Exosomal hsa_circ_0007047 Attenuates Post-myocardial Infarction Remodeling by Promoting Angiogenesis via miR-1178-3p/PDPK1 Axis

Shuai MAO (maoshuai@gzucm.edu.cn)
Stanford University School of Medicine

Yubin Liang
Stanford University School of Medicine

Ling Yu
Stanford University School of Medicine

Minzhou Zhang
Stanford University School of Medicine

Phillip C. Yang
Stanford University School of Medicine

Aleksander Hinek
Hospital for Sick Children

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Abstract

Emerging studies indicate that exosomes and their inner noncoding RNAs, especially circular RNAs (circRNAs), play key roles in gene regulatory network and cardiovascular repair. However, our understanding of exosomal circRNAs on cardiac remodeling after myocardial infarction (MI) remains limited. In the present study, exosomes were harvested from the serum of patients with and without postinfarction cardiac remodeling. The results showed that the level of hsa_circ_0007047 was significantly downregulated in serum exosome of patients with the adverse cardiac remodeling when compared with those without post-MI remodeling or normal subjects. Loss-of-function approaches in vitro established that exosomal hsa_circ_0007047 robustly promoted angiogenesis and stimulated of cultured human vascular smooth muscle cells proliferation and migration. Accordingly, overexpression of exosomal hsa_circ_0007047 in mice significantly attenuated MI-induced myocardial fibrosis and left ventricular dysfunction, accompanied by a larger functional capillary network at the border zone. Further exploration of the downstream target gene indicated that hsa_circ_0007047 acts as a competing endogenous RNA by directly binding to miR-1178-3p and thereby inducing transcription of its target gene phosphoinositide-dependent kinase-1 (PDPK1), a critical positive regulatory factor of angiogenesis. Together, our results revealed that exosomal hsa_circ_0007047 attenuated detrimental post-MI remodeling via miR-1178-3p/PDPK1 axis, which facilitated revascularization, ultimately improved the cardiac function.

Introduction

Despite current pharmacological and technological treatment strategies, progression to heart failure occurs in up to one-third of myocardial infarction (MI) patients because of adverse cardiac remodeling. Pathological left ventricular (LV) remodeling after MI is one of the most important risk factors for the development of complicated ventricular arrhythmia, congestive heart failure and even ultimate cardiogenic death. On the other hand, it has been showed that amplified myocardial angiogenesis, facilitating neo-vascularization is mainly responsible for structural preservation of infarcted myocardium and maintaining its basic function. Therefore, promotion of myocardial angiogenesis has been proposed as one of efficient therapeutic approaches for cardiac dysfunction and deleterious remodeling after MI.

Meaningfully, recent studies aimed at this phenomenon explored whether exosomes, the membranous vesicular bodies typically 30–100 nm in diameter, might be also involved in its regulation. Exosomes contain multiple proteins, mRNAs and noncoding RNAs, contributing to the cellular communication and regulation of the multiple processes. The number and content of heterogenous exosomes not only affect the physiological state of different normal cells, but they could be also linked to many pathological pathways. Importantly, it has been already demonstrated that exosomes play crucial roles in the development of cardiovascular diseases, specifically, in the regulation of the post-MI cardiac remodeling. Thus, it has been shown that the CD4-activated exosomes promote the post-ischemic cardiac fibrosis,
through miR-142-3p/Wnt signaling cascade-mediated activation of interstitial myofibroblasts. This finding suggested that pharmacologic targeting of miR-142-3p in CD4-activated exosomes may hold promise for alleviation of the post-MI cardiac remodeling. Moreover, application of exosomes isolated from the plasma of ischemia-conditioned rats improved cardiac function and angiogenesis after MI through targeting the 70-kDa heat shock protein. Song et al., also showed that application of exosomes derived from the umbilical blood mesenchymal stem cells attenuated myocardial injury by inhibiting ferroptosis in mice with experimental MI. In addition, the use of certain exosome-derived noncoding RNAs, such as the miRNAs and IncRNAs has been proven beneficial for healing of human post-MI myocardium. For example, exosomal IncRNA AK139128 derived from hypoxic cardiomyocytes promoted apoptosis and inhibited proliferation of cardiac fibroblasts. LncRNA KLF3-AS1 isolated from human mesenchymal stem cells-derived exosomes ameliorates pyroptosis of cardiomyocytes and alleviated the outcome of MI through the induction of the miR-138-5p/sirtuin1 axis. Moreover, exosomes isolated from coronary serum of patients with MI promote myocardial angiogenesis through the miRNA-143/insulin-like growth factor-1 receptor pathway. Together, the above-mentioned results indicate that non-coding RNAs may indeed play crucial roles in the regulation of different processes contributing to the final clinical outcome of patients afflicted with MI.

More precisely, the circular non-coding RNAs (circRNAs), which are widely present in eukaryotic cells and participate in the regulation of the transcription and post-transcriptional expression of multiple genes contributing to normal cardiac functions, might also regulate the development of certain cardiac pathologies. Meaningfully, it has been proposed that certain changes of circRNAs levels might be considered as a potential biomarker of the therapeutic efficiency of cardiovascular diseases. Indeed, circRNA HIPK3 was demonstrated to contribute to cardiac regeneration after experimental MI in mice by binding to Notch1 and miR-133a. Elevation of circRNA 010567 also associated with the improvement of cardiac function and alleviation the myocardial fibrosis of MI rats through inhibiting transforming growth factor β1. Moreover, circRNA Ttc3 in cardiomyocytes counteracted the hypoxia-induced ATP depletion and the deterioration of cardiac dysfunction, by sponging miR-15b in a rat model of MI. Exosomal circRNA 0001273 derived from human umbilical cord mesenchymal stem cells remarkably inhibited the occurrence of myocardial cell apoptosis and subsequently promoted MI repair in ischemic environment. However, other putative regulatory mechanism in which exosome-derived circRNAs would contribute the postinfarct cardiac remodeling, remain to be better explored.

To provide a basis for the further study of the molecular pathogenesis of LV remodeling after MI and to identify potential therapeutic targets for this disease, we compared the expression profiles of exosomal circRNAs in patients with and without cardiac remodeling using a high-throughput RNA sequencing. First, we tested function of exosomal hsa_circ_0007047, which has already been related to LV remodeling, in vitro and in vivo using silencing and overexpression strategies. Then, we explored the mechanisms underlying the actions of exosomal hsa_circ_0007047 during the LV remodeling after MI. We anticipated that the obtained results would improve our understanding of exosomal circRNAs regulation during development of cardiovascular diseases.
Results

Exosome isolated from serum of patients with post-MI cardiac remodeling contains the significantly downregulated levels of hsa_circ_0007047

Our initial whole transcriptome sequencing analysis was aimed at the exploration of circRNAs expression in exosomes derived from sera of patients with and without postinfarct cardiac remodeling. The obtained results indicated that three differentially expressed exosomal circRNAs (hsa_circ_0000212, hsa_circ_0089282 and hsa_circ_0007047) could be detected in the comparison between postinfarct cardiac remodeling (CR) vs. control groups and also between CR vs. non-postinfarct cardiac remodeling (N-CR) group (Fig. 1A, Supplementary Table 4).

