Topic: Heart Failure (Basic), Molecular Cardiology

The Mechanism of Hey2 Expression in Cardiac Development

Dai Ihara,^{1,2} Yusuke Watanabe,² Daily Seya,² Yukihiro Harada,^{1,2} Osamu Nakagawa² and Teruhisa Kawamura¹

1. Department of Life Science, Ritsumeikan University, Kyoto, Japan; 2. Department of Molecular Physiology, National Cerebral and Cardiovascular Center, Osaka, Japan

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Hairy/enhancer-of-split related with YRPW motif proteins, known as Hey family, were reported to encode a downstream transcription factor of Notch and ALK1 signalling pathways. Among the three Hey family members (Hey1, Hey2, HeyL), Hey2 null mice show postnatal lethality with various cardiac malformations, ventricular septum defect, right ventricular hypoplasia, and tricuspid atresia. Hence, it is clear that Hey2 is crucial during heart formation. Hey1 and Hey2 are expressed in the atrium and ventricle, respectively, throughout the cardiac development. Thus, we focused on the ventricular specific expression of Hey2 to reveal the molecular transcription mechanism.

Enhancer activity was investigated by multistep analysis. First, Hey2 enhancer regions were predicted by *in silico* ChIP-seq

analysis including cardiac transcription factor bindings and histone modification. Second, the response of the enhancers were examined by Luciferase assay against cardiac transcription factors; involvement Gata4, Nkx2.5 and Tbx5. Finally, enhancer activity was confirmed with transgenic (Tg) mice LacZ reporter system using the minimal promoter. The analysis of Tg mouse embryo heart showed that two enhancers had region specific activity respectively.

In this study, we found Hey2 ventricular specific expression was not only regulated by one enhancer, but by multiple enhancers specific to each region. This novel finding on Hey2 expression regulation is significant to understand cardiac development. ■