Discovery of Novel Small Molecules for Heart Failure Therapy Using Cultured Cardiomyocyte by High Throughput Screening Assay

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Citation: *European Cardiology Review* 2021;16:e66. **DOI:** https://doi.org/10.15420/ecr.2021.16.PO10

Introduction: Heart failure (HF) is one of the leading causes of death in the world. Although pharmacological therapies for HF, such as angiotensin 2 receptor blockers, angiotensin-converting enzyme inhibitors and β-blockers, are established, the mortality of patients with severe HF remains still high. The developing of the agent for HF therapy is strongly required. To identify a pharmacological therapy for HF, we developed a high throughput screening system using primary neonatal rat cardiomyocytes.

Methods: Primary culture of neonatal rat cardiomyocytes were isolated and cultured on 48 well plates for 36 hours. These cells were treated with a library of 268 small molecules for 2 hours and stimulated with 30 μM PE for 48 hours. Cardiomyocytes were stained with an anti-α-actinin antibody, and nuclei were stained with Hoechst 33258. Immuno-fluorescence was detected and cardiomyocyte surface area was measured using ArrayScan® system. Inhibition rate was calculated using the following formula: Inhibition rate: (PE(+) – compound) / (PE(+) – PE(–)). Compounds of 50%-150% inhibition were classified as first hits. The already reported ones were excluded. In the second screening, the mRNA levels of hypertrophic response genes, such as ANF and BNP, were quantified by RT-PCR.

Results: ArrayScan® system could automatically selected and evaluated the area of α-actinin-positive cardiomyocytes. 35 compounds were hits as inhibitor as cardiomyocyte hypertrophy. Eight compounds suppressed PE-induced hypertrophic response gene activations.

Conclusion: We discovered eight small molecule inhibitors of cardiomyocyte hypertrophy by high throughput screening. Further animal examinations are needed to clarify the effects of these compounds on HF. ❑