Biomedical Scientists find Effective Way to Induce Maturation of Human and Mouse Stem Cell-Derived Heart Cells

A research group led by Dr. Patrick C. H. Hsieh, a Research Fellow at the Institute of Biomedical Science (IBMS), has recently confirmed a new effective way to induce the maturation of human and mouse stem cell-derived cardiomyocytes (heart cells) by using a defined combination of four microRNAs. A large amount of mature cardiomyocytes are needed for various research into cardiovascular diseases such as drug screening, toxicity studies and cell-based therapy, as well as for the development of stem cell-based therapies and personalized medicine. The related article was selected as the cover story of the 29th September, 2015 issue of *Cell Reports*.

For more than half a century, death from cardiovascular diseases has been the number one cause of mortality in the world. In Taiwan, cardiovascular disease is the second most frequent cause of death, after cancer, but is still the most prevalent cause of death from a single organ. Patients suffering from myocardial infarction (MI) may die due to the death of a large number of cardiomyocytes and lack of adequate blood supply to the body; however, even with non-fatal myocardial infarction, heart function will never recover to pre-disease levels. This can eventually result in heart failure in patients even after anticoagulant treatments or catheterization and mean that patients have to undergo heart transplantation in order to survive. In clinical practice, chemotherapy in cancer patients can often cause cardiac toxicity as a side effect which will lead to the death of cardiomyocytes and increase of morbidity of cancer patients.

Since cardiomyocytes cannot replicate, scientists are striving to find ways to induce embryonic stem cells (ES) or induced pluripotent stem cells (iPS) to differentiate into cardiomyocytes that can contract and beat in the same way as normal cardiomyocytes. However, the induced cells are still immature and lack mature sarcomeric structures and expression of the protein Connexin-43. As a result, they display fetal-like electrophysiological properties and a weaker contraction force, both of which are necessary for mature cardiomyocyte functioning. These immature cardiomyocytes cannot be used for pharmacological testing or toxicity testing, nor are they suitable for use as the source of cardiomyocytes for cell therapy, since they pose a risk of inducing arrhythmia (irregular heart beat) after implantation. Thus, the question of how to obtain a large population of structurally and functionally mature cardiomyocytes *in vitro* is an urgent matter that needs to be resolved.

Dr. Hsieh's team used a combination of four microRNAs to promote the maturation of human and mouse embryonic-stem cell-derived cardiomyocytes (h/mES-CMs). This was accomplished using technology developed through collaboration with biomedical scientists at the University of Washington, Seattle. Cardiomyocytes were differentiated with 80% efficiency using this method.

The maturation of mouse cardiomyocytes could be achieved in three days and human cardiomyocytes in 21 days.

They found that coculturing of ES-CMs with endothelial cells sped up their maturation process as indicated by the improved sarcomeric alignment and calcium handling. Four microRNAs (miR-125b, miR-199a, miR-221 and miR-222) were upregulated during coculture, and the delivery of the combination of these four microRNAs into mouse and human ES-CMs also resulted in a more negative resting membrane potential and increased expression of cardiomyocyte maturation markers. Interestingly, all four miRNAs targeted ErbB4 and siRNA knockdown of ErbB4 partially recapitulated the effects of miR-combo.

The first author of the paper is Ms. Desy Lee is a Ph.D. student at National Cheng Kung University. Research team members included Academician Shu Chien, and Dr. Charles E. Murry of the University of Washington, Seattle. The paper was supported by Ministry of Science and Technology, Ministry of Health and Welfare, National Health Research Institute, National Cheng Kung University and Academia Sinica.

The complete article is available at the *Cell Reports* journal website at:
http://www.cell.com/cell-reports/abstract/S2211-1247%2815%2900927-4