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Pre-conditioning with Nicotinamide-mononucleotide Enhances Cardioprotective Potentials of Umbilical Cord-derived Mesenchymal Stem Cells in Diabetes: Role of Autophagy Flux

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Abstract

Background:

in vitro *in vivo*. Here, we have investigated the e ects of preconditioning of human

of autophagy þux in diabetic rats.

Methods:

throughout the experiment. Cardiac injury was assessed using the measurement of infarct size and CK-mB release via planimetry and ELISA methods. Mitochondrial function was evaluated by þuorometric assays. Autophagy-related protein expression was evaluated using immunoblotting.

Results: Administration of NMN or MSCs alone had no signiýcant protective e ects. NMN-preconditioned MSCs signiýcantly reduced myocardial infarction and CK-mB levels, restored IR-induced mitochondrial reactive oxygen

diabetic rats. IR-induced overexpression of proteins Beclin-1, P62, and LC3-II and reduction of LC3-II/LC3-I ratio were signiýcantly reversed following treatment with preconditioned-MSCs. The administration of chloroquine, an autophagy þux inhibitor, abolished these cardioprotective e ects.

Conclusion: Therefore, NMN serves as a good preconditioning modality to increase the cardioprotective e cacy of MSCs in diabetic rats and the improvement of autophagy þux may play a signiýcant role.

Keywords: GLIPR1; Astroglioma; Proliferation; Migration; Invasion; CD63

In addition, NMN boosts the expansion of adult stem cells derived from di erent tissues like bone marrow, pancreatic cells, intestine, and umbilical cord [18]. It has been reported that this compound promotes cell survival and positively in uences the di erentiation of MSCs into neuronal and cardiac lineages [18,19]. Also, mitochondrial NAD+ replenishment via NMN supplementation delays stem cell senescence and facilitates their reprogramming [20]. As a result, it is hypothesized

CQ co-administration signi cantly reversed the protective e ects of NMN preconditioned-MSCs on mitochondrial function (P <0.05).

Promotion of autophagy by NMN preconditioned-MSCs and its reversal by CQ

e expression of autophagy-related proteins Beclin-1, LC3-I, and LC3-II was signi cantly increased following myocardial IR injury of diabetic hearts compared to the Sham group (P <0.05 to P <0.001) (Figures 4a-4f). e protein expression of P62, as the LC3 substrate, has also been upregulated signi cantly, indicating the possible reduction of autophagy ux in diabetic IR hearts. e e ects of alone treatment witahagy by

Improvement of mitochondrial function by NMN preconditioned-MSCs and its reversal by CQ

e production of mitochondrial ROS and mitochondrial membrane depolarization were signi cantly increased while the level of cellular ATP was reduced following IR injury as compared with the Sham group (P <0.01) (Figures 3a-3c). Although alone administration of NMN or MSCs tended to restore these changes, other di erences were not signi cant from the IR group except for changes in mitochondrial membrane potential by MSCs. However, injection of preconditioned-MSCs to IR diabetic hearts signi cantly reduced mitochondrial ROS (P <0.05) and membrane depolarization (P <0.01), and increased ATP level in comparison to those of the IR group (Figure 3). e e ects of preconditioned-MSCs on mitochondrial membrane potential and ATP level were statistically di erent from IR+nico and IR+MSC groups, respectively (P <0.05). Additionally, inhibition of autophagy ux using

of NMN preconditioned-MSCs on the regulation of Becline-1, LC3, and P62 expressions ($P < 0.05$), con rming the reduction of autophagy ux in IR hearts.

interactions between LC3 and ubiquitinated proteins [8]. e higher ratio of LC3-II/LC3-I and concomitant downregulation of P62 in the hearts of rats receiving NMN preconditioned-MSCs indicates that autophagy is triggered and the fusion of autophagosome to lysosomes and thereby the autophagosome clearance is promoted. e administration of CQ reversed these actions. is nding demonstrates that treatment of diabetic IR hearts with NMN preconditioned-MSCs corrected IR-induced dysregulation of autophagy and thereby prompted autophagosome degradation and autophagy ux, and ultimately exerted e ective cardioprotection. Identifying the contribution of other survival mechanisms in MSCs preconditioning-induced cardioprotection will help us to nd the central therapeutic cellular targets.

Conclusion

Preconditioning of human umbilical cord-derived MSCs with NMN signi cantly restored the cardioprotective e ects of MSCs in diabetic hearts subjected to IR injury via restricting infarct size, reducing CK release, improving mitochondrial function, and modifying the expression of the Beclin/LC3/P62 autophagy pathway. Progression of autophagy ux had a positive role in this cardioprotection. erefore, enhancing MSCs responsiveness with appropriate preconditioning stimuli can overcome the negative impacts of diabetes on cardioprotection.

Con icts of Interest

e authors have no con ict of interest to declare.

Authors' Contributions

All authors designed the project, performed the experimentations, analyzed, and interpreted the data. QW was the major contributor in writing the manuscript. All authors read and approved the nal manuscript.

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