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miR-878靶向调控Pim1促进线粒体分裂导致心肌细胞缺氧/复氧损伤*

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摘要 **目的** 心肌缺血/再灌注（MI/R）损伤是导致急性心肌梗死患者不良心血管结局的重要原因。然而，目前对MI/R损伤的分子机制仍不明确。本文旨在确定微小RNA-878（miR-878）对MI/R损伤的影响及其分子机制。**方法** 在H9c2细胞中建立缺氧/复氧（H/R）模型。采用CCK-8法检测细胞活力。采用生化试剂盒检测乳酸脱氢酶（LDH）含量。流式细胞术分析细胞凋亡水平。采用免疫荧光法及激光共聚焦显微镜分析线粒体形态。采用免疫荧光法检测线粒体活性氧（mtROS）水平。使用双荧光素酶报告基因实验研究miR-878与Pim1的结合位点。RNA免疫沉淀（RIP）实验验证miR-878与Pim1的结合关系。实时荧光定量PCR（RT-qPCR）和蛋白质印迹法（Western blot）检测基因的表达水平。**结果** 与对照组相比，miR-878在H/R处理的H9c2细胞中表达显著升高((1.00±0.25) *vs* (9.70±2.63)，P<0.01)。在H/R诱导的细胞中，转染miR-878抑制剂能够显著增加细胞活力((46.67±3.00) *vs* (74.62±4.08)，P<0.000 1)，并降低LDH释放量((358.58±41.71) *vs* (179.09±15.59)，P<0.000 1)及细胞凋亡率((43.41±0.72) *vs* (27.42±4.48)，P<0.01)。同时，下调miR-878表达能够显著抑制DRP1介导的线粒体过度分裂及mtROS产生((6.60±0.57) *vs* (4.32±0.91)，P<0.000 1)。机制研究显示，miR-878能够靶向结合Pim1 mRNA的3'-UTR区域并抑制Pim1的表达水平。挽救实验证明，下调Pim1表达能够显著逆转miR-878抑制剂抗H9c2细胞损伤的作用（均P<0.01），并出现线粒体过度分裂及mtROS产生增加（均P<0.05）。**结论** 在H/R条件下，miR-878通过靶向抑制Pim1表达而促进DRP1介导的线粒体过度分裂，最终导致心肌细胞损伤。

关键词 miR-878，Pim1，发动蛋白相关蛋白1，心肌缺血/再灌注损伤
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急性心肌梗死（acute myocardial infarction，AMI）是目前世界范围内发病率和死亡率较高的疾病之一[1-2]。改善AMI患者预后的关键治疗手段是及时再灌注治疗[3-4]，然而，随着心肌再灌注的进行会发生心肌细胞不可逆性损伤，即心肌缺血/再灌注（myocardial ischemia/reperfusion，MI/R）损伤，表现为心肌梗死面积增加、诱发心律失常和心功能障碍等[5-6]。临床中尚缺乏针对MI/R的有效防治措施，因此深入探究MI/R发生发展的分子机制具有重要意义[7]。

线粒体动力学在MI/R损伤的病理机制中起重要作用。其中，发动蛋白相关蛋白1（dynamin-related protein 1，DRP1）在MI/R损伤过程中扮演关键角色。MI/R条件下，DRP1从胞质向线粒体转位，引发线粒体过度分裂[8-9]、线粒体活性氧（mitochondrial reactive oxygen）产生增加、膜电位失衡、线粒体膜通透性转换孔开放，最终导致心肌细胞凋亡损伤[10]。最近研究表明，Pim1属于丝氨酸/苏氨酸蛋白激酶Pim家族中的一种蛋白质激酶，能够调节DRP1在Ser637位点的磷酸化，降低

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DRP1的GTPase活性，从而阻碍DRP1形成聚合物切割线粒体，抑制线粒体过度分裂损伤[11]。因此，深入探究Pim1/DRP1在调控线粒体分裂方面的信号机制，有助于开发新的MI/R损伤防治靶点。

既往研究发现，微小RNAs（miRNAs）在MI/R损伤过程中存在着异常表达，并对心肌细胞命运转归具有重要影响[12-13]。已有研究证实，通过下调miRNA-34a[14]、miR-143-3p[15]、miR-205[16]、miR-423-5p[17]表达可导致线粒体功能障碍，增加心肌细胞凋亡。而过表达miR-19b[18]、miR-140[19]、miRNA-210[20]、miR-124[21]可改善线粒体功能，减少心肌细胞凋亡损伤。然而，在MI/R损伤中，对于参与调控线粒体分裂的miRNAs研究较少。本研究前期在预实验中发现，miR-878在MI/R损伤中存在表达变化，但对于miR-878的作用及相关分子机制还有待研究。

本研究拟通过在H9c2细胞中建立体外MI/R损伤模型来研究miR-878对线粒体分裂介导心肌细胞凋亡损伤的影响，以期为MI/R的治疗提供新靶点。

1 材料与方法

1.1 仪器与试剂

仪器：CO_2培养箱HERAcell VIOS 160i（ThermoFisher Scientific Inc）、生物安全柜（ThermoFisher Scientific Inc）、倒置相差显微镜（Olympus）、LSM880激光共聚焦显微镜（Zeiss）、酶标仪infinite M200PRO（Tecan）、PCR热循环仪S100（Bio-Rad）、QuantStudio 5实时荧光定量PCR系统（Applied Biosystems）、超微量紫外可见分光光度计（Quawell）。

试剂：CCK-8（cell counting kit-8）试剂盒（Biosharp）、乳酸脱氢酶试剂盒（北京索莱宝科技有限公司）、Lipofectamine 3000（Invitrogen）、MitoSOX红色线粒体超氧化物试剂盒（Invitrogen）、BCA蛋白质浓度测定试剂盒（北京索莱宝科技有限公司）、PVDF膜（Millipore）、Pim-1 Rabbit mAb（Cell Signaling Technology）、DRP1 Rabbit mAb（Cell Signaling Technology）、Horseradish Peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG（H+L）（Proteintech Group）、Phospho-DRP1（Ser637）Antibody（Cell Signaling Technology）、Tom20 Rabbit mAb（Cell Signaling Technology）、Dual-Lumi双萤光素酶报告基因检测试剂盒（上海碧云天生物技术有限公司）、PCR引物设计（上海生工生物工程技术服务有限公司）、PCR试剂盒（上海生工生物工程技术服务有限公司、ThermoFisher Scientific Inc）、cDNA逆转录试剂盒（上海生工生物工程技术服务有限公司、ThermoFisher Scientific Inc）、Pim1特异性shRNA及阴性对照设计（上海吉凯基因医学科技股份有限公司）。

1.2 细胞培养及H/R模型的建立

细胞培养：大鼠H9c2心肌细胞系购自中国医学科学院基础医学研究所细胞资源中心（北京）。培养于含10%胎牛血清、100 U/ml青霉素和100 mg/L链霉素的DMEM培养液中，在37℃、5% CO_2的恒温、密闭培养箱中培养，用显微镜观察其状态并记录，每隔1~2 d向培养皿中更换培养液。

