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Discovery of Potential Therapeutic miRNA Targets in Cardiac Ischemia-Reperfusion Injury

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Abstract

Background: A highly efficient approach to select microRNA (miRNA) targets is a key to develop a miRNA-based therapeutic approach to cardiac ischemia–reperfusion (I/R). To reverse the change induced by disease, I/R in this case, is the traditional strategy to develop therapeutic drugs. However, examples show that it will not always serve the purpose. In this study, we demonstrate an additional approach of selecting miRNA targets with therapeutic potential following cues from cardioprotection-induced changes rather than by reversing disease-induced changes in cardiac I/R.

Methods: Isolated perfused rat hearts subjected to I/R were treated with 50 µmol/L sodium hydrosulfide (NaHS) or 10 nmol/L urocortin 2 (UCN2). Cardiac miRNA regulations were determined by miRNA array. Functional screening of selected miRNA mimics, assessed by WST (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) activity and lactate dehydrogenase (LDH) release, was performed in H9c2 and neonatal rat ventricular myocytes (NRVMs) with hypoxia/reoxygenation. RNA-induced silencing complex (RISC)-loaded miRNAs caused by mimic transfection were quantified following argonaute-2 immunoprecipitation. Gene regulations of 1 selected miRNA were determined by quantitative polymerase chain reaction and Western blot.

Results: Treatment with NaHS and UCN2 significantly improved cardiac function and reduced LDH release. The miRNA array indicated a panel of commonly up- and downregulated miRNAs. Among them, 10 upregulated miRNAs with antiapoptotic and antiautophagy potentials were selected for further screening. Mimics of miRNA-221, -150, and -206 were protective in both H9c2 and NRVM. RISC-loaded miRNAs were up by ∼20-fold above. To further prove the feasibility of this approach, miRNA-221 was studied. It reduced I/R-induced caspase 3/7 activity and LC3-II (microtubule-associated protein 1 light chain 3). Measuring genes predicted to regulate apoptosis and autophagy, miRNA-221 mimic decreased Ddit4, TP53inp1, and p27 at both messenger RNA (mRNA) and protein levels, and reduced mRNA of Bak1 and Puma and proteins of Bim and Bmf.

Conclusion: Mimicking miRNA changes caused by cardioprotective agents, combined with functional screening, enables investigators to efficiently identify novel miRNAs with therapeutic potential in cardiac I/R.

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