FKBP prolyl isomerase 4: a potential target for heart failure and cardioprotective effects via leonurine-pretreated mesenchymal stem cell-derived exosomes

Zhaoyi Li[1], De-Chong Zheng[1], Ruixiang Wang[2], Qian Ding[3] and Yi Zhun Zhu[3,4,5*]

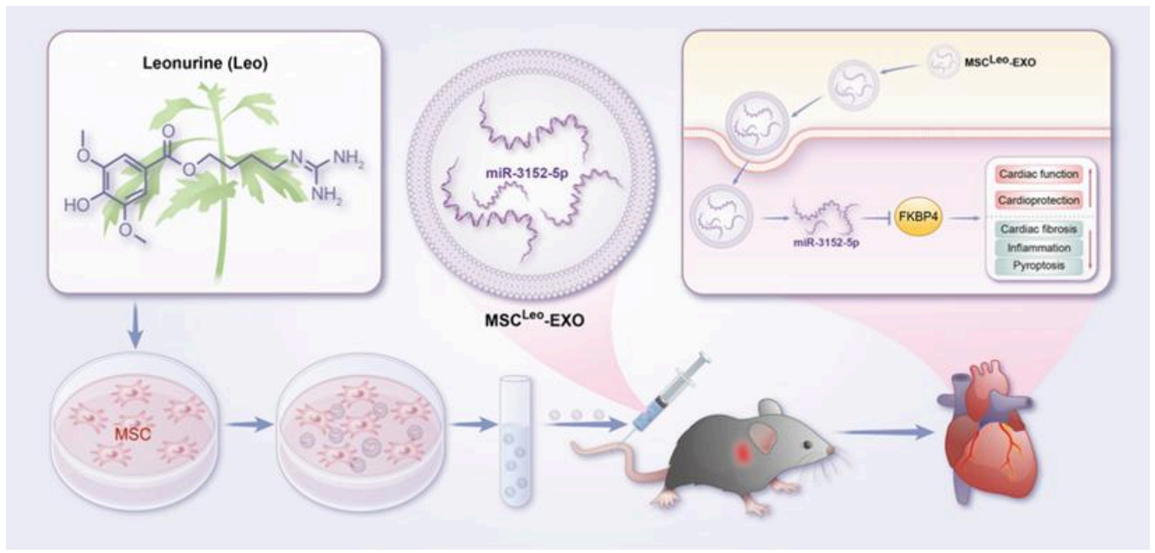
1 School of Pharmacy, Faculty of Medicine & State Key Laboratory of Quality Research in Chinese Medicines, Macau University of Science and Technology, Macau 999078, China.
2 State Key Laboratory of Quality Research in Chinese Medicines, Macau University of Science and Technology, Macau SAR, 999078, China.
3 Laboratory of Drug Discovery from Natural Resources and Industrialization, School of Pharmacy, Macau University of Science and Technology, Macau 999078, China.
4 Shanghai Key Laboratory of Bioactive Small Molecules, Department of Pharmacology, School of Pharmacy, Fudan University, Shanghai, 201203, China.

Corresponding author Chair Prof. Yi Zhun Zhu; Email: yzzhu@must.edu.mo

HIGHLIGHTS:

The use of mesenchymal stem cell-derived exosomes (MSC-EXOs) is a promising strategy for treating heart failure. Pretreatment of MSCs with cardioprotective agents, such as leonurine, has the potential to augment the therapeutic efficacy of their EXO activities. However, the cardioprotective potential of EXOs derived from MSCs pretreated with leonurine (MSC^{Leo}-EXO) remains unexplored. This study offers the first proof that leonurine-pretreated MSC-derived exosomes increase miR-3152-5p expression, which in turn causes specific suppression of FKBP4. This process results in a reduction in cardiac fibrosis, cell pyroptosis, and inflammatory responses, thereby enhancing cardioprotection, improving cardiac function postheart failure, and promoting myocardial repair. Our findings offer a novel strategy for optimizing the application of exosome therapy in cardiac repair and establish a research foundation for the future utilization of exosome therapy in this field.

