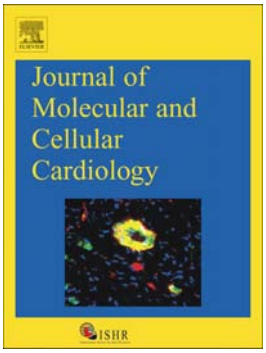

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Characterization of circular RNAs in human, mouse and rat hearts

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Abstract

Deep sequencing techniques and advanced data analysis methods recently enabled the characterization of thousands of circular RNA isoforms (circRNAs) from a number of tissues and organisms. There is emerging evidence that some circRNAs may have important biological functions or serve as diagnostic biomarkers in disease conditions.
In order to analyze circRNA expression in the heart and its changes in different conditions we performed RNA-Seq analysis of ribosome-depleted libraries from rats (neonatal and adult), mice (sham or after transverse aortic constriction, TAC) and humans (failing, non-failing). All samples were sequenced after a treatment with exonuclease RNase R or a mock treatment and >9,000 candidate circRNAs were detected for each species.
Additionally, we performed separate isolation of nuclear and cytoplasmic RNA and co-immunoprecipitated RNA interacting with endogenous argonaute 2 (Ago2) in primary cardiac myocytes. We found circRNAs to be significantly enriched in the cytoplasm compared to linear transcripts and to have a similar level of association with Ago2.
Notably in all three species we observed dozens of circRNAs arising from the titin (Ttn) gene, which is known to undergo highly complex alternative splicing during heart maturation. Correspondingly we observed extensive differential regulation of Ttn circRNAs between neonatal and adult rat hearts, suggesting that circRNA formation could be involved in the regulation of titin splicing.
We expect that our inventory of cardiac circRNAs, as well as the information on their conservation and differential expression will provide an important basis for further studies addressing their function and suitability as biomarkers.

Keywords
Circular RNA; circRNA; heart; titin