Molecular Analysis of Long-Term Cultured Cardiac Stem Cells for Cardiac Regeneration

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A c-Kit (CD117) is a well-known cell surface marker for adult somatic stem cells. We harvested c-Kit-positive cardiac stem cells (CSCs) from adult rat hearts by performing magnetic-activated cell sorting (MACS) and subjected them to long-term bulk culture more than 40 times. We made 11 attempts to obtain c-Kit-positive cells from adult (6–8-month-old) rats. Our initial expectation was of obtaining cells with homogenous cardiac phenotypes. However, each CSC bulk culture expressed varying degrees of the genes and cell surface markers belonging to cardiac and other mesenchymal lineages. The results suggested that these CSCs retained multiple developmental potential to some extent. Consequently, we investigated these CSCs in detail, hoping to establish the regeneration method by using c-Kit-positive cardiac cells [1–12].

- CSC-21E maintained the cell shape, yielding spherical aggregates under a culture condition. The aggregate shape did not facilitate cell adherence to the dish surface. Interestingly, the proteomic analysis of these two morphological statuses revealed the drastic change of the protein profiles with the spherical aggregates showing protein profiles characteristic of stem cells and the flat cells

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showing the profile indicative of differentiated cells, especially of the distinct differences in stress proteins and metabolic enzymes [2, 3, 7].

- CSC5 differentiated into cells with a myocyte/adipocyte mixed phenotype. Members of the transforming growth factor (TGF)-β superfamily were identified as significant regulators of the differentiation of these cells into either adipocytes or myocytes [1–6, 9, 10].
- CSC4A exhibited the ability for sustained contractibility shown by cardiomyocytes that were cocultured with a membrane filter separating cardiomyocytes from CSC4A. This suggests that the CSC4A cells release factors that support cardiomyocyte contraction. Among the cytokines measured in the cocultured medium, insulin-like growth factor-1 (IGF-1) levels appeared to correlate with cardiomyocyte sustenance. However, CSC4A cells do not express IGF-1. This suggests that some other unknown factors are released from CSC4A that can induce IGF expression in cardiomyocytes [1–6, 8, 12].

References

1. Kawaguchi N, Hatta, K, Nakanishi T. 3D-Culture system for heart regeneration and cardiac medicine. BioMed Res. 2013;Int Article ID 895967.
2. Kawaguchi N, Machida M, Hatta K et al. Cell shape and cardiosphere differentiation: A revelation by proteomic profiling. Biochem Res. 2013;Int Article ID:730874.
3. Kawaguchi N, Nakanishi T. Cardiomyocyte regeneration using stem cells. Cells. 2013;2: Article ID 6782.
4. Kawaguchi N, Hayama E, Furutani Y, et al. Prospective in vitro disease model for cardiomyopathies and cardiochannelopathies. Stem Cell. 2012;Int Article ID:439219.
5. Kawaguchi N. Differentiation and survival regulators on adult cardiac derived stem cells. Vitam Horm Stem Cell Regul. 2012;87:111–25.
6. Kawaguchi N. Stem cells for cardiac regeneration and possible roles of the transforming growth factor (TGF)-β family. Biomol Concepts. 2011;3:99–106.
7. Machida M, Takagaki Y, Matsuoka R, et al. Proteomic comparison of floating spherical aggregates and dish-attached cells of cardiac stem cells. Int J Cardiol. 2011;153:296–305.
8. Kawaguchi N, Smith A, Waring C, et al. Gata4^{high} CSCs foster cardiac myocyte survival. PLoS One. 2010;5:e1429.
9. Kawaguchi N, Nakao R, Yamaguchi M. TGF-β Superfamily regulates a switch that mediates differentiation either into adipocytes or myocytes in