缺氧/复氧（hypoxia/reoxygenation，H/R）模型建立：采用本课题组既往实验方法[22]建立H/R模型。在缺氧阶段，向HeraCell VIOS 160i培养箱中充满含有1% O_2、5% CO_2和94% N_2气体，混合物模拟缺氧条件，同时使用不含葡萄糖、血清的DMEM培养液，模拟能量供应中断3 h。在复氧阶段，将细胞置于常规培养基和正常培养环境6 h。

1.3 细胞转染

H9c2细胞培养至60%~70%汇合度后进行转染。使用Lipofectamine 3000试剂，分别将抑制剂的阴性对照（negative control inhibitors，NC inh）、miR-878的抑制剂（miR-878 inihibitors，miR-878 inh）、miR-878的模拟物（miR-878 mimics）、模拟物的阴性对照（negative control mimics，NC mimics）、Pim1的质粒转染H9c2细胞，温度37°C孵育细胞2~4 d，随后分析转染细胞mRNA的表达水平。

转染序列如下。NC inh：5'-CAGUACUUUU-GUGUAGUACAA-3'。miR-878 inh：5'-UCUACC-CAGUAUGGUGUCAUGC-3'。NC mimics：sense 5'-UUCUCCGACGUGUCACGUTT-3'；antisense：5'-ACGUGACACGUUCGGAGAATT-3'。miR-878 mimics：sense 5'-GCAUGACACCAUACUGGGUA-GA-3'；antisense：5'-UACCCAGUAUGGUGUCA-UGCUU-3'。shPim1(1)：5'-CCGGCCGCGG-CGAACTCAAACTCATCTCGAGATGAGTTTGAG-TTCGCCGCGGTTTTT-3'；shPim1(2)：5'-CCG-GGTTCGAGAGGCCCGATAGTTTCTCGAGAAA-CTATCGGGCCTCTCGAACTTTTT-3'；shPim1(3)：

5'-CCGGAATCCAGAACCATCCGTGGATCTCGA-GATCCACGGATGGTTCTGGATTTTTTT-3'。

1.4 CCK−8法

将H9c2细胞制成悬液加入96孔板，在培养箱内孵育过夜。按照实验分组处理后，每孔加入10 μl CCK-8溶液，在37℃培养箱内孵育。用酶标仪在450 nm处测定各孔吸光度（*A*），计算细胞活力（%）。

1.5 乳酸脱氢酶测定

采集细胞培养上清，离心后按照乳酸脱氢酶（lacate dehydrogenase，LDH）检测试剂盒说明书测量LDH活性。采用酶标仪在450 nm处测定各孔吸光度值。

1.6 细胞凋亡的检测

采用Annexin V-FITC/PI凋亡检测试剂盒及流式细胞术分析H9c2细胞的凋亡情况。H9c2细胞用冷PBS洗涤2次，然后在含有5 μl Annexin V-FITC和10 μl PI的100 μl结合缓冲液中黑暗悬浮。孵育15 min后，使用流式细胞仪分析细胞悬液。

1.7 线粒体形态的检测

H9c2细胞经转染后接种至激光共聚焦培养皿上，室温下采用4%多聚甲醛固定及通透处理后，使用Tom20抗体继续孵育24 h，加入免疫荧光二抗避光孵育2 h，并使用激光共聚焦显微镜进行观察拍照（63×）。通过Image J软件对线粒体网络形态进行统计。

1.8 线粒体活性氧(mtROS)的测定

收集H9c2细胞，加入PBS洗涤2次。加入1 ml MitoSOX Red工作液，室温孵育30 min后，使用荧光显微镜进行观察并拍照。

1.9 双荧光素酶报告基因检测

构建Pim1野生型质粒（pmirGLO-Pim1-WT）和突变型质粒（pmirGLO-Pim1-MUT），将H9c2细胞接种在24孔板中。用Lipofectamine 3000将pmirGLO-Pim1-WT、pmirGLO-Pim1-MUT分别与miR-878 mimics和NC mimics共转染于H9c2细胞中。48 h后应用化学发光仪检测荧光素酶与海肾酶活性。

1.10 RNA免疫沉淀(RIP)实验

收集H9c2细胞，经PBS清洗后加入RIPA裂解液重悬细胞，制备细胞裂解液。用5 μg抗Ago2抗体或IgG阴性对照抗体孵育磁珠。然后取细胞裂解液与相应抗体包被的磁珠共孵育，获得RIP-蛋白质复合物沉淀，并用蛋白酶K缓冲液重悬沉淀。最后使用Trizol试剂提取共沉淀复合物的RIP，应用逆转录定量聚合酶链式反应（reverse transcription quantitative polymerase chain reaction，RT-qPCR）法检测miR-878和Pim1的表达水平。

1.11 RT−qPCR

收集转染后的H9c2细胞，通过Trizol试剂提取总RNA。测定RNA的浓度和纯度后，分别使用miRNA荧光定量PCR试剂盒（染料法）进行反转录合成cDNA。采用PCR扩增仪进行RT-qPCR，检测各组细胞RNA表达水平。

引物序列如下。miR-878：5'-GCATGACA-CCATACTGGGTAGA-3'；U6：5'-CTCGCTTCGG-CAGCACATA-3'；Pim1 F：5'-GCTGCTCAAGGA-CACAGTCTACAC-3'，R：5'-CGTGGTAGCGATG-GTAGCGAATC-3'；β-actin：F：5'-CTGTGTGGAT-TGGTGGCTCTA-3'，R：5'-GAAAGGGTGTAAA-ACGCAGCT-3'。

1.12 蛋白质印迹法(Western blot)检测

采用含有1% PMSF和1%磷酸酶抑制剂混合物的RIPA缓冲液中裂解细胞，使用BCA蛋白质测定试剂盒测定蛋白质浓度；用SDS聚丙烯酰胺凝胶电泳（SDS-PAGE）（10%~15%）分离蛋白质，随后转移到PVDF上，用5%脱脂奶粉或牛血清白蛋白封闭2 h；分别加入相应一抗在4℃条件下孵育过夜。然后，将PVDF膜与辣根过氧化物酶HRP标记亲和纯化山羊抗兔IgG（H+L）二抗在室温下孵育1 h；用ECL Kit检测膜上的蛋白质条带。凝胶图像使用Universal Hood II采集，并用Image J进行条带灰度值分析。

1.13 统计学分析

计量资料以均数±标准差表示。两组间比较采用独立样本*t*检验。以双侧$P<0.05$为差异有统计学意义。绘图和统计分析采用GraphPad Prism 7.00软件进行。

2 结　果

2.1 miR−878在H9c2的H/R模型中表达上调

用RT-qPCR分析miR-878在H9c2细胞（图1a）中的表达变化，结果显示，与对照（Ctl）组比较，H/R显著升高了miR-878表达（（1.00±0.25）*vs*（9.70±2.63），$P<0.01$）。

为确定miR-878在MI/R损伤中的作用，采用miR-878 inh进行基因敲减实验（图1b）。RT-qPCR结果发现，miR-878 inh能够显著降低miR-878表达（（1.00±0.16）*vs*（0.41±0.43），$P<0.01$）。