Importantly, we fund that while the expression of hsa_circ_0000212 and hsa_circ_0089282 was significantly higher, the expression of hsa_circ_0007047 was downregulated in both CR vs. N-CR group, and CR vs. N-CR groups (Fig. 1B and C). In order to confirm these results, qRT-PCR was performed to detect the expression of these circRNAs by using divergent primers in the serum exosomes of patients with and without post-MI cardiac remodeling. The isolated exosomes were identified by TEM and NTA. The results showed that the diameter of exosomes is between 100–150 nm, which presented the typical exosomal morphology (Fig. 1D). Then, we additionally found that the exosomal biomarkers, CD6, CD9 and TSG101 were also highly detected in all groups (Fig. 1E). Consistent with the transcriptome analysis, qRT-PCR results showed that the expression of hsa_circ_0000212 and hsa_circ_0089282 was significantly elevated while the expression of hsa_circ_0007047 was downregulated in the comparison between both the CR vs. N-CR groups and in CR vs. control groups (Fig. 1F).

Characteristics of hsa_circ_0007047

To learn whether hsa_circ_0007047 functions act as circRNA during the postinfarct cardiac remodeling, first the ring structure of hsa_circ_0007047 was detected (Fig. 2A). The specific products of hsa_circ_0007047 were probed with the divergent and convergent primers. The agarose electrophoresis assay demonstrated that hsa_circ_0007047 could be amplified from both cDNA and gDNA template by using convergent primers while it only could be amplified from cDNA template by using divergent primers (Fig. 2B). However, the GAPDH could only be amplified by the convergent primers, using cDNA and gDNA as the template.

Moreover, the reverse shear site of hsa_circ_0007047 was confirmed by Sanger sequencing of the amplified product of hsa_circ_0007047 (Fig. 2C). Finally, qRT-PCR was performed with cDNA as templates after the RNA was digested by RNA exonuclease R before reverse transcription, GAPDH as the negative control. The results showed that the expression of hsa_circ_0007047 was not changed in RNase R(+) group compared to the RNase R(-) group. Meaningfully, the expression of GAPDH was significantly downregulated in RNase R(+) group compared to the RNase R(-) group, indicating that hsa_circ_0007047 had a relatively stable structure (Fig. 2D).
The hsa_circ_0007047 promote the new angiogenesis, migration and proliferation of cultured VSMCs

It has been previously demonstrated that angiogenesis plays crucial roles in the postinfarct cardiac remodeling. Therefore, we now explored whether hsa_circ_0007047 would affect angiogenesis of CMECs and the proliferation and migration of VSMCs. The gain or loss function mutations of hsa_circ_0007047 were achieved by co-incubation with the exosomes harboring with overexpression (Over-) or suppression (Sh-) vector of hsa_circ_0007047. The qRT-PCR analysis showed that the expression of hsa_circ_0007047 was successfully overexpressed or suppressed respectively (Fig. 3A). Meaningfully, we have noticed that the numbers of capillary-like structures were significantly decreased in Sh-hsa_circ_0007047-treated cultures and increased in the Over-hsa_circ_0007047-treated group, when compared to the NC group (Fig. 3B). Further analysis showed that the CMECs-formed tubes were significantly shorter in Sh-hsa_circ_0007047 group than that in the NC group, but elongated in the Over-hsa_circ_0007047 group. These results indicated that the hsa_circ_0007047 could promote angiogenesis of CMECs. Results of additional CCK-8 assay also demonstrated that the cell viability of VSMCs was significantly increased in the Over-hsa_circ_0007047 group, as compared to the NC group, while it was significantly suppressed in cultures treated with the Sh-hsa_circ_0007047 at 48 h and 72 h, respectively (Fig. 3C). Results of cell cycle analysis showed that the number of VSMCs staged in the G1 phase was significantly decreased in the Over-hsa_circ_0007047-treated cultures, while the number of VSMCs progressed to the S and G2 phases was significantly increased in comparison with the NC group. Also, the treatment with Sh-hsa_circ_0007047 induced just opposite results when compared to the NC group (Fig. 3D). In addition, we found that while Over-hsa_circ_0007047 promoted VSMCs migration, while inhibiting the migration by Sh-hsa_circ_0007047 (Fig. 3E). Jointly, our novel results suggested that overexpression of hsa_circ_0007047 promoted the angiogenesis of CMECs, as well as increased the proliferation and migration of VSMCs.

hsa_circ_0007047 acted as a molecular sponge for miR-1178-3p

We used the CircInteractome program to predict with which miRNA the hsa_circ_0007047 would interact. The results showed that there is a binding site of miR-1178-3p in the 3'UTR, suggesting that the expression of hsa_circ_0007047 might be regulated by miR-1178-3p (Fig. 4A). To confirm this assumption, dual luciferase reporter analysis was performed. The results showed that the relative luciferase activity was significantly reduced in the hsa_circ_0007047-WT-pmiGLO group compared to the pmiGLO group treated with miR-1178-3p mimics. However, no significant changes were found in the hsa_circ_0007047-Mut-pmiGLO group, when compared with the pmiGLO group (Fig. 4B). Further analysis showed that the expression of miR-1178-3p was significantly elevated in the exosomes isolated from serum of patients with postinfarct cardiac remodeling, when compared with non-postinfarct cardiac remodeling and control group (Fig. 4C). In addition, we have noticed that the expression of miR-1178-3p was significantly decreased in Over-hsa_circ_0007047 VSMCs while increased in the Sh-
hsa_circ_0007047 (Fig. 4D). These results demonstrated that hsa_circ_0007047 is a direct target of miR-1178-3p.

**miR-1178-3p inhibited the angiogenesis of CMECs and the proliferation and migration of VSMCs**

Considering the demonstrated interaction between the hsa_circ_0007047 and the miR-1178-3p, we next confirmed the potential role of miR-1178-3p in the CMECs angiogenesis and the proliferation and migration of VSMCs by assessing the loss and gain of miR-1178-3p function mutations. Results of qRT-PCR analysis showed that the expression of miR-1178-3p was successfully overexpressed or suppressed (Fig. 5A). Tube formation analysis showed that the number of capillary-like structures was significantly decreased in miR-1178-3p mimics group while increased in miR-1178-3p inhibitor group compared to the NC group. Further quantification analysis showed that the tube length of CMECs was significantly shorter in miR-1178-3p mimics group than that in the NC group, while it was longer in CMECs treated with miR-1178-3p inhibitor (Fig. 5B). The above results jointly indicated that miR-1178-3p could inhibit angiogenesis of CMECs.

Results of the CCK-8 assay demonstrated that the viability of VSMCs was significantly decreased in the miR-1178-3p mimics group, when compared to the NC group, while it was dramatically elevated in miR-1178-3p inhibitor group (Fig. 5C). Cell cycle analysis showed that the number of VSMCs entering the G1 phase was significantly increased in miR-1178-3p mimics-treated group, while the number of VSMCs entering the S and G2 phase was significantly decreased, as compared to the NC group. Meaningfully, just opposite results were detected in cultures of VSMCs treated with miR-1178-3p inhibitor (Fig. 5D). Furthermore, we showed that overexpressing the miR-1178-3p in cultured VSMCs inhibited their migration. In contrast, suppressing of the miR-1178-3p expression promoted migration of cultured VSMCs (Fig. 5E). In addition, we found that the inhibitory the effects of miR-1178-3p on the angiogenesis of CMECs, and the proliferation and migration of VSMCs could be partially reversed by the co-incubation of these cultured cells with the exosomes harboring the Over-expressed hsa_circ_0007047 or Sh-hsa_circ_0007047 plasmid in miR-1178-3p mimics or inhibitor groups, respectively (Fig. 5B-E). These findings suggested that overexpression of miR-1178-3p inhibited CMECs angiogenesis, and VSMCs proliferation and migration.

