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MICRORNA-223 PREVENTS CARDIOMYOCYTE HYPERTROPHY BY TARGETING CARDIAC TROPONIN I-INTERACTING KINASE

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Objectives Our study was designed to investigate the role of microRNA-223 (miR-223) and its direct target gene, cardiac troponin I-interacting kinase (TNNI3K), in regulating cardiomyocyte hypertrophy.

Methodology Neonatal rat cardiomyocytes (CMs) were cultured from one to two days old Sprague–Dawley rats. Cardiomyocyte hypertrophy was induced by endothelin-1

(ET-1). Expression of miR-223 in CMs was detected by real-time PCR. miR-223 mimics transfection was performed to achieve overexpression of miR-223 in CMs. Cell size was measured via surface area calculation under fluorescence microscopy after anti- α -actinin staining. Expression levels of ANP, α -actinin, Myh6, Myh7, as cardiac hypertrophy related marker genes, were detected by RT-PCR. The expression of TNNI3K protein was analysed by western blot. Luciferase assay was performed to confirm the direct binding of miR-223 to the 3'UTR of TNNI3K mRNA.

Results In ET-1 induced hypertrophic CMs, expression of miR-223 was lower than that in normal CMs (Normal CMs: 1.00 ± 0.08 vs Hypertrophic CMs: 0.62 ± 0.16 , $p < 0.05$). Under stimulation of ET-1, miR-223 overexpressed CMs showed alleviated hypertrophic phenomenon, which characterised by less cell surface area (miR-223 group: $2590 \pm 781 \text{ mm}^2$ vs ET-1 only group: $4680 \pm 1040 \text{ mm}^2$, $p < 0.01$) and lower expression of ANP, α -actinin, Myh6, Myh7, when compared with ET-1 stimulation only CMs. In miR-223 overexpressed CMs, the expression of TNNI3K protein was significantly decreased (miR-223 group: 0.39 ± 0.05 vs Control: 0.03 ± 0.01). Co-transfection of a miR-223 expression vector with pMIR-TNNI3K led to the reduced activity of luciferase in a dual-luciferase reporter gene assay, suggesting that TNNI3K is a direct target gene of miR-223.

Conclusion All these results suggest that TNNI3K, a novel cardiac-specific kinase gene, is a direct target of miR-223. miR-223 plays an important role as suppressor in cardiomyocyte hypertrophy and could be used in clinical treatment of hypertrophy in future.