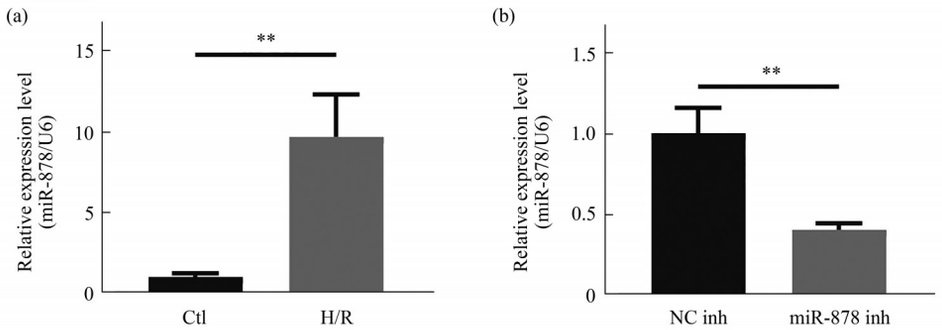

Fig. 1　miR−878 expression is upregulated in the H/R model

(a) RT-qPCR was used to detect the expression changes of miR-878 in the control group and after H/R-treated group. (b) RT-qPCR was used to detect the expression changes of miR-878 transfected with NC inhibitor and miR-878 inhibitors. Data are expressed as *mean*±*SD* (*n*=3). $^{**}P$<0.01. U6 was the internal reference.

2.2　下调miR−878表达能够缓解H/R诱导的细胞损伤

通过CCK-8法检测细胞活力，结果显示（图2a），H9c2细胞经H/R处理后，细胞活力下降（（100.00±5.77）*vs*（47.52±4.62），*P*<0.000 1），表明H/R细胞模型制作成功，给予miR-878 inh显著减轻了H/R诱导的细胞损伤，且对细胞无明显毒性作用（（46.67±3.00）*vs*（74.62±4.08），*P*<0.000 1）。

采用LDH检测H9c2细胞损伤程度，结果显示（图2b），H9c2细胞经H/R处理后，LDH释放量显著增加（(118.26±8.18) *vs* (352.01±37.25)，*P*<0.000 1），给予miR-878 inh显著减少了H/R诱导的LDH释放（（358.58±41.71）*vs*（179.09±15.59），*P*<0.000 1）。

采用Annexin V-FITC/PI凋亡检测试剂盒及流式细胞术分析H9c2细胞凋亡，结果提示（图2c），H/R处理后，细胞凋亡率增加（（4.54±0.41）*vs*（43.55±1.92），*P*<0.000 1），而给予miR-878 inh可显著减少细胞凋亡率（（43.41±0.72）*vs*（27.42±4.48），*P*<0.01）。

2.3　下调miR−878表达能够抑制DRP1介导的线粒体过度分裂

使用激光共聚焦显微镜进行线粒体形态测定，结果显示（图3a），与Ctl组相比，H/R损伤引起线粒体计数增加（（1.00±0.22）*vs*（1.39±0.26），*P*<0.01）、周长减小（（1.00±0.21）*vs*（0.72±0.06），*P*<0.01）、面积减少（（1.00±0.27）*vs*（0.51±0.08），*P*<0.000 1），而给予miR-878 inh后，线粒体计数减少（（1.44±0.12）*vs*（1.077±0.19），*P*<0.001），周长增大（（0.60±0.11）*vs*（0.82±0.07），*P*<0.001），面积增加（（0.53±0.04）*vs*（0.73±0.06），*P*<0.000 1）。

荧光显微镜观察mtROS的荧光强度，结果显示（图3b），与Ctl组相比，H/R损伤引起mtROS产生增加（（1.00±0.05）*vs*（6.80±0.32），*P*<0.000 1），给予miR-878 inh后，mtROS产生减少（（6.60±0.57）*vs*（4.32±0.91），*P*<0.000 1）。

Western blot检测p-DRP1蛋白表达水平，结果显示（图3c），与Ctl组相比，H/R组p-DRP1表达下降（（1.00±0.01）*vs*（0.46±0.17），*P*<0.01），而给予miR-878 inh可增加p-DRP1表达水平（（0.45±0.14）*vs*（0.79±0.06），*P*<0.05）。

2.4　miR−878靶向Pim1调控DRP1表达水平

为进一步探索miR-878的下游机制，使用DIANA tools生物信息学数据库预测miR-878的靶基因，结果显示（图4a），miR-878可能结合Pim1的3'-UTR区。

将构建的pmirGLO-Pim1-WT、pmirGLO-Pim1-MUT（图4b）与miR-878 mimics、NC mimics转染至细胞中，测量荧光素酶活性（图4c），结果显示，miR-878抑制了含Pim1野生型质粒细胞的荧光素酶活性，组间差异有统计学意义（1.00±0.06 *vs* 0.39±0.03，*P*<0.000 1），而对含Pim1突变型质粒细胞的荧光素酶活性无影响（*P*>0.05）。双荧光素酶报告基因实验结果表明，miR-878和Pim1存在直接结合位点。

RIP实验检测miR-878和Pim1之间的结合，结果显示（图4d），与IgG组比较，miR-878和Pim1均显著被Ago2抗体富集（(1.00±0.25) *vs* (94.67±34.41)和（1.00±0.29）*vs*（66.14±20.57），均*P*<0.01）。