**PDPK1 is identifies as the target of miR-1178-3p**

To identify the downstream regulator of miR-1178-3p, three online databases, TargetScan7.2, miRwalk and miRDB, were used to predict its targets (**Supplementary Table 5**). As shown in Fig. 6A, we found three common targets from the three databases, including cholinergic receptor nicotinic beta 4 (CHRNB4), Stonin 2 (STON2) and PDPK1. The subsequent qPCR and western blotting demonstrated that the expression of PDPK1 was significantly downregulated while the expression of CHRNB4 and STON2 was upregulated in the serum of CR group compared with N-CR and control group (Fig. 6B and C). The expression of PDPK1 was most downregulated among the three genes and was selected for further
study. Furthermore, the consecutive results of the luciferase reporter assays, indicated that co-
transfection of the PDPK1-WT pmiGLO reporter plasmids and miR-1178-3p mimic predominantly reduced
the luciferase activity. Conversely, co-transfection of the PDPK1-Mut pmiGLO reporter plasmids with miR-
1178-3p mimic showed no obvious effect on the luciferase activity (Fig. 6D). In addition, we found that
miR-1178-3p overexpression reduced PDPK1 expression while miR-1178-3p suppression increased
PDPK1 expression both at mRNA and protein levels (Fig. 6E and 6F). These results indicated that PDPK1
is targeted by miR-1178-3p.

miR-1178-3p-mediated PDPK1 regulate CMECs angiogenesis and VSMCs proliferation and migration

The next set of experiments was aimed at exploring how the loss or gain of PDPK1 expression would
affect the functions of the cultured cells. Results of qRT-PCR and western blotting analysis showed that
the PDPK1 could be successfully overexpressed or suppressed in cultured CMECs (Fig. 7A and B). The
consecutive tube formation analysis indicated that overexpression PDPK1 significantly increased the
number of capillary-like structures and the tube length, while suppression PDPK1 greatly decreased the
number of capillary-like structures and the CMECs tube length as compared to the control group (Fig. 7C).

The CCK-8 assay demonstrated that the cell viability of VSMCs was significantly elevated in the Over-
PDPK1 group compared to the NC group, while it was dramatically decreased in Si-PDPK1 group at 48 h
and 72 h incubations, respectively (Fig. 7D). Cell cycle analysis further indicated that the number of
VSMCs being in the G1 phase was significantly decreased in the Over-PDPK1 group while the number of
VSMCs that entered the S and G2 period was significantly increased as compared to the NC group.
Meaningfully, just the opposite results were detected in the Si-PDPK1 group compared to the NC group
(Fig. 7E). Furthermore, we showed that the overexpression of PDPK1 could promote the migration of
VSMCs, while the suppression of PDPK1 inhibited migration of VSMCs (Fig. 7F). In addition, we found
that the effects of PDPK1 on the angiogenesis of CMECs, proliferation and migration of VSMCs could be
reversed by adding miR-1178-3p mimics to the over-PDPK1 group, or by treatment of the si-PDPK1 group
with the miR-1178-3p inhibitors (Fig. 7C-F). Altogether, these results indicated that miR-1178-3p-induced
PDPK1 regulated the angiogenesis of CMECs and the proliferation and migration of VSMCs.

hsa_circ_0007047 alleviated postinfarct cardiac remodeling in vivo

To further confirm the beneficial effects of the exosomal hsa_circ_0007047 in the postinfarct ventricular
remodeling, exosomes containing overexpressing or suppressing hsa_circ_0007047 plasmid were
injected into the tail vein of mice after induction of their experimental MI. Importantly, we recorded that
the injection of exosomes containing Over-hsa_circ_0007047 significantly decreased theirs LVEDD and
LVESD, and improved LVEF and LVFS (P < 0.05). In contrast, the injection of exosomes containing Sh-
hsa_circ_0007047 to the parallel MI mice induced the opposite effects (Fig. 8A). Even the initial
comparison of the myocadiac sections from all experimental groups, stained with the H&E, clearly
illustrated the exclusive beneficial effects of treatment with the Over-hsa_circ_0007047. Analysis of the
parallel histologic sections, stained with the Masson's method or with the Sirius Red further confirmed a highly reduced degree of cardiac fibrosis in the myocardium of the Over-hsa_circ_0007047 Exo-treated group, as compared to the untreated MI group. In contrast, we have noticed that administration of the exosome of Sh-hsa_circ_0007047 aggravated the postinfarct ventricular remodeling (Fig. 8B).

Further histologic analysis also demonstrated that treatment with the Over-hsa_circ_0007047 exosomes led to a significant increase in capillary density as compared to the untreated MI group. In contrast, the density of capillaries was significantly decreased in the Sh-hsa_circ_0007047 exosomes group (Fig. 8B). Results of western blotting analysis and immunofluorescence staining assay of the parallel tissue samples additionally demonstrated that the expression of HIF-1α, VEGF and VEGFR were significantly elevated in the Over-hsa_circ_0007047 exosomes-treated group, while decreased in the Sh-hsa_circ_0007047 exosomes-treated group as compared with the NC group (Fig. 8C & D). These results were also confirmed by immunofluorescence staining assays. These results demonstrated that hsa_circ_0007047 functions as an effective suppressor of the post-MI deleterious ventricular remodeling.

The next series of experiments was aimed to investigate whether hsa_circ_0007047 could affect the expression of miR-1178-3p and PDPK1, in heart tissues. Meaningfully, we found that administration of exosomes containing overexpressing hsa_circ_0007047 plasmid greatly decreased levels of miR-1178-3p and increased PDPK1 levels. However, treatment with the exosomes containing siRNA of hsa_circ_0007047 significantly increased the expression of miR-1178-3pm and decreased PDPK1 levels in vivo (Fig. 9A & B). The additional western blotting and immunofluorescence staining also confirmed the already mentioned expression changes of PDPK1 in different groups (Fig. 9C & D). Therefore, these results demonstrated that hsa_circ_0007047 attenuated the post-MI ventricular remodeling via regulation of miR-1178-3p-PDPK1 axis in vivo.

**Discussion**

In the present study, exosomal hsa_circ_0007047 was identified as a novel regulator of the postinfarct ventricular remodeling. Function and mechanism analysis revealed that exosomal hsa_circ_0007047 alleviated adverse postinfarct cardiac remodeling by promoting angiogenesis via regulating miR-1178-3p/PDPK1 axis.

The cardiac remodeling after MI refers to a cascade of structural and functional changes in cardiomyocytes and intercellular substance. An extremely complex pathogenesis of this process includes myocardial hypertrophy, fibrosis, inflammation, autophagy and metabolic malfunction. It has been recently demonstrated that exosomes are considered as the main mediators of intercellular communication in the myocardium, which may attenuate the development of detrimental structural changes and the consequent heart failure after MI. Thus, the presence of exosomes likely contributes to inhibition of fibrosis, promotion of angiogenesis and alleviation of inflammation and pyroptosis, by translocation of intercellular molecules, proteins, and organelles. Particular attention has been paid recently to the exosomal circRNAs, after the discovery that the circular form of the non-coding RNA, such
as circFndc3b, circRNA HIPK3 and circRNA CDR1, could contribute to beneficial regulation of post-MI ventricular remodeling\textsuperscript{21,22}. Further studies exploring the mechanistic involvement of the diverse exosomes in the myocardial healing demonstrated that hypoxia-elicited mesenchymal stem cell derived exosomes facilitated cardiac repair through miR-125b-mediated amelioration of apoptosis and pyroptosis of cardiomyocytes within the infarct region of murine MI model\textsuperscript{23}.