Graphical Abstract:



1. MSC^{Leo}-EXOs effectively protected cardiomyocytes from hypoxia-induced damage

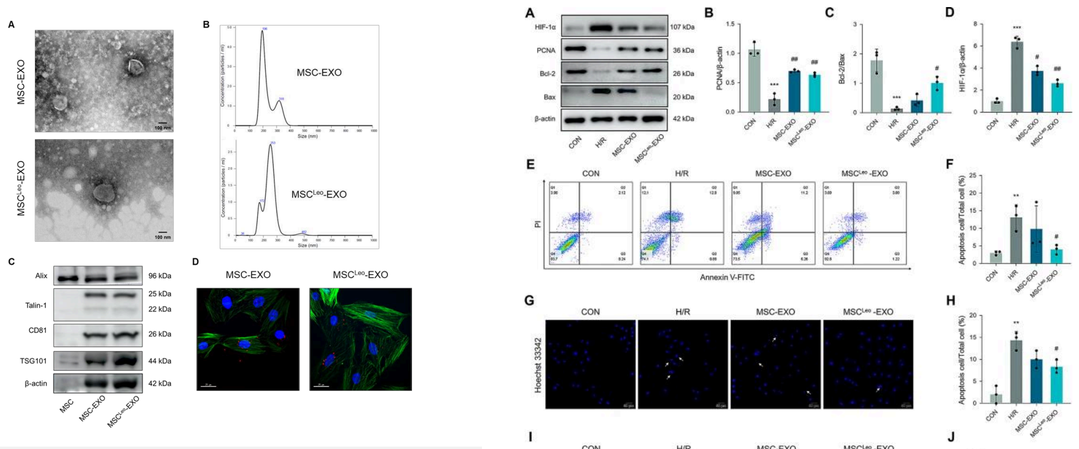


Fig 1. (A) Representative western blot images showing the expression levels of PCNA, Bcl-2, Bax, and HIF-1α in H9c2 cells treated with MSC-EXOs and MSCLeo-EXOs under hypoxia. **(B)** Quantification of PCNA expression (n = 3). **(C)** Quantification of Bcl-2/Bax expression (n = 3). **(D)** Quantification of HIF-1α expression (n = 3). **(E)** Dot plots of apoptosis in H9c2 cells treated with MSC-EXOs or MSCLeo-EXOs under hypoxia detected by flow cytometry and **(F)** quantification (n = 3). **(G)** Representative images of Hoechst 33342 nuclear staining in H9c2 cells treated with MSC-EXOs or MSCLeo-EXOs under hypoxia and **(H)** quantification (n = 3). White arrows = damaged nuclei. Scale bar = 50 μm. **(I)** Representative Mitro Red fluorescence images showing mitochondrial activation in H9c2 cells treated with MSC-EXOs or MSCLeo-EXOs under hypoxia and **(J)** quantification (n = 3). Scale bar = 50 μm. **p < 0.01, ***p < 0.001, vs. CON. #p < 0.05, ##p < 0.01, vs. H/R. (Figure in right)

2. MSC^{Leo}-EXOs reduced TGF-induced cardiac fibrosis, inflammation and alleviated pyroptosis by preventing NLRP3 inflammasome activation