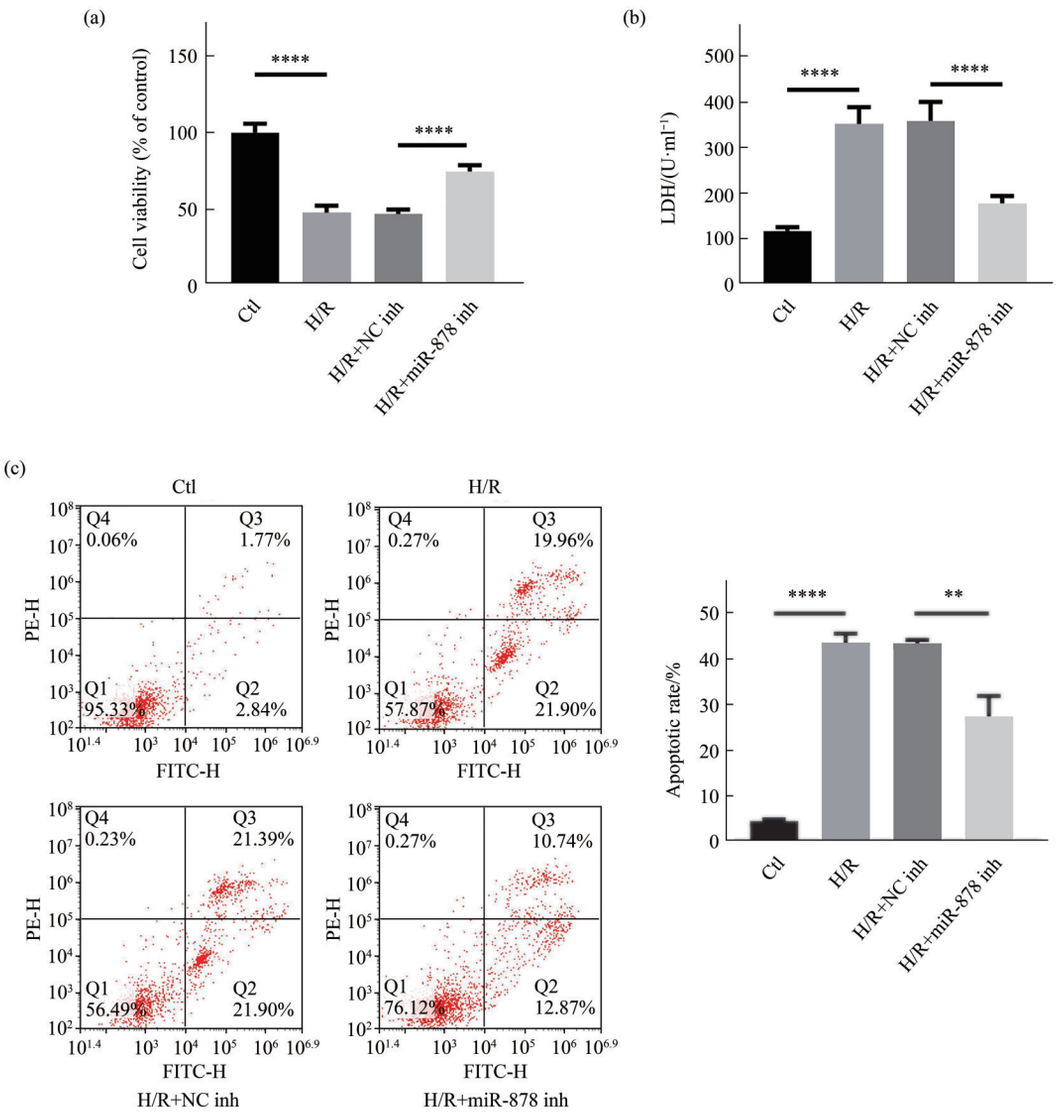

Fig. 2　Down-regulating miR-878 expression alleviated H/R-induced cell damage

(a) CCK-8 assay was used to detect the viability of H9c2 cells. (b) LDH level in H9c2 cells. (c) Apoptosis of H9c2 cells was detected by Annexin V-FITC/PI double staining. Data are expressed as *mean*±*SD* (*n*=3). $^{**}P$<0.01; $^{****}P$<0.000 1.

采用RT-qPCR分析miR-878和Pim1之间表达水平关系，结果显示（图4e），过表达或沉默miR-878均显著改变Pim1的表达水平。转染miR-878 mimics后Pim1表达丰度减少（(1.00±0.13) *vs* (0.38±0.03)，*P*<0.01），而转染miR-878 inh后Pim1表达丰度增加（(1.00±0.03) *vs* (3.19±0.68)，*P*<0.01）。

Western blot结果显示（图4f），在H/R诱导细胞损伤时，Pim1表达减少（(1.00±0.11) *vs* (0.64±0.03)，*P*<0.01），低表达miR-878能够增加Pim1表达水平，组间差异均有统计学意义（(0.61±0.01) *vs* (0.92±0.09)，*P*<0.01），提示miR-878影响Pim1表达丰度。

2.5　Pim1介导miR-878促进H/R损伤的作用

分别将shPim1(1)、shPim1(2)、shPim1(3)、NC shRNA质粒载体转染H9c 2细胞，结果显示（图5a），与NC shRNA组相比，shPim1(1)组的Pim1表达下降最显著（(1.00±0.18) *vs* (0.61±0.08)，*P*<0.05）。因此选择shPim1(1)进行后续功能实验。

CCK-8法检测细胞活力，结果显示（图5b），H/R+miR-878 inh组和H/R+NC inh组相比，细胞活力增加（(49.55±3.35) *vs* (81.61±1.90)，*P*<0.000 1），

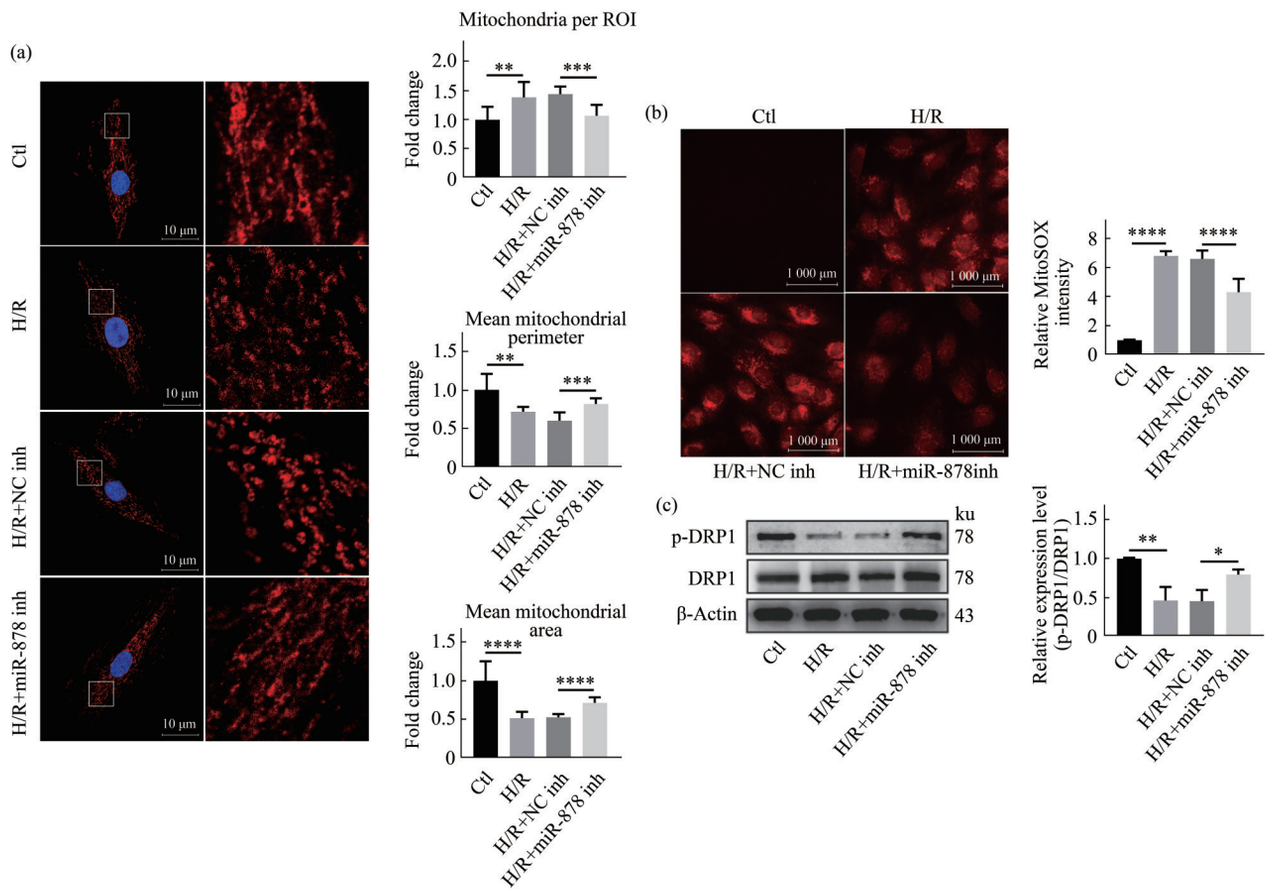

Fig. 3　Down−regulation of miR−878 expression can inhibit DRP1−mediated mitochondrial hyperdivision

(a) Confocal laser microscopy was employed to examine the morphology of mitochondria, and ImageJ software was utilized to analyze the mitochondrial network morphology. Red fluorescence indicates the presence of Tom20 antibody labeling, while blue fluorescence signifies DAPI staining. (b) Fluorescence microscope was used to observe the fluorescence intensity of mtROS. (c) Western blot examined the protein level of DRP1 and p-DRP1. Data are expressed as *mean*±*SD* (*n*=3). $^{*}P$<0.05; $^{**}P$<0.01; $^{***}P$<0.001; $^{****}P$<0.000 1. β-Actin was the internal reference.