Results of other studies also indicated that angiogenesis plays a crucial role in ameliorating the adverse myocardial remodeling, thereby, contributing to the improvement of cardiac function and preventing the heart failure\textsuperscript{24}. Silencing of angiogenesis inhibiting factor epiregulin disrupted the ERK1/2 signaling and promoted LV remodeling\textsuperscript{25}. Inhibition of miR-17 prevented high glucose induced impairment of angiogenesis in diabetic mice, thereby improving their cardiac function after MI by targeting VEGFA\textsuperscript{26}. Moreover, vildagliptin, granulocyte colony stimulating factor (G-CSF) and oncostatin M (OSM) have been listed as the angiogenic factors contributing to the improvement of cardiac function after MI\textsuperscript{27,28}.

On the other hand, numerous studies demonstrated that exosomes play a regulatory role in angiogenesis during cardiac remodeling after MI, by harboring certain non-coding RNAs or metabolic proteins\textsuperscript{29}. Thus, exosomes derived from Akt-modified human umbilical cord mesenchymal stem cells improve cardiac regeneration and promote angiogenesis via activating platelet-derived growth factor D\textsuperscript{30}. Exosomal miR-132 and miR-146a delivered by mesenchymal stem or adipose-derived stem cells attenuated the ischemic myocardial damage by inducing angiogenesis in MI, respectively\textsuperscript{31,32}. Importantly, results of our present study revealed that the newly identified exosomal hsa_circ_0007047, which promoted angiogenesis, proliferation, and migration of vascular smooth muscle cells in vitro and could alleviate ventricular remodeling in murine MI model.

HIF-1α, VEGF and VEGFR have been previously indicated as the angiogenetic factors after MI\textsuperscript{33,34}. We found that the expression of these factors in the murine myocardium subjected to experimental MI was significantly elevated after treatment with Over-hsa_circ_0007047 exosomes but decreased after treatment with Sh-hsa_circ_0007047 exosomes. Therefore, these results demonstrated that hsa_circ_0007047 functions as a potential suppressor of the adverse postinfarct cardiac remodeling by promoting angiogenesis.

In the present study, bioinformatics analysis and dual luciferase reporter system demonstrated that miR-1178-3p not only binds to hsa_circ_0007047 but also to PDPK1, the leucyl-specific aminopeptidase activated factor, contributing to the VEGF-dependent activation of S6K, and the ultimate stimulation of endothelial cell proliferation and angiogenesis\textsuperscript{35}. Interestingly, we have also established that the overexpression of miR-1178-3p inhibited angiogenesis, proliferation, and migration of VSMCs, suggesting that miR-1178-3p could inhibit the overzealous angiogenesis in cardiac remodeling after MI. On the other hand, it has been shown that the ablation of PDPK1 in cultured vascular endothelial cells enhanced insulin sensitivity and suppressed angiogenesis\textsuperscript{36}. These results demonstrated that PDPK1 act as a positive regulator in angiogenesis. Consistent with these findings, we revealed that overexpression of
PDPK1 promoted angiogenesis, proliferation and migration of VSMCs. In addition, we found that the function of miR-1178-3p and PDPK1 on the angiogenesis, proliferation and migration of VSMCs could be reversed by hsa_circ_0007047 and miR-1178-3p, respectively. Altogether, these results indicated that hsa_circ_0007047 alleviated postinfarct ventricular remodeling by promoting angiogenesis via miR-1178-3p-mediated PDPK1 expression.

In this present study, we identified a novel exosomal hsa_circ_0007047, which was profoundly downregulated in patients with detrimental ventricular remodeling after MI. Meaningfully, we have further established that exosomal hsa_circ_0007047 alleviated postinfarct remodeling by promoting angiogenesis and expanding collateral network via regulating miR-1178-3p/PDPK1 axis, that consequently preserved function of the border zone of infarcted myocardium. These findings suggest that overexpressing or application of exosomal hsa_circ_0007047 should be further investigated as a putative therapeutic target in ischemic heart failure patients.

**Methods**

**Clinical specimens and ethical statement**

The clinical serum specimens were obtained from MI patients with (n = 10) or without (n = 10) cardiac remodeling at Guangdong Provincial Hospital of Chinese Medicine following the inclusion and exclusion criteria (Supplementary Table 1). The group of healthy individuals (n = 10) respectively matching with our patients, served as an additional control. All participants of our study have signed informed consent, and the entire project has been approved by the ethics committee of Guangdong Provincial Hospital of Chinese Medicine (B2015-129-01) and conducted in accordance with the Declaration of Helsinki and its text revisions. The clinical parameters of each group were summarized in Supplementary Table 2.

**Isolation and identification of human exosomes**

Exosomes were isolated from human serum by using ExoQuick exosome precipitation solution (SBI, CA, USA) following the user manual. Briefly, serum was centrifuged at 3000 g for 15 min and then add the 63 µL ExoQuick exosome precipitation solution and refrigerate the mixture 30 min at 4°C. And then the exosome was resuspended by 100 µL of sterile PBS after centrifugation at 1500 g for 30 min. Particle size, morphology and the total amount of the exosomes were then identified by transmission electron microscope (TEM) (Philips TECNAI 20, Netherland) and by the nanoparticle tracking analysis (NTA), respectively. Exosome protein markers CD6, CD9 and tumor susceptibility gene 101 (TSG101) were identified by western blot assay.

**Cell cultures and transfection of human VSMCs and CMECs**

Human vascular smooth muscle cells (VSMCs) and cardiac microvascular endothelial cells (CMECs) were purchased from the American Type Culture Collection (ATCC, Bethesda, MD, USA). VSMCs were cultured in the 90% Dulbecco's modified Eagle's medium (DMEM, 12430054, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 100ug/mL penicillin and 100ug/mL streptomycin in a 5%
CO₂-contained incubator under 95% saturation humidity at 37°C. For the function analysis of hsa_circ_0007047, the overexpressing or suppressing plasmids of hsa_circ_0007047 were co-incubated with the serum exosomes and then co-incubated with VSMCs. For further analysis of the phosphoinositide-dependent kinase-1 (PDPK1) or miR-1178-3p effects, the (60–80%) confluent cell cultures were transfected with the overexpressing or suppressing of pCDNA3.1-PDPK1 plasmids, and with the miR-1178-3p mimics or its inhibitor, using the Lipofectamine™ 2000 transfection reagents (52887; Invitrogen, USA). The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and western blots were additionally used to detect the overexpression or silencing efficiency.

**Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)**

The expression of hsa_circ_0007047, PDPK1 and miR-1178-3p in cells or tissues were detected by qRT-PCR. Total RNA from tissues and cells were extracted by the TRIzol reagent (15596026, Invitrogen, Carlsbad, CA, USA). For the identification of hsa_circ_0007047, 2 µg samples of total RNA were incubated for 30 min at 37°C with or without 2.5 U of RNase R (Epicentre Technologies, Madison, WI). Then, the reverse transcription reactions were performed using cDNA using HiScript III RT SuperMix for qPCR (+ gDNA wiper) (R323-01, Vazyme, Nanjing, China) (2 µg RNA samples) according to the manufacturer’s instructions. The qRT-PCR reactions were also performed using an ABI 7500 instrument (ABI7500, ABI, USA) with SYBR Green Mix (4913914001, Roche) in a 20-µL reaction system containing 9 µL SYBR Mix, 0.5 µL of each primer (10 µM), 2 µL cDNA template and 8 µL RNase free H₂O. The following programs were used: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, 60°C for 1 min, and 72°C for 45s. Relative expression levels of targeted genes were calculated using the 2^−ΔΔCt methods, which was normalized to GAPDH (for PDPK1 and hsa_circ_0007047) and to U6 (for miR-1178-3p). The nuclear and cytoplasmic RNA were extracted using nuclear and cytoplasmic RNA purification kit (Fisher scientific). The samples of DNA or cDNA were amplified by divergent or convergent primers of hsa_circ_0007047, respectively. The amplification process were run as follows: 95°C for 5 min, followed by 32 cycles at 94°C for 15 s, 60°C for 30 min, and 72°C for 30s. The final products were then detected by 1% Agarose gel electrophoresis. All primers used in the present study were synthesized by General Biol company (General Biol Co., Ltd, Anhui, China), and the detailed information is listed in Supplementary Table 3.

**Western blotting**

Total protein was extracted from the serum or heart tissues using RIPA lysis buffer (P0013, Beyotime, Shanghai, China) according to the manufacturer’s instructions as described previously. Twenty grams of protein aliquots derived from each experimental samples, were assessed with the BCA protein assay kit (70-PQ0012, MultiSciences, China). They were boiled at 100°C for 5 min, separated using 10–12% SDS-PAGE electrophoresis and transferred onto PVDF membranes. The membranes were then blocked with 5% lipid-free milk/TBST buffer for 2 h at room temperature, and then incubated with anti-PDPK1 antibody (ab234064, 1: 1000, Abcam, UK ), with anti-HIF1α (20960-1-AP, 1: 200, Proteintech, USA), with anti-VEGF (19003-1-AP, 1: 1000, Proteintech, USA), with anti-VEGFR (26415-1-AP, 1: 1000, Proteintech, USA).
USA), with anti-CD6 (ab109217, 1: 1000, Abcam, UK), with anti-CD9 (ab92726, 1: 500, Abcam, UK), with anti-TSG101 (ab125011, 1: 1000, Abcam, UK) and with anti-GAPDH (ab32391, 1: 1000, Abcam, Cambridge, UK) at 37°C for 2 h, respectively. Then, all blotting membranes were subsequently incubated with secondary anti-mouse IgG antibodies (ab205719, 1:20000, Abcam, Cambridge, UK) for 1–2 h. All the immuno-complexes were finally detected using ECL after washing with TBST and analyzed using the Image-Pro Plus 6.0 software.

**Cell proliferation and cell cycle assays**

The cell proliferation ability of VSMCs was evaluated by the CCK-8 Counting Kit (A311-02, Vazyme, Nanjing, China) according to the manufacturer’s instructions. Briefly, VSMCs transfected for 48 h were isolated by the enzymatic digestion and seeded in 96-well plates for 24, 48 and 72 hours, respectively. Cultures were then incubated with medium containing 10 µL CCK-8 marker solution for 2 h at 37°C. Absorbance was detected at 450 nm using a microplate reader. Cell cycle progressions were detected in cultures, fixed overnight in 70% ethanol at 4°C by Flow cytometry (FACSCalibur, Becton) at 488 nm, after staining with propidium iodide (PI, KeyGEN Biotech, Nanjing, China). Obtained results were then analyzed with ModFit LT software (Verity software House).

**Cell migration assays**

VSMCs from different groups were harvested after culturing in serum-free medium for 16 h, resuspended in serum-free medium and transferred to two-layered Transwell chambers pre-coated with Matrigel (BD Biosciences) and incubated at 37°C for the following 24 h. Both membranes were then fixed with 100% methanol and stained with 1% toluidine blue. The numbers of cells attached to each membrane were counted under a light microscope (Zeiss710, Germany).

**Tube formation assay**

The growth factor reduced Matrigel (BD Biosciences) was thawed on ice and the 300 µL samples of this preparation were plated into 24-well plates and incubated for 30 min at 37°C to allow polymerization. CMECs were suspended in 0.2% endothelial growth basal medium (EBM), and 5×10^4 cells of CMECs were added to Matrigel-coated wells. To assess the potential influence of hsa_circ_0007047, miR-1178-3p and PDPK1 in VSMCs, cultures of CMECs were co-incubated with exosomes contained overexpressing or silencing hsa_circ_0007047, with miR-1178-3p mimics/inhibitors or overexpressing/silencing the PDPK1 plasmids. Then, the influence of these diverse preparations on the initiation of cellular tubes formation was monitored for 12 h at 37°C under a phase contrast microscope (×4) (Nikon TS100). Tube lengths were quantified using the Image J software (National Institutes of Health).

**Luciferase reporter assay**

For hsa_circ_0007047, the miRNA targets of hsa_circ_0007047 was predicted by CircInteractome. For the downstream regulator of miR-1178-3p, online databases TargetScan7.2, miRwalk and miRDB were used to predict the targets of miR-1178-3p. And then the fragment of hsa_circ_0007047 or PDPK1 were cloned into the pmiGLO vector containing the wild type sequence and mutant binding sequence, respectively. The
hsa_circ_0007047 or PDPK1 pmiGLO vector and miR-1178-3p mimics were co-transfected into the VSMCs when the confluence reached at 60–70% by using the Lipofectamine™ 2000 transfection reagents (52887, Invitrogen, USA). Cells were washed twice with phosphate buffered saline and lysed using the passive lysis buffer after cultured for 48 h after transfection. The luciferase activity was evaluated using the Dual-Luciferase Reporter Assay System (Promega). The primers used for vector construction were listed in Supplementary Table 3.

**Model of experimental MI in mice**

Wild-type male C57BL/6J mice (10–12 weeks weighing 24-27g) were purchased from the Experimental Animal Center of Guangdong Province (Guangzhou, China) and maintained in the SPF Animal Center of Guangdong Provincial Hospital of Chinese Medicine under 12-h light-dark cycles at 21 ± 2°C with a humidity of 50–80%. They were randomly divided into three groups with 15 mice per group. Then left anterior descending branches of their coronary artery were ligated to induce the acute experimental MI model as previously described. The coronary arteries of mice from the sham group were only surgically stranded without ligation. Echocardiographic and histopathological changes were analysed to assess the cardiac remodeling 28 days after surgical procedures. Exosomes containing the overexpressing or suppressed hsa_circ_0007047 plasmids were isolated and injected via a tail vein of mice from all different groups, 24 hours after the surgical induction of theirs MI. Then, levels of their hsa_circ_0007047 transfection's efficiency were assessed by qRT-PCR. The project was approved by the ethics committee of the Guangdong Provincial Hospital of Chinese Medicine.

**Echocardiography**

Echocardiographic assessment was performed as previously described assess the relevant heart's actions. Briefly, the LV function in C57BL/6J mice was conducted just before sacrifice using the Acuson Sequoia C512 system equipped with a 15L8 linear array transducer with 30 MHz. Mice were anesthetized with 1.5% isoflurane mixed with oxygen and placed in a supine position on a heating pad. Short-axis measurements were used to capture M-mode tracing at the level of the papillary muscles with a 25-mm signal depth. Three to six consecutive cardiac cycles were measured using M-mode tracings with the accompanying software.

