statistical analysis was performed to compare differences in cell migration ability among different treatment groups.

Statistical Analysis All statistical analyses and graphic presentation were performed using GraphPad Prism 10.0 (GraphPad Software, USA). Results were presented as mean $\pm$ standard deviation (SD) or mean $\pm$ standard error of mean (SEM). Student's t test was performed in data comparison of two groups. One-way ANOVA was performed in data comparison of three groups or more. Log-rank test was performed on survival rates. To assess the associations of indications, Spearman correlation analysis was applied to assess the correlations of continuous variables, and Point-biserial correlations analysis was applied to assess correlations between dichotomous and continuous variables. Results were considered significant at $p < 0.05$.

Abbreviations

AMI	Acute myocardial infarction
BAX	Bcl-2-associated X protein
BCL-2	B-cell lymphoma 2
CCK-8	Cell counting kit-8
Col	Colchicine
CPQ	Carboxypeptidase Q
CVD	Cardiovascular disease
DMEM	Dulbecco's Modified Eagle Medium
ECL	Enhanced chemiluminescence
EOGT	EGF domain-specific O-linked N-acetylglucosamine transferase
FDA	Food and Drug Administration

FBS	Fetal bovine serum
GBP5	Guanylate binding protein 5
GBP5-KD	GBP5 knockdown
GEO	Gene Expression Omnibus
GO	Gene Ontology
H ₂ O ₂	Hydrogen peroxide
HE	Hematoxylin and eosin
IFN- γ	Interferon- γ i κ ba: inhibitor of kappa B alpha
LAD	Left anterior descending artery
LPS	Lipopolysaccharide
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVFS	Left ventricular fractional shortening
MI	Myocardial infarction
M0-Migs	M0 macrophage-derived migrasomes
M1-Migs	M1 macrophage-derived migrasomes
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NDST1	N-deacetylase/N-sulfotransferase 1
PBS	Phosphate-buffered saline
PI	Propidium iodide
PIGK	Phosphatidylinositol glycan anchor biosynthesis class K
PTPRC	Protein tyrosine phosphatase receptor type C
ROCK1	Rho-associated coiled-coil containing protein kinase 1
scRNA-seq	Single-cell RNA sequencing
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TSPAN4	Tetraspanin 4
TTC	Triphenyl tetrazolium chloride
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UMAP	Uniform manifold approximation and projection
WGA	Wheat germ agglutinin

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Qingfu Zhang, Aolin Du, and Zhichao Li contributed equally to this work; they jointly conceived and designed the study, performed the majority of experiments, collected and analyzed key data, and drafted the initial version of the manuscript. Hui Gu and Wanqi Huang provided critical experimental support by performing specific experiments, and they also assisted in the interpretation of experimental data. Ying Li and Su Han participated in the analysis and verification of research data, and contributed to the revision of the manuscript—including refining figures and optimizing descriptions of experimental procedures. Chuanhe Wang and Zhijun Sun co-supervised the study, provided overall research direction and funding support, revised the manuscript critically for important intellectual content, and approved the final version for publication. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All animal experiments were performed according to the protocol approved by the Experimental Animal Ethics Committee of Shengjing Hospital Affiliated to China Medical University (2023PS1249K). The clinical studies were approved by the Medical Ethics Committee of Shengjing Hospital Affiliated to China Medical University (2025PS1577K). Human material Peripheral blood samples were provided by 12 healthy individuals, 12 AMI patient. The diagnostic criteria for AMI patients were according to the ESC/AHA/ACC guidelines. The study participants were patients with AMI based on electrocardiographic diagonal evidence, chest pain, and diagnostic cardiac catheterization at Shengjing Hospital Affiliated to China Medical University. The exclusion criteria were: (1) diabetes, (2) infectious diseases, and (3) other contraindications including tumors and nephritic or hepatic diseases. This experiment was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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