与 H/R+miR-878 inh+NC shRNA 组相比，H/R+miR-878 inh+NC shPim1 组的细胞活力下降（(80.12±0.92) *vs* (61.91±3.10)，*P*<0.000 1）。

LDH水平的检测发现（图5c），H/R+miR-878 inh组和H/R+NC inh组相比，LDH释放量显著减少（(319.35±119.35) *vs* (157.25±35.57)，*P*<0.000 1），与 H/R+miR-878 inh+NC shRNA 组相比，H/R+miR-878 inh+NC shPim1组的LDH释放量显著增加（(153.32±34.15) *vs* (245.25±81.55)，*P*<0.000 1）。

使用激光共聚焦显微镜进行线粒体形态测定，结果显示（图5d），与 H/R+miR-878 inh+NC shRNA 组相比，H/R+miR-878 inh+shPim1 组周长减小（(1.00±0.16) *vs* (0.61±0.12)，*P*<0.001）、线粒体计数增加（(1.00±0.21) *vs* (1.37±0.14)，*P*<0.01）、面积减少（(1.00±0.09) *vs* (0.32±0.08)，*P*<0.001）。

荧光显微镜观察mtROS的荧光强度，结果显示（图5e），与H/R+miR-878 inh+NC shRNA组相比，H/R+miR-878 inh+shPim1组mtROS产生增加（(1.00±0.12) *vs* (2.41±0.12)，*P*<0.001）。

Western blot检测p-DRP1蛋白表达水平，结果显示（图5f），与H/R+miR-878 inh+NC shRNA组相比，H/R+miR-878 inh+shPim1组p-DRP1表达下降（(1.00±0.15) *vs* (0.59±0.06)，*P*<0.05）。

上述结果表明，下调miR-878表达可减少细胞损伤。同时抑制Pim1表达会促进线粒体分裂，加重细胞损伤。说明miR-878通过靶向抑制Pim1表达而促进DRP1介导的线粒体过度分裂，最终导致细胞损伤。

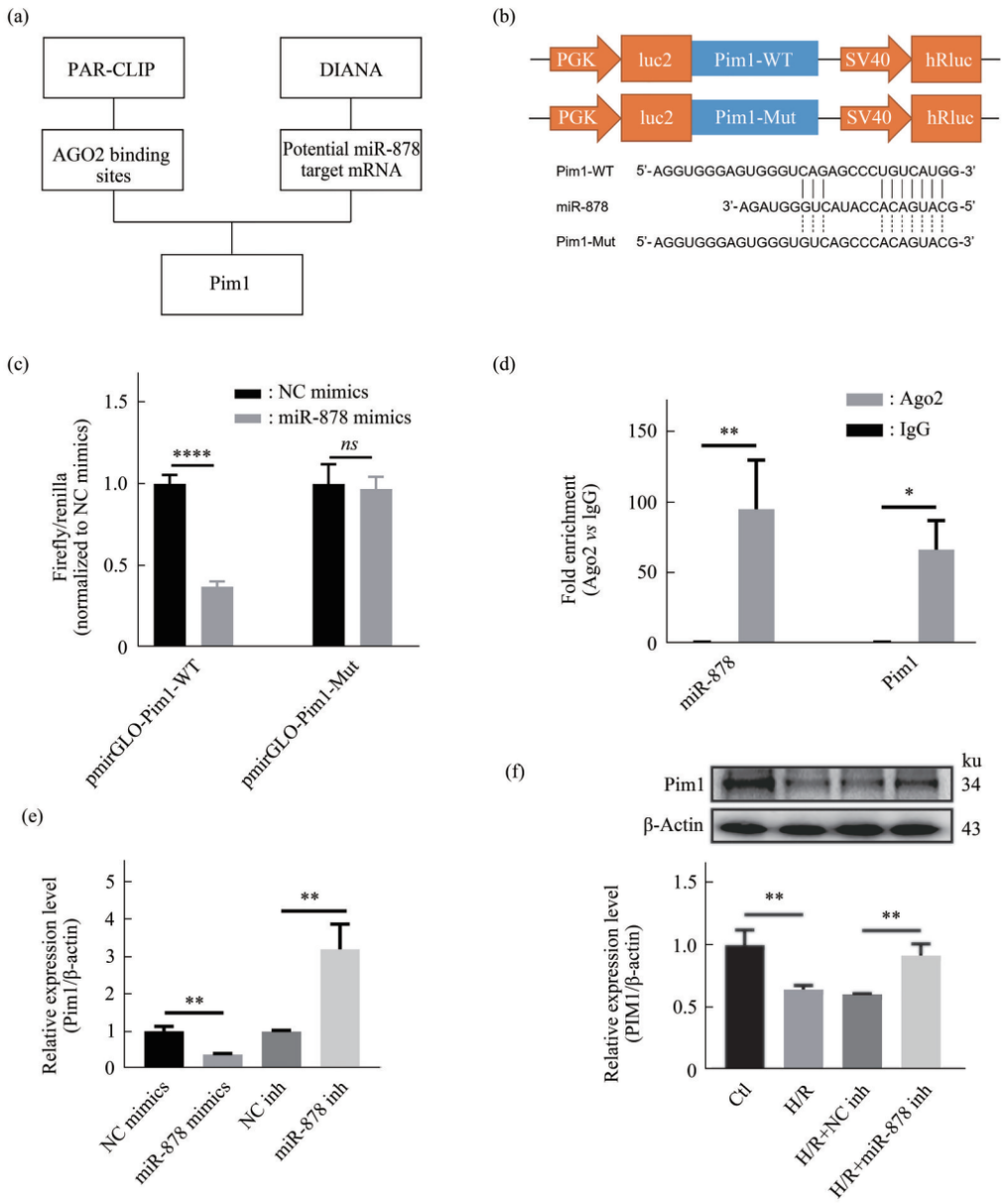

Fig. 4　MiR−878 regulates DRP1 expression by targeting Pim1

(a) DIANA tools database predicts the miR-878 combination site in Pim1 and Ago2 protein. (b) The miR-878 sequence and location of the Pim1 wild and mutant binding sites were cloned to the pmirGLO luciferase report carrier. (c) Dual luciferase reporter assay was used to determine the interaction sites between miR-878 and Pim1 3'-UTR. (d) RT-qPCR analysis of miR-878 and Pim1 enrichment by Ago2 antibody and IgG antibody in RNA immunoprecipitation experiments. (e) Relative level of Pim1 expression transfected by the mimics and inhibitors of miR-878 and the corresponding negative control (NC). (f) Western blot examined the protein level of Pim1. Date are expressed as *mean*±*SD* (*n*=3). *ns*, no significance; $^{*}P<0.05$; $^{**}P<0.01$; $^{****}P<0.000\ 1$. β-Actin was the internal reference.