**Histopathological changes analysis**

Hematoxylin and eosin (H&E), Masson and Sirius red staining were performed to evaluate the histopathological changes in vivo as described previously. Briefly, heart tissue was sliced into 8-µm-thickness sections and fixed with 4% paraformaldehyde at room temperature. The tissue sections were then stained with H&E Staining Kit (C0105S, Beyotime, China), Masson staining solution (R20379, YuanYe Biotech, Shanghai, China) and Sirius-Red staining (2610-10-8, Maokang Biotech, Shanghai, China), respectively. The sections were dehydrated and sealed with neutral gum and subsequently washed with running water and imaged under a microscope (Olympus, Japan). Three infarct areas were taken from each mouse from more than three independent mice.
Immunofluorescence staining

Immunofluorescence staining of the parallel histologic slides was used to detect the expression of proteins involved in angiogenesis, including hypoxia-inducible factor 1 (HIF-1α), vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR). Briefly, 4-µm serial microtome sections of the paraffin embedded heart tissues were generated and placed in 3% catalase for 15 min and blocked with 50 µL goat serum for 20 min at room temperature. Subsequently, the serial sections were incubated with anti-PDPK1 antibody (ab234064, 1:1000, Abcam, UK), with anti-HIF1α (20960-1-AP, 1:200, Proteintech, USA), with anti-VEGF (19003-1-AP, 1:1000, Proteintech, USA), with anti-VEGFR (26415-1-AP, 1:1000, Proteintech, USA) at 37°C for 2 h, respectively. The parallel sections were consecutively incubated with the FITC conjugated goat anti-rabbit IgG (ab6785, Abcam, Cambridge, UK, 1:1000) at 37°C for 1 h, then with 50 µl of DAPI dye in the dark at room temperature for 5 min for the nuclei counterstain. The sections were accessed using an XSP-36 microscope (Boshida Optical Co., Ltd., Shenzhen, China). Three infarct areas were taken from each mouse from more than three independent mice.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) from at least three independent experiments performed in triplicate. Analyses were performed using Prism 8.1.2 (GraphPad Software Inc.) by unpaired t-test and one-way analysis of variance (ANOVA) between two groups and more than two groups, respectively. P values < 0.05 was defined as the level of statistical significance.

Abbreviations

3'UTR, 3'-Untranslated regions; ACAP2, ArfGAP with coiled-coil, ankyrin repeat and Ph domains 2; CCK-8, cell counting kit-8; cDNA: first strand DNA; ceRNA, competing endogenous RNA; CHRNA4, cholinergic receptor nicotinic beta 4; circRNAs, circular RNAs; CMECs, cardiac microvascular endothelial cells; CR, cardiac remodeling; ERK1/2, extracellular signal-regulated protein kinase 1/2; G-CSF, granulocyte colony stimulating factor; HIF-1α, hypoxia-inducible factor 1; HSP70, 70-kDa heat shock proteins; IGF-IR, insulin-like growth factor-I receptor; IncRNAs, long non-coding RNA; LV, left ventricular; miRNAs, microRNA; MI, myocardial infarction; NC, negative control; N-CR, non-postinfarct cardiac remodeling; OSM, oncostatin M; PARP, Poly [ADP-ribose] polymerase 1; PDPK1, phosphoinositide-dependent kinase-1; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RIPA, radioimmunoprecipitation assay; Sirt1, sirtuin 1; STON2, stonin 2; TEM, transmission electron microscope; TGF-β1, transforming growth factor β1; TSG101, tumor susceptibility gene 101; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VSMCs, vascular smooth muscle cells.

Declarations

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**AUTHORS’ CONTRIBUTIONS**

SM and AH drafted this manuscript; SM, YL and MZZ performed the experiments, LY made statistical analysis; PY made a critical revision of the manuscript and contributed to the rationalization of the study. All authors read and approved the final manuscript.

**COMPETING INTEREST**

The authors declare no conflict of interest.

**References**

1. Jenča D, et al. Heart failure after myocardial infarction: incidence and predictors. *ESC Heart Fail* **8**, 222-237 (2021).

2. De Luca L. Established and Emerging Pharmacological Therapies for Post-Myocardial Infarction Patients with Heart Failure: a Review of the Evidence. *Cardiovasc Drugs Ther* **34**, 723-735 (2020).

3. Vandoorne K, et al. Chronic Akt1 deficiency attenuates adverse remodeling and enhances angiogenesis after myocardial infarction. *Circ Cardiovasc Imaging* **6**, 992-1000 (2013).

4. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* **367**, (2020).

5. Gurunathan S, Kang MH, Kim JH. A Comprehensive Review on Factors Influences Biogenesis, Functions, Therapeutic and Clinical Implications of Exosomes. *Int J Nanomedicine* **16**, 1281-1312 (2021).

6. Henning RJ. Cardiovascular Exosomes and MicroRNAs in Cardiovascular Physiology and Pathophysiology. *J Cardiovasc Transl Res* **14**, 195-212 (2021).

7. Cai L, et al. Activated CD4(+) T cells-derived exosomal miR-142-3p boosts post-ischemic ventricular remodeling by activating myofibroblast. *Aging (Albany NY)* **12**, 7380-7396 (2020).
8. Chen Q, Huang M, Wu J, Jiang Q, Zheng X. Exosomes isolated from the plasma of remote ischemic conditioning rats improved cardiac function and angiogenesis after myocardial infarction through targeting Hsp70. *Aging (Albany NY)* **12**, 3682-3693 (2020).

9. Song Y, *et al.* Human umbilical cord blood-derived MSCs exosome attenuate myocardial injury by inhibiting ferroptosis in acute myocardial infarction mice. *Cell Biol Toxicol* **37**, 51-64 (2021).

10. Wang L, Zhang J. Exosomal IncRNA AK139128 Derived from Hypoxic Cardiomyocytes Promotes Apoptosis and Inhibits Cell Proliferation in Cardiac Fibroblasts. *Int J Nanomedicine* **15**, 3363-3376 (2020).

11. Mao Q, Liang XL, Zhang CL, Pang YH, Lu YX. LncRNA KLF3-AS1 in human mesenchymal stem cell-derived exosomes ameliorates pyroptosis of cardiomyocytes and myocardial infarction through miR-138-5p/Sirt1 axis. *Stem Cell Res Ther* **10**, 393 (2019).

12. Geng T, Song ZY, Xing JX, Wang BX, Dai SP, Xu ZS. Exosome Derived from Coronary Serum of Patients with Myocardial Infarction Promotes Angiogenesis Through the miRNA-143/IGF-IR Pathway. *Int J Nanomedicine* **15**, 2647-2658 (2020).

13. Altesha MA, Ni T, Khan A, Liu K, Zheng X. Circular RNA in cardiovascular disease. *J Cell Physiol* **234**, 5588-5600 (2019).

14. Si X, *et al.* circRNA Hipk3 Induces Cardiac Regeneration after Myocardial Infarction in Mice by Binding to Notch1 and miR-133a. *Mol Ther Nucleic Acids* **21**, 636-655 (2020).

15. Bai M, Pan CL, Jiang GX, Zhang YM. CircRNA 010567 improves myocardial infarction rats through inhibiting TGF-β1. *Eur Rev Med Pharmacol Sci* **24**, 369-375 (2020).

16. Cai L, *et al.* Circular RNA Ttc3 regulates cardiac function after myocardial infarction by sponging miR-15b. *J Mol Cell Cardiol* **130**, 10-22 (2019).