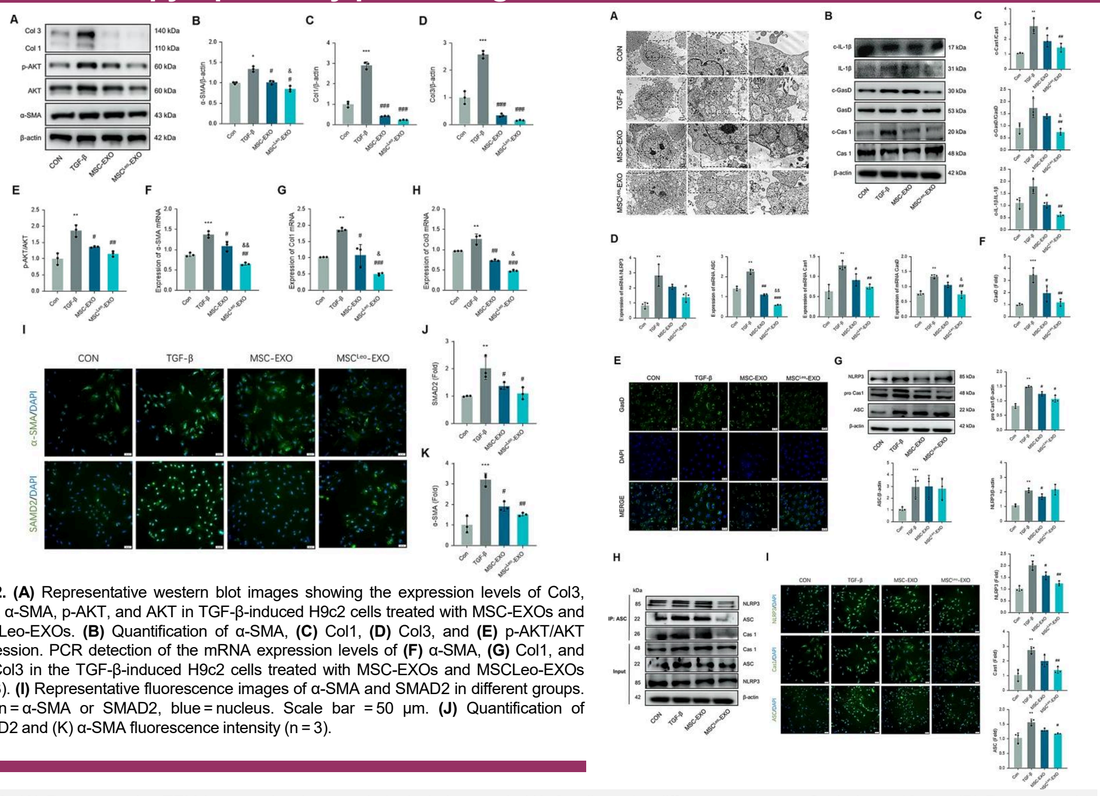


Fig 2. (A) Representative western blot images showing the expression levels of Col3, Col1, α-SMA, p-AKT, and AKT in TGF-β-induced H9c2 cells treated with MSC-EXOs and MSCLeo-EXOs. **(B)** Quantification of α-SMA, **(C)** Col1, **(D)** Col3, and **(E)** p-AKT/AKT expression. PCR detection of the mRNA expression levels of **(F)** α-SMA, **(G)** Col1, and **(H)** Col3 in the TGF-β-induced H9c2 cells treated with MSC-EXOs and MSCLeo-EXOs (n = 3). **(I)** Representative fluorescence images of α-SMA and SMAD2 in different groups. Green = α-SMA or SMAD2, blue = nucleus. Scale bar = 50 μm. **(J)** Quantification of SMAD2 and (K) α-SMA fluorescence intensity (n = 3).

Fig 3. (A) Representative TEM images showing morphological changes in H9c2 cells under different treatments. Scale bar = 10 μm. **(B)** Representative western blot images showing the expression levels of IL-1β, c-IL-β, GasD, c-GasD, Cas1, and c-Cas1 in TGF-β-induced H9c2 cells after MSC-EXO and MSC^{Leo}-EXO treatment and **(C)** quantification of c-Cas1/Cas1, c-Cas1/Cas1, and c-IL-1β/IL-1β expression (n = 3). **(D)** PCR detection of the mRNA expression levels of NLRP3, ASC, Cas1, and GasD in TGF-β-induced H9c2 cells after MSC-EXO or MSC^{Leo}-EXO treatment (n = 3). **(E)** Representative fluorescence images of GasD in different groups and (F) its quantification (n = 3). **(G)** Representative western blot images showing the expression levels of the NLRP3 inflammasome complex (pro Cas1, ASC, and NLRP3) in TGF-β-induced H9c2 cells after treatment with MSC-EXOs or MSC^{Leo}-EXOs and their quantification (n = 3). **(H)** Co-IP was used to verify the interaction between the NLRP3 inflammasome complex in H9c2 cells after different treatments. **(I)** Representative fluorescence images of NLRP3, ASC, and NLRP3 in different groups and their quantification (n = 3).