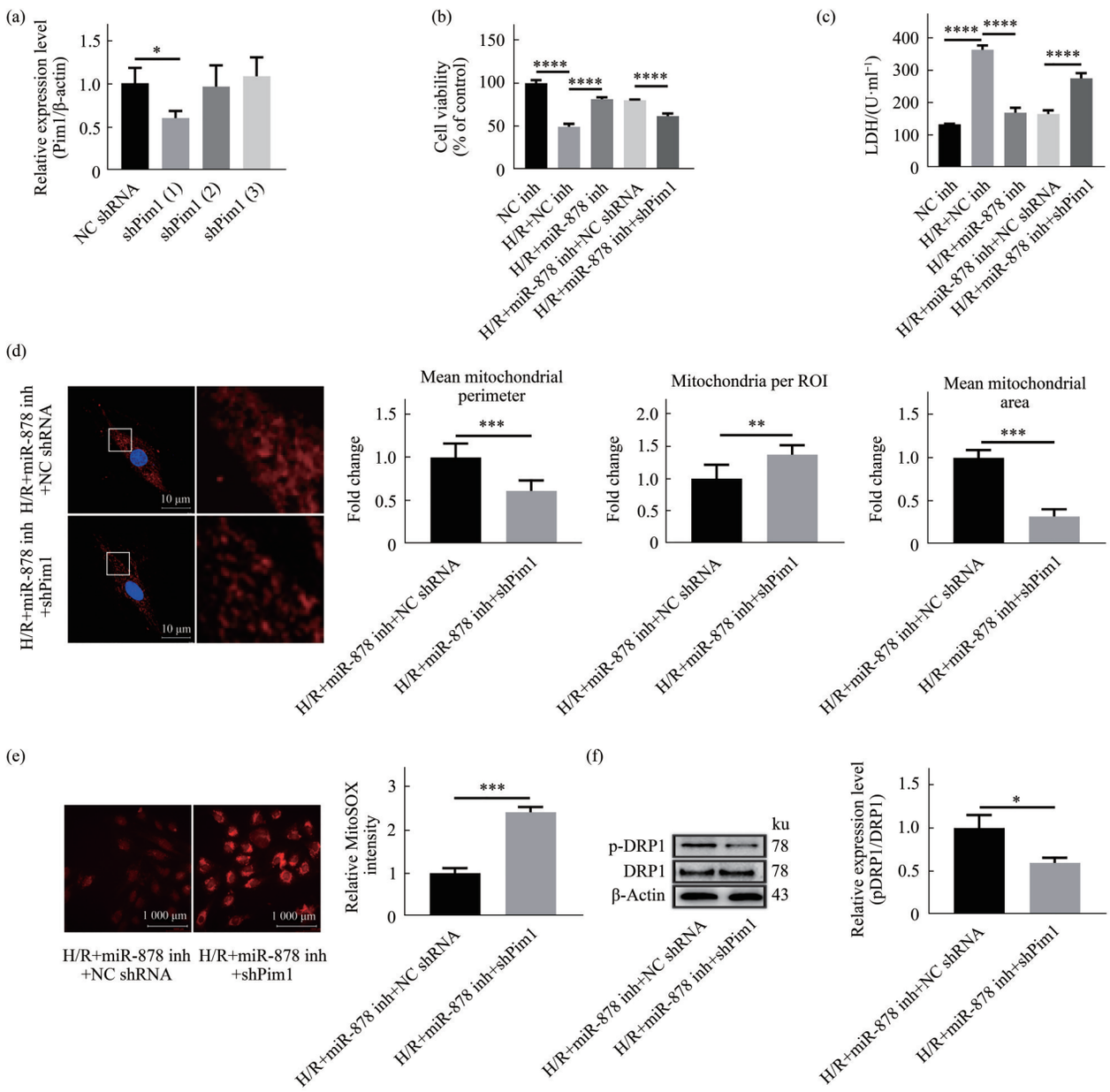

Fig. 5　Pim1 mediates miR−878 to promote H/R injury

(a) RT-qPCR detected the relative level of Pim1 expression in H9c2 cells. (b) The cell viability of H9c2 cells was detected by CCK-8 assay. (c) LDH level in H9c2 cells. (d) Confocal laser microscopy was used to determine the morphology of mitochondria and to analyze the morphology of mitochondrial network. red fluorescence indicates Tom 20 antibody dyed; blue fluorescent indicates DAPI staining; (e) Fluorescence microscope was used to observe the fluorescence intensity of mtROS. (f) Western blot examined the protein level of DRP1 and p-DRP1. Data are expressed as *mean*± *SD* (*n*=3). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.000\ 1$. β-Actin was the internal reference.

3　讨　　论

研究表明，miR-878参与调控多种疾病的发生发展。据报道，miR-878在肝癌中表达下调，参与调控肿瘤细胞增殖、侵袭及转移过程[23]。此外，miR-878在肝硬化和肝纤维化等肝病中严重下调，其显著影响肝细胞的代谢、凋亡及炎症反应[24]。而且最近一项研究发现，miR-878在牙周疾病进展机制中起重要作用[25]。然而，对miR-878在心脏疾病中的作用知之甚少。本研究发现，miR-878在H/R处理的H9c2细胞中表达升高。通过敲低miR-878表达可抑制线粒体分裂并减少细胞损伤。这表

明，miR-878参与了MI/R损伤过程的进展。DRP1介导线粒体分裂的过程已被发现受到miRNAs的精细调控[26]。通过升高miR-29a-3p表达，可以降低DRP1水平，抑制线粒体过度分裂而抑制心肌细胞肥大[27]。此外，通过升高miR-376b-3p[28]及miR-485-5p[29]表达水平抑制线粒体分裂，延缓心肌细胞肥厚病理进展。在MI/R损伤进程中，抑制线粒体分裂目前被认为是治疗MI/R损伤的核心靶标[10]。本次研究发现，敲低miR-878表达会通过Pim1蛋白显著影响DRP1介导的线粒体分裂。

目前已有广泛的研究表明，Pim1在心脏中发挥重要的作用。Pim1通过调控细胞周期进展、信号转导、抑制心肌细胞损伤以及保护线粒体完整性等多种机制，具有强大的心脏保护作用[30]。Pim1和DRP1通过蛋白质-蛋白质相互作用结合在一起，促使Pim1的激酶活性区域靠近底物蛋白DRP1，并将磷酸基团从ATP转移到底物蛋白DRP1上的Ser637氨基酸残基，从而直接磷酸化DRP1蛋白[8]。研究显示[31]，H/R处理心肌细胞后，Pim1表达降低。通过慢病毒或转基因操作促进Pim1过表达，可抑制线粒体的过度分裂并减少心肌细胞凋亡。而敲除Pim1基因的表达，加重线粒体过度分裂、碎片化形成，细胞凋亡增加。在MI/R损伤模型中，给予选择性Pim1激酶抑制剂II，能够减少心肌梗死面积[32]。七氟醚通过增加Pim1在细胞质和线粒体中的表达，抑制DRP1向线粒体外膜聚集，从而保持线粒体的完整性并减少心肌梗死面积[33]。本次研究发现了Pim1的上游调节机制：miR-878在转录后水平调控Pim1表达，且miR-878与Pim1基因的3'-UTR存在着结合位点，回复实验进一步证实miR-878促进H/R损伤的作用依赖于Pim1蛋白。这些结果表明，miR-878/Pim1/DRP1信号通路可能在MI/R损伤的进展过程中发挥着重要作用。