17. Li CX, Song J, Li X, Zhang T, Li ZM. Circular RNA 0001273 in exosomes derived from human umbilical cord mesenchymal stem cells (UMSCs) in myocardial infarction. *Eur Rev Med Pharmacol Sci* **24**, 10086-10095 (2020).

18. Wu X, Reboll MR, Korf-Klingebiel M, Wollert KC. Angiogenesis after acute myocardial infarction. *Cardiovasc Res* **117**, 1257-1273 (2021).

19. Galli A, Lombardi F. Postinfarct Left Ventricular Remodelling: A Prevailing Cause of Heart Failure. *Cardiol Res Pract* **2016**, 2579832 (2016).

20. Qi Z, *et al.* The pluripotent role of exosomes in mediating non-coding RNA in ventricular remodeling after myocardial infarction. *Life Sci* **254**, 117761 (2020).
21. Garikipati VNS, et al. Author Correction: Circular RNA CircFndc3b modulates cardiac repair after myocardial infarction via FUS/VEGF-A axis. *Nat Commun* **11**, 2234 (2020).

22. Mester-Tonczar J, et al. Association between Circular RNA CDR1as and Post-Infarction Cardiac Function in Pig Ischemic Heart Failure: Influence of the Anti-Fibrotic Natural Compounds Bufalin and Lycorine. *Biomolecules* **10**, (2020).

23. Zhu LP, et al. Hypoxia-elicited mesenchymal stem cell-derived exosomes facilitates cardiac repair through miR-125b-mediated prevention of cell death in myocardial infarction. *Theranostics* **8**, 6163-6177 (2018).

24. Sánchez-Alonso S, Alcaraz-Serna A, Sánchez-Madrid F, Alfranca A. Extracellular Vesicle-Mediated Immune Regulation of Tissue Remodeling and Angiogenesis After Myocardial Infarction. *Front Immunol* **9**, 2799 (2018).

25. Cai Y, Xie KL, Wu HL, Wu K. Functional suppression of Epiregulin impairs angiogenesis and aggravates left ventricular remodeling by disrupting the extracellular-signal-regulated kinase1/2 signaling pathway in rats after acute myocardial infarction. *J Cell Physiol* **234**, 18653-18665 (2019).

26. Yan M, et al. Glucose impairs angiogenesis and promotes ventricular remodelling following myocardial infarction via upregulation of microRNA-17. *Exp Cell Res* **381**, 191-200 (2019).

27. Li M, Wang Z, Xia H, Yu L, Hu Z. Vildagliptin and G-CSF Improved Angiogenesis and Survival after Acute Myocardial Infarction. *Arch Med Res* **50**, 133-141 (2019).

28. Zhang X, et al. OSM Enhances Angiogenesis and Improves Cardiac Function after Myocardial Infarction. *Biomed Res Int* **2015**, 317905 (2015).

29. Suzuki E, Fujita D, Takahashi M, Oba S, Nishimatsu H. Therapeutic Effects of Mesenchymal Stem Cell-Derived Exosomes in Cardiovascular Disease. *Adv Exp Med Biol* **998**, 179-185 (2017).

30. Ma J, et al. Exosomes Derived from Akt-Modified Human Umbilical Cord Mesenchymal Stem Cells Improve Cardiac Regeneration and Promote Angiogenesis via Activating Platelet-Derived Growth Factor D. *Stem Cells Transl Med* **6**, 51-59 (2017).

31. Ma T, et al. MicroRNA-132, Delivered by Mesenchymal Stem Cell-Derived Exosomes, Promote Angiogenesis in Myocardial Infarction. *Stem Cells Int* **2018**, 3290372 (2018).

32. Pan J, Alimujiang M, Chen Q, Shi H, Luo X. Exosomes derived from miR-146a-modified adipose-derived stem cells attenuate acute myocardial infarction-induced myocardial damage via downregulation of early growth response factor 1. *J Cell Biochem* **120**, 4433-4443 (2019).

33. Du Y, et al. Hypoxia-Inducible Factor 1 alpha (HIF-1α)/Vascular Endothelial Growth Factor (VEGF) Pathway Participates in Angiogenesis of Myocardial Infarction in Muscone-Treated Mice: Preliminary
34. Oduk Y, et al. VEGF nanoparticles repair the heart after myocardial infarction. *Am J Physiol Heart Circ Physiol* **314**, H278-h284 (2018).

35. Yamazaki T, Akada T, Niizeki O, Suzuki T, Miyashita H, Sato Y. Puromycin-insensitive leucyl-specific aminopeptidase (PILSAP) binds and catalyzes PDK1, allowing VEGF-stimulated activation of S6K for endothelial cell proliferation and angiogenesis. *Blood* **104**, 2345-2352 (2004).

36. Tawaramoto K, et al. Ablation of 3-phosphoinositide-dependent protein kinase 1 (PDK1) in vascular endothelial cells enhances insulin sensitivity by reducing visceral fat and suppressing angiogenesis. *Mol Endocrinol* **26**, 95-109 (2012).

37. Mao S, Wang Y, Zhang M, Hinek A. Phytoestrogen, tanshinone IIA diminishes collagen deposition and stimulates new elastogenesis in cultures of human cardiac fibroblasts. *Exp Cell Res* **323**, 189-197 (2014).

38. Mao S, Vincent M, Chen M, Zhang M, Hinek A. Exploration of Multiple Signaling Pathways Through Which Sodium Tanshinone IIA Sulfonate Attenuates Pathologic Remodeling Experimental Infarction. *Front Pharmacol* **10**, 779 (2019).

39. Mao S, Chen P, Li T, Guo L, Zhang M. Tongguan Capsule Mitigates Post-myocardial Infarction Remodeling by Promoting Autophagy and Inhibiting Apoptosis: Role of Sirt1. *Front Physiol* **9**, 589 (2018).

40. Mao S, Ma H, Chen P, Liang Y, Zhang M, Hinek A. Fat-1 transgenic mice rich in endogenous omega-3 fatty acids are protected from lipopolysaccharide-induced cardiac dysfunction. *ESC Heart Fail*, (2021).

**Figures**
hsa_circ_0007047 was significantly downregulated in the serum exosome of patients with postinfarct cardiac remodeling. A, Venn diagram to present the number of differentially expressed circRNAs among the serum exosomes from patients with and without postinfarct cardiac remodeling using whole transcriptome sequencing. B and C, Heatmap and volcano plot to present the expression of differentially expressed circRNAs, respectively. D, Phenotype of exosomes isolated from serum from patients with and without postinfarct cardiac remodeling by transmission electron microscope, Bar=100 μm the particle size of exosomes identified by nanoparticle tracking analysis. E, The protein expression of CD6, CD9 and TSG101 detected using western blotting. F, The expression of hsa_circ_0000212, hsa_circ_0089282 and hsa_circ_0007047 in exosomes from patients with and without postinfarct cardiac remodeling by using divergent primers, GAPDH served act as control. Data are mean±SD. **P<0.01, N-CR group vs Control group; ##P<0.01, CR group vs N-CR group. CR, postinfarct cardiac remodeling; N-CR, non-postinfarct cardiac remodeling.
Figure 2