3. MSC^{Leo}-EXOs effectively protected cardiac function in ISO-induced heart failure mice

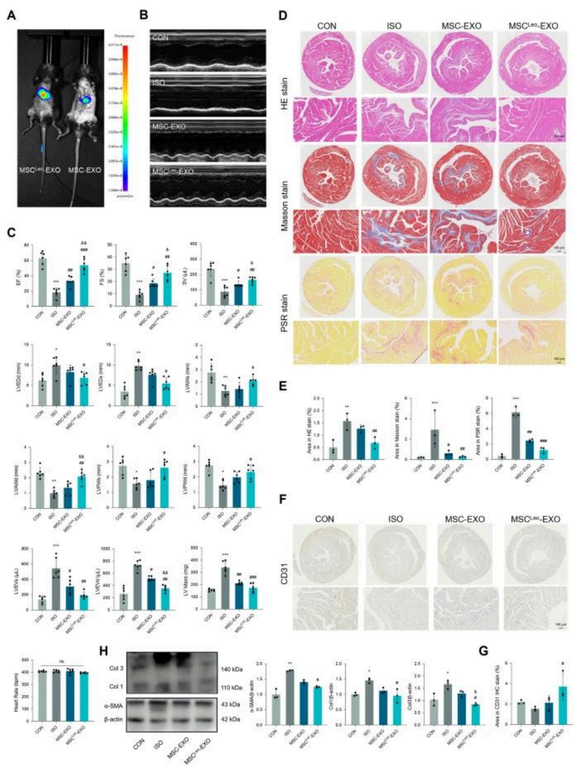
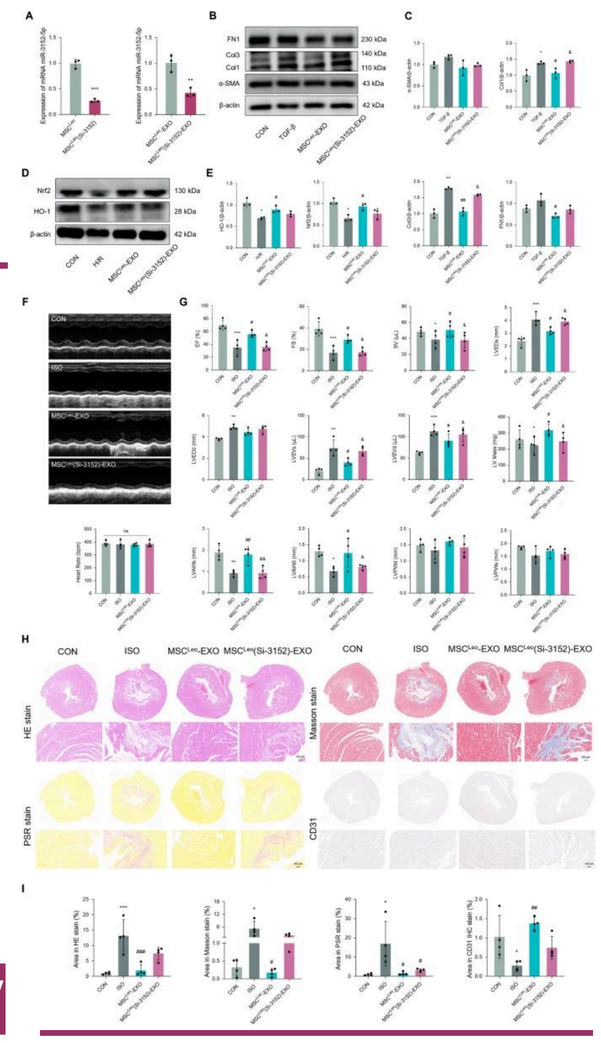