综上所述，miR-878可以靶向调控Pim1，进而促进DRP1介导的线粒体分裂，加剧H/R诱导的细胞损伤。本次研究为探索MI/R损伤的分子机制及防治MI/R损伤提供了新的思路。

4 结　论

本研究发现，miR-878在H/R处理的H9c2细胞中表达升高，而抑制miR-878表达水平可以缓解心肌细胞损伤。同时，下调miR-878表达能够显著抑制DRP1介导的线粒体分裂及mtROS产生。miR-878的作用机制为靶向结合Pim1基因的3'-UTR区并抑制Pim1表达。

参　考　文　献

[1] Li Y, Cao G Y, Jing W Z, *et al*. Global trends and regional differences in incidence and mortality of cardiovascular disease, 1990-2019: findings from 2019 global burden of disease study. Eur J Prev Cardiol, 2023, **30**(3): 276-286

[2] Abbas A, Raza A, Ullah M, *et al*. A comprehensive review: epidemiological strategies, catheterization and biomarkers used as a bioweapon in diagnosis and management of cardio vascular diseases. Curr Probl Cardiol, 2023, **48**(7): 101661

[3] Sobhy M, Elshal A, Ghanem N, *et al*. Development of primary percutaneous coronary intervention as a national reperfusion strategy for patients with ST-elevation myocardial infarction and assessment of its use in Egypt. CVIA, 2020, **4**(4): 269-278

[4] Heusch G. Myocardial ischaemia-reperfusion injury and cardioprotection in perspective. Nat Rev Cardiol, 2020, **17**(12): 773-789

[5] Heusch G. Cardioprotection: chances and challenges of its translation to the clinic. Lancet, 2013, **381**(9861): 166-175

[6] Yellon D M, Hausenloy D J. Myocardial reperfusion injury. N Engl J Med, 2007, **357**(11): 1121-1135

[7] Bai M, Zhang J, Chen D, *et al*. Insights into research on myocardial ischemia/reperfusion injury from 2012 to 2021: a bibliometric analysis. Eur J Med Res, 2023, **28**(1): 17

[8] Fonseca T B, Sánchez-Guerrero Á, Milosevic I, *et al*. Mitochondrial fission requires DRP1 but not dynamins. Nature, 2019, **570**(7761): E34-E42

[9] Crola Da Silva C, Baetz D, Védère M, *et al*. Isolated mitochondria state after myocardial ischemia-reperfusion injury and cardioprotection: analysis by flow cytometry. Life, 2023, **13**(3): 707-721

[10] Zerihun M, Sukumaran S, Qvit N. The Drp1-mediated mitochondrial fission protein interactome as an emerging core player in mitochondrial dynamics and cardiovascular disease therapy. Int J Mol Sci, 2023, **24**(6): 5785-5814

[11] Din S, Mason M, Völkers M, *et al* . Pim-1 preserves mitochondrial morphology by inhibiting dynamin-related protein 1 translocation. Proc Natl Acad Sci USA, 2013, **110**(15): 5969-5974

[12] Khan I, Siraj M. An updated review on cell signaling pathways regulated by candidate miRNAs in coronary artery disease. Noncoding RNA Res, 2023, **8**(3): 326-334

[13] Ao X, Ding W, Li X, *et al*. Non-coding RNAs regulating mitochondrial function in cardiovascular diseases. J Mol Med (Berl), 2023, **101**(5): 501-526

[14] Li Q H, Ge Z W, Xiang Y, *et al*. Upregulation of microRNA-34a enhances myocardial ischemia-reperfusion injury *via* the mitochondrial apoptotic pathway. Free Radic Res, 2022, **56**(3-4): 229-244

[15] Lu C H, Chen D X, Dong K, *et al*. Inhibition of miR-143-3p

alleviates myocardial ischemia reperfusion injury *via* limiting mitochondria-mediated apoptosis. Biol Chem, 2023, **404**(6): 619-631

[16] Xu Y, Guo W, Zeng D, *et al*. Inhibiting miR-205 alleviates cardiac ischemia/reperfusion injury by regulating oxidative stress, mitochondrial function, and apoptosis. Oxid Med Cell Longev, 2021, **2021**: 9986506

[17] Zhu X, Lu X. MiR-423-5p inhibition alleviates cardiomyocyte apoptosis and mitochondrial dysfunction caused by hypoxia/reoxygenation through activation of the wnt/β-catenin signaling pathway *via* targeting MYBL2. J Cell Physiol, 2019, **234**(12): 22034-22043

[18] Zhang P, Guan P, Ye X, *et al*. SOCS6 promotes mitochondrial fission and cardiomyocyte apoptosis and is negatively regulated by quaking-mediated miR-19b. Oxid Med Cell Longev, 2022, **2022**: 1121323

[19] Yang S, Li H, Chen L. MicroRNA-140 attenuates myocardial ischemia-reperfusion injury through suppressing mitochondria-mediated apoptosis by targeting YES1. J Cell Biochem, 2019, **120**(3): 3813-3821

[20] Song R, Dasgupta C, Mulder C, *et al*. MicroRNA-210 controls mitochondrial metabolism and protects heart function in myocardial infarction. Circulation, 2022, **145**(15): 1140-1153

[21] Guo L, Liu C, Jiang C, *et al*. MiR-124 inhibits cardiomyocyte apoptosis in myocardial ischaemia-reperfusion injury by activating mitochondrial calcium uniporter regulator 1. Mol Med Rep, 2023, **28**(2): 144

[22] Niu X, Pu S, Ling C, *et al*. LncRNA Oip5-as1 attenuates myocardial ischaemia/reperfusion injury by sponging miR-29a to activate the SIRT1/AMPK/PGC1α pathway. Cell Prolif, 2020, **53**(6): e12818

[23] Lu B, Sheng Y, Zhang J, *et al*. The altered microRNA profile in andrographolide-induced inhibition of hepatoma tumor growth. Gene, 2016, **588**(2): 124-133

[24] Roderburg C, Luedde T. The role of miRNAs in animal models of liver fibrosis. Drug Discov Today Dis Models, 2013, **10**(3): e121-e126

[25] Maruyama T, Kobayashi T, Sugiura Y, *et al*. Association between serum miRNAs and gingival gene expression in an obese rat model. J Periodontal Res, 2022, **57**(3): 502-509

[26] Mokhtari B, Hosseini L, Høilund-Carlsen PF, *et al*. The additive effects of nicotinamide mononucleotide and melatonin on mitochondrial biogenesis and fission/fusion, autophagy, and microRNA-499 in the aged rat heart with reperfusion injury. Naunyn Schmiedebergs Arch Pharmacol, 2023, **396**(8): 1701-1711

[27] Xie Y, Hu J, Zhang X, *et al*. Neuropeptide Y induces cardiomyocyte hypertrophy *via* attenuating miR-29a-3p in neonatal rat cardiomyocytes. Protein Pept Lett, 2020, **27**(9): 878-887