Characteristics of hsa_circ_0007047. A, Schematic diagram of the ring structure of hsa_circ_0007047; B, Agarose electrophoresis assay to amplified the specific products of hsa_circ_0007047 from both cDNA and gDNA template by using convergent and divergent primers, GAPDH served as control; C, The reverse shear site of hsa_circ_0007047 was confirmed by Sanger sequencing of the amplified product of hsa_circ_0007047; D, qRT-PCR was used to detect the expression of hsa_circ_0007047 by using cDNA as templates with the RNA was digested by RNA exonuclease R before reverse transcription, GAPDH served as the negative control. Data are mean±SD. **P <0.01, RNase R+ group vs RNase R- group.
Figure 3

hsa_circ_0007047 promote angiogenesis of CMECs, proliferation and migration of VSMCs. A, qRT-PCR analysis was used to detect the expression of hsa_circ_0007047 in the coincubation with exosomes harboring with Over-hsa_circ_0007047 or Sh-hsa_circ_0007047, GAPDH served as control; B, Capillary-like structures analysis by tube formation assay in the Over-hsa_circ_0007047 or Sh-hsa_circ_0007047 group compared with the NC group, Bar=200μm; C, The CCK-8 assay was used to detect the cell viability of VSMCs coincubation with exosomes harboring with Over-hsa_circ_0007047 or Sh-hsa_circ_0007047 at 24 h, 48 h and 72 h, respectively; D, Flow cytometry was used to detect the cell cycle of VSMCs coincubation with exosomes harboring with Over-hsa_circ_0007047 or Sh-hsa_circ_0007047; E, Transwell analysis was used to detect the migration of VSMCs coincubation with exosomes harboring with Over-hsa_circ_0007047 or Sh-hsa_circ_0007047, Bar=200μm. Data are mean±SD. **p<0.01, Over-hsa_circ_0007047 group vs NC group; ##, p<0.01, Sh-hsa_circ_0007047 vs NC group.

Figure 4

hsa_circ_0007047 acts as a molecular sponge for miR-1178-3p. A, The miRNA targets of hsa_circ_0007047 was predicted by CircInteractome; B, Dual luciferase reporter system analysis to detect
the regulatory of miR-1178-3p to hsa_circ_0007047; C, The expression of miR-1178-3p in the exosome of serum from patients with and without postinfarct cardiac remodeling detected using qRT-PCR. Data are mean±SD. **P<0.01, N-CR group vs Control group; ##P<0.01, CR group vs N-CR group. CR, postinfarct cardiac remodeling; N-CR, non-postinfarct cardiac remodeling. D, The expression of miR-1178-3p detected in the VSMC cells coincubation with exosomes harboring with Over-hsa_circ_0007047 or Sh-hsa_circ_0007047, U6 served as control. Data are mean±SD. **P<0.01, Over-hsa_circ_0007047 group vs NC group; ##, P <0.01, Sh-hsa_circ_0007047 vs NC group.

Figure 5

miR-1178-3p inhibited angiogenesis of CMECs, proliferation and migration of VSMCs A, The expression of miR-1178-3p was detected using qRT-PCR in CMECs treated with miR-1178-3p mimics or inhibitors; B, Tube formation analysis to detect the capillary-like structures in miR-1178-3p mimics group or miR-1178-3p inhibitor group and rescued by hsa_circ_0007047, Bar=200μm; C, The CCK-8 assay was used to detect the cell viability of VSMCs in the miR-1178-3p mimics or miR-1178-3p inhibitor group and rescued by hsa_circ_0007047 at 24h, 48 h and 72 h, respectively; D, Flow cytometry was used to detect the cell cycle of VSMCs in the miR-1178-3p mimics or miR-1178-3p inhibitor group and rescued by hsa_circ_0007047; E, Migration of VSMCs was detected by Transwell assay when overexpressing or suppressing miR-1178-3p and rescued by hsa_circ_0007047, Bar=200μm. Data are mean±SD. **P<0.01, miR-1178-3p mimics group vs NC group; ##, P<0.01, miR-1178-3p inhibitor group vs Control group.
Figure 6

PDPK1 is the target of miR-1178-3p. A, The targets of miR-1178-3p was predicted by online databases TargetScan7.2, miRwalk and miRDB; B and C, The qPCR and western blotting analysis was used to detect the expression of PDPK1, CHRN34 and STON2 in the serum of patients with and without postinfarct cardiac remodeling, respectively, GAPDH served as control. Data are mean±SD. **P<0.01, N-CR group vs Control group; ##P <0.01, CR group vs N-CR group. CR, postinfarct cardiac remodeling; N-CR, non-postinfarct cardiac remodeling. D, Luciferase reporter assays to verify the binding of miR-1178-3p to PDPK1 at 3’UTR; E and F, The expression of PDPK1 was detected using the qRT-PCR and western blotting in the VSMCs with miR-1178-3p mimics or inhibitors at mRNA and protein levels, respectively. Data are mean±SD. **P<0.01, miR-1178-3p mimics group vs NC group; ##P <0.01, miR-1178-3p inhibitor group vs NC group.
miR-1178-3p-mediated PDPK1 regulated angiogenesis of CMECs, proliferation and migration of VSMCs. A and B, The expression of PDPK1 was detected by qRT-PCR and western blotting by overexpressing or suppressing of PDPK1 in CMECs, respectively, GAPDH served as control; C, Tube formation analysis was used to detect the capillary-like structures in the overexpression or suppression of PDPK1 group and rescued by miR-1178-3p, Bar=200 μm; D, The CCK-8 assay was used to detect the cell viability of VSMCs in the overexpressing or suppressing of PDPK1 group and rescued by miR-1178-3p at 24 h, 48 h and 72 h, respectively; E, Flow cytometry was used to detect the cell cycle of VSMCs in the overexpression or suppressing of PDPK1 group and rescued by miR-1178-3p; F, Migration of VSMCs was detected by Transwell assay when overexpressing or suppressing PDPK1 and rescued by miR-1178-3p, Bar=200 μm. Data are mean±SD. **P<0.01, Over-PDPK1 group vs NC group; ##P<0.01, Sh-PDPK1 group vs NC group.
Figure 8

hsa_circ_0007047 alleviated postinfarct cardiac remodeling  

A, qRT-PCR was used to verify the expression of hsa_circ_0007047 in the heart tissue treatment with exosomal hsa_circ_0007047, GAPDH served as control;  

B, Echocardiographic measurements to detect the in the model group compared with sham group;  

C, Pathological changes analysis detected by HE, Masson and Sirius Red staining in the heart tissue treatment with exosome hsa_circ_0007047, Bar=50μm;  

D, Western blotting analysis to detect the expression of HIF-1α, VEGF and VEGFR in the Over-circa-Exo group or Sh-circa-Exo group. GAPDH served act as control;  

E, Immunofluorescence staining was used to detect the expression of HIF-1α, VEGF and VEGFR in the Over-circa-Exo group or Sh-circa-Exo group. DAPI was used to stain the nuclei; Bar=50μm. Data are mean±SD. ** p<0.01, Over-circ-Exo group vs Model-NC group; ##, p<0.01, She-circ-Exo group vs Model-NC group.
Figure 9

The expression changes of miR-1178-3p and PDPK1 in vivo treatment with exosomes harboring hsa_circ_0007047. A, The expression of miR-1178-3p was detected by qRT-PCR, U6 act as control; B and C, The expression of PDPK1 was detected by qRT-PCR and western blotting, respectively, GAPDH served as control; D, Immunofluorescence staining was used to detect the expression of PDPK1 in heart tissues.
DAPI was used to stain the nuclei; Bar=50μm. Data are mean±SD. **P<0.01, Over-circ-Exo group vs Model-NC group; ##P<0.01, She-circ-Exo group vs Model-NC group.

**Supplementary Files**

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- SupplementaryTable12.docx
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