Single cell PCR study of rat mesenchymal stem cells cocultured with cardiomyocytes

Qiuying Liu[1,2], Zhen Ma[2], Lina Wei[2,3], Yifei Wang[1], Bin Xu[4], Xiaoming Guo[4], and Bruce Z Gao[2*]

[1]Biomedical Research & Develop Center of Jinan University, Guangzhou, China 510632

[2]Clemson University, Clemson, SC 29634

[3]Center for Biomedical Materials and Tissue Engineering, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China 100871

[4]Grandhope Biotech Co., Ltd., Guangzhou Science Park, Guangzhou, China 510663

[*]Corresponding author: zgao@clemson.edu

Statement of Purpose: Bone marrow-derived mesenchymal stem-cell (MSC) transplantation to regenerate injured tissues is a promising new treatment with the potential of using the patient's own cells to treat disease. For example, it is demonstrated that the transdifferentiation of MSCs into cardiomyocytes, can improve the cardiac function[1]. The potential of adult stem cells to function as cellular therapy in chronic cardiac diseases relies on their ability to self-renewal and to replenish multiple cell types within the tissue they reside in. Studies have demonstrated that MSCs co-culture with cardiomyocytes will differentiate into cardiomyocytes *in vitro*[2]. In many aspects, individual cells exhibit a high degree of variability and may response to identical stimuli very differently even in a seemingly homogeneous population[3]. It is necessary to measure gene expression at single cell levels rather than in bulk cells, in which individual differences or heterogeneity could be lost[4]. In our studies, the MSC and the cardiomyocyte were cocultured at single cell level in microfabricated microwells with modified surfaces. These identical microwells, each with only one MSC and one cardiomyocyte formed a biochip for statistical study of individual differentiating MSCs. Here we report gene expression study conducted with single cell real-time PCR at mRNA level. The results can be used to investigate the subphenotypical variations in the differentiation from MSCs to cardiomyocytes and the inter-activation of the coculture.

Methods: Rat MSCs were purchased through ScienCell Inc. and characterized by immunofluorescent method with antibodies to CD73, CD90, CD105. They were labeled with DiO before coculture. Neonatal cardiomyocytes were isolated from the hearts of 3-day-oldneonatal rats. With soft lithographic techniques, a biochip was microfabricated with multiple identical microwells for MSC-cardiomyocyte coculture at single cell level. Cells were laser deposited into each microwell. Cytoplasm of each single cell was captured using motor controlled micropipette into a think-well PCR tube after of 2d, 4d, and 6d coculture. GATA4, Nkx2.5, Connxin43 gene expression of the single MSC or cardiomyocyte were analyzed by single cell real-time PCR.

Results and Conclusions: MSC-cardiomyocyte pairs can be cocultured in the developed biochip. When the two cells contacted with each other, tendency of MSC cardiomyogenic differentiation has been observed morphologically and electrophysiologically. Single cell RT-PCR has been shown to be a powerful technique for analyzing gene expression variations in individual cells.

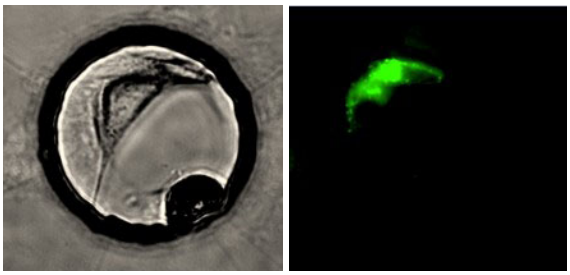

Figure 1. Cardiomyocyte-MSC pair cocultured in one of the microwells of the biochip. MSC was green labeled with DiO fluorescence marker.

Figure 2. Micropipette cytoplasm extraction

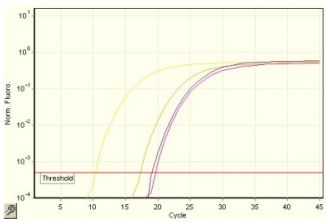

Figure 3. GATA4, Nkx2.5, Connxin43 and β-actin gene expression in the cocultured stem cell.

References:

[1] Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001,410:702-705

[3] Jihyun Yoon, Wan Joo Shim, Young Moo Ro, et al. Transdifferentiation of mesenchymal stem cells into cardiomyocytes by direct cell-to-cell contact with neonatal cardiomyocyte but not adult cardiomyocytes. Ann Hematol. 2005, 84:715-721

[4] Anders Ståhlberg, Martin Bengtsson. Single-cell gene expression profiling using reverse transcription quantitative real-time PCR. Methods. 2010,50:282-288

[5] Yongzhong Li, Hansa Thompson, Courtney Hemphil, et al. An improved one-tube RT-PCR protocol for analyzing single-cell gene expression in individual mammalian cells. Anal Bioanal Chem. 2010,397:1853-1859