[28] Sun Y L, Li S H, Yang L, *et al*. MiR-376b-3p attenuates mitochondrial fission and cardiac hypertrophy by targeting mitochondrial fission factor. Clin Exp Pharmacol Physiol, 2018, **45**(8): 779-787

[29] Zhao Y, Ponnusamy M, Liu C, *et al*. MiR-485-5p modulates mitochondrial fission through targeting mitochondrial anchored protein ligase in cardiac hypertrophy. Biochim Biophys Acta Mol Basis Dis, 2017, **1863**(11): 2871-2881

[30] Din S, Mason M, Völkers M, *et al*. Pim-1 preserves mitochondrial morphology by inhibiting dynamin-related protein 1 translocation. Proc Natl Acad Sci USA, 2013, **110**(15): 5969-5974

[31] Liu N, Wang B J, Broughton K M, *et al*. PIM1-minicircle as a therapeutic treatment for myocardial infarction. PLoS One, 2017, **12**(3): e0173963

[32] Stumpner J, Tischer-Zeitz T, Lotz C, *et al*. The second window of desflurane-induced preconditioning is mediated by STAT3: role of Pim-1 kinase. Acta Anaesthesiol Scand, 2016, **60**(1): 103-116

[33] Liu J D, Chen H J, Wang D L, *et al*. Pim-1 kinase regulating dynamics related protein 1 mediates sevoflurane postconditioning-induced cardioprotection. Chin Med J (Engl), 2017, **130**(3): 309-317

MiR-878 Aggravates Hypoxia/Reoxygenation Injury in H9c2 Cardiomyocytes by Inducing Pim1-mediated Mitochondrial Fission*

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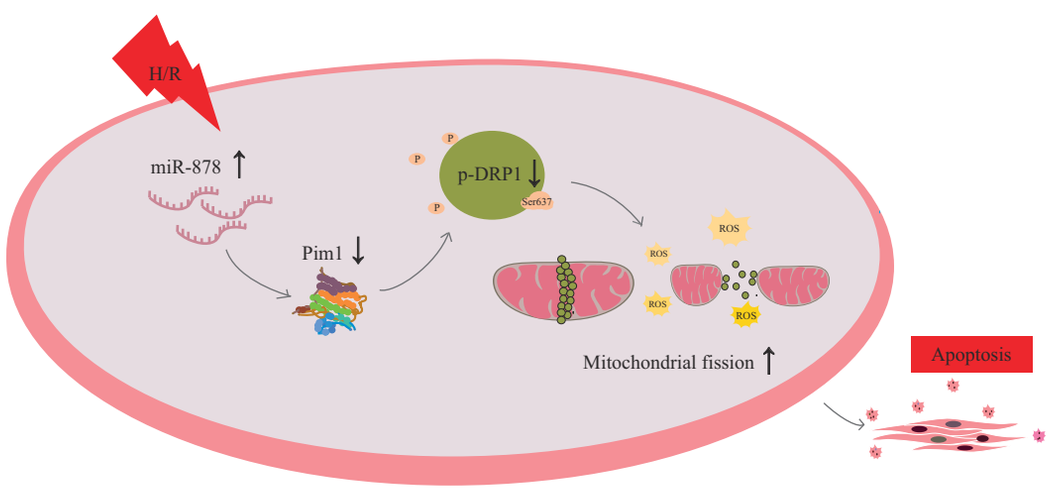
Graphical abstract



Abstract　Objective　Acute myocardial infarction (AMI) is a highly prevalent and deadly disease globally, with its incidence continuing to rise in recent years. Timely reperfusion therapy is crucial for improving the prognosis of AMI patients. However, myocardial reperfusion can lead to irreversible myocardial ischemia/reperfusion (MI/R) injury, which is associated with adverse cardiovascular outcomes following AMI. Studies have shown that microRNAs (miRNAs) are abnormally expressed during MI/R injury and play an important role in the fate of cardiomyocytes. Effective preventive and therapeutic strategies against MI/R injury remain lacking in clinical practice, necessitating elucidation of the molecular mechanisms underlying MI/R onset and progression. This

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study investigated the role of microRNA-878 (miR-878) in the regulation of mitochondria-mediated apoptosis in MI/R injury. **Methods** The H9c2 cells were flushed with a gas mixture containing 1% O_2, 5% CO_2 and 94% N_2 for 3 h. Then the cells were incubated in complete culture medium under 5% CO_2 and 95% air for 6 h to mimic *in vivo* hypoxia/reoxygenation (H/R) injury. Cell viability were detected by CCK-8 assay. The concentrations of lactate dehydrogenase (LDH) were then measured. The level of apoptosis was analyzed by flow cytometry. The morphology of mitochondria was analyzed by immunofluorescence and laser confocal microscopy. The levels of mitochondrial reactive oxygen species (mtROS) were detected by immunofluorescence. Dual luciferase reporter gene assay was used to study the binding site of miR-878 and Pim1. RNA immunoprecipitation (RIP) assay was used to verify the binding relationship between miR-878 and Pim1. The gene expression levels were detected by real-time fluorescent quantitative PCR (RT-qPCR) and Western blot. **Results** The study found that compared with the control group, the expression of miR-878 in H/R-treated H9c2 cells was significantly increased ((1.00±0.25) *vs* (9.70±2.63), $P<0.01$). In H/R-induced cells, transfection of miR-878 inhibitor significantly increased cell viability ((46.67±3.00) *vs* (74.62±4.08), $P<0.000\ 1$), and decreased LDH release ((358.58±41.71) *vs* (179.09±15.59), $P<0.000\ 1$) and cell apoptosis rate ((43.41±0.72) *vs* (27.42±4.48), $P<0.01$). At the same time, downregulation of miR-878 expression significantly inhibited DRP1-mediated mitochondrial overdivision and mtROS production ((6.60±0.57) *vs* (4.32±0.91), $P<0.000\ 1$). The mechanism study showed that miR-878 could target and bind Pim1 and inhibit the expression level of Pim1 ((1.00±0.13) *vs* (0.38±0.03), $P<0.01$). Rescue experiments confirmed that down-regulation of Pim1 expression significantly reversed the anti-injury effect of miR-878 inhibitor in H9c2 cells ($P<0.01$), promoted mitochondrial overdivision and mtROS production ((1.00±0.12) *vs* (2.41±0.12), $P<0.01$), and decreased the expression level of p-DRP1 ((1.00±0.15) *vs* (0.59±0.06), $P<0.05$). **Conclusion** The present study demonstrates that miR-878 expression is upregulated in H9c2 cardiomyocytes subjected to H/R injury. Inhibition of miR-878 expression alleviates H/R-induced cardiomyocyte damage. Notably, downregulation of miR-878 significantly inhibits DRP1-mediated mitochondrial fission and mitigates mtROS production. Mechanistically, miR-878 targets and binds to the 3'-UTR of the Pim1 gene, thereby suppressing Pim1 protein expression. Collectively, these findings suggest that under H/R conditions, miR-878 promotes excessive mitochondrial fragmentation through DRP1 activation by targeting Pim1, ultimately contributing to cardiomyocyte injury. Modulation of the miR-878/Pim1 axis may represent a potential therapeutic strategy for mitigating MI/R-induced cardiac damage.

Key words miR-878, Pim1, dynamin-related protein 1, myocardial ischemia/reperfusion injury
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