Fig 4. (A) Representative in vivo imaging images of the MSC-EXO and MSC^{Leo}-EXO groups on day 29. **(B)** Representative M-mode echocardiograms of the hearts of mice in different groups on day 29 after heart failure and **(C)** cardiac function parameters, including EF, FS, SV, LVEDd, LVEDs, LVAWs, LVAWd, LVPWs, LVPWd, LVEVs, LVEVd, and heart rate (n = 6). **(D)** Representative images of HE staining, Masson staining, and PSR staining of heart cross sections from mice in different groups. Scale bar = 100 μm. **(E)** Statistics of the different staining areas in **(D)** (n = 3). **(F)** CD31 staining of heart cross sections from the mice in each group and **(G)** quantification (n = 3). Scale bar = 100 μm. **(H)** Representative western blot images showing the expression levels and quantification of α-SMA, Col1, and Col3 in the myocardial tissues of each group (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001, vs. CON. #p < 0.05, ##p < 0.01, ###p < 0.001, vs. ISO. &p < 0.05, &&p < 0.01, vs. MSC-EXOs.

4. miR-3152-5p mediated the cardioprotective effects of MSC^{Leo}-EXOs



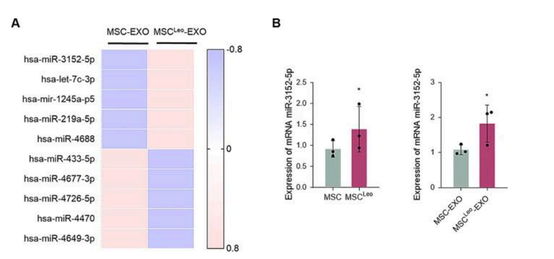


Fig 5. (A) Expression levels of miR-3152-5p in $MSCs^{Leo}$ and EXOs after knockdown of miR-3152-5p (n = 3). **p < 0.01, ***p < 0.001, vs. MSC^{Leo} or MSC^{Leo}-EXO. **(B)** Representative western blot images showing the expression levels of α-SMA, Col1, Col3, and FN1 in H9c2 cells induced by TGF-β after treatment with MSC^{Leo}-EXOs and MSC^{Leo} (Si-3152)-EXOs and **(C)** their quantification (n = 3). **(D)** Representative western blot images showing the expression levels of Nrf2 and HO-1 in H9c2 cells under H/R conditions after treatment with MSC^{Leo}-EXOs and MSC^{Leo} (Si-3152)-EXOs and **(E)** their quantification (n = 3). **(F)** Representative M-mode echocardiograms of the hearts of mice in different groups on day 29 after heart failure and **(G)** cardiac function parameters, including EF, FS, SV, LVEDd, LVEDs, LVAWs, LVAWd, LVPWs, LVPWd, LVEVs, LVEVd, and heart rate (n = 4). **(H)** Representative images of HE staining, Masson staining, and PSR staining of the hearts of the mice in different groups and **(I)** statistics of the size of the stained area (n = 3). Scale bar = 100 μm. *p < 0.05, **p < 0.01, ***p < 0.001, vs. CON. #p < 0.05, ##p < 0.01, ###p < 0.001, vs. TGF-β, H/R, or ISO. &p < 0.05, &&p < 0.01, &&&p < 0.001, vs. MSC^{Leo}-EXOs

5. miR-3152-5p protected cardiomyocytes by targeting FKBP4

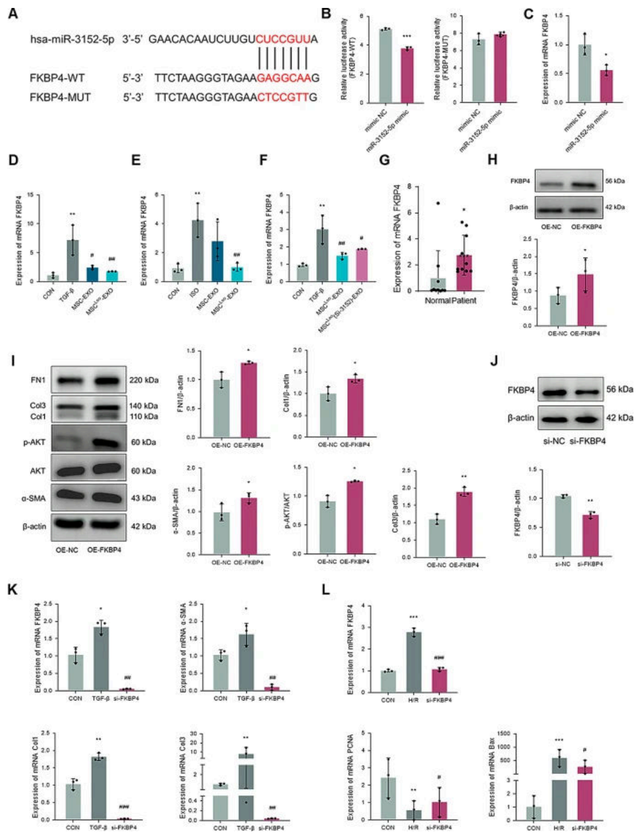


Fig 6. (A, B) Dual luciferase reporter assay for the binding sites of miR-3152-5p and FKBP4 (n = 3). ***p < 0.001, vs. mimic NC. **(C)** PCR assay for FKBP4 levels in H9c2 cells treated with miR-3152-5p and NC (n = 3). *p < 0.001, vs. mimic NC. **(D)** mRNA levels of FKBP4 in H9c2 cells treated with CON, TGF-β, MSC-EXOs, or MSCLeo-EXOs (n = 3). **(E)** mRNA levels of FKBP4 in the myocardial tissues of mice treated with CON, ISO, MSC-EXOs, or MSC^{Leo}-EXOs (n = 3). (F) mRNA levels of FKBP4 in H9c2 cells treated with CON, TGF-β, MSC^{Leo}-EXO, or MSC^{Leo} (Si-3152)-EXO (n = 3). **p < 0.01, vs. CON. #p < 0.05, ##p < 0.01, vs. TGF-β or ISO. **(G)** FKBP4 mRNA levels in the blood of healthy people and patients with heart failure (n = 10). **(H)** Representative western blot images showing the expression level of FKBP4 in H9c2 cells transfected with the FKBP4 overexpression plasmid or its NC and its quantification (n = 3). *p < 0.05, vs. OE-NC. **(I)** Representative western blot images showing the expression and quantification of α-SMA, Col1, Col3, FN1, p-AKT, and AKT in H9c2 cells transfected with the FKBP4 overexpression plasmid or its NC (n = 3). *p < 0.05, *p < 0.01, vs. OE-NC. **(J)** Representative western blot images showing the expression level of FKBP4 in H9c2 cells transfected with si-FKBP4 or its NC and its quantification (n = 3). **p < 0.01, vs. si-NC. **(K)** mRNA expression levels of FKBP4, α-SMA, Col1 and Col3 in H9c2 cells treated with CON, TGF-β and si-FKBP4 (n = 3). *p < 0.05, **p < 0.01, vs. si-NC. ##p < 0.01, ###p < 0.001, vs. TGF-β. (L) mRNA expression levels of FKBP4, PCNA, and Bax in H9c2 cells treated with CON, HR, or si-FKBP4 (n = 3). **p < 0.01, ***p < 0.001, vs. si-NC. #p < 0.05, ###p < 0.001, vs. H/R. Abbreviations: NC: negative control; OE: overexpression

Conclusion

Leonurine-pretreated MSC-derived exosomes increase miR-3152-5p expression, which in turn causes specific suppression of FKBP4. This process results in a reduction in cardiac fibrosis, cell pyroptosis, and inflammatory responses, thereby enhancing cardioprotection, improving cardiac function post heart failure, and promoting myocardial repair.

Contact Person: Zhaoyi Li **Supervisor: Chair Prof. Yi Zhun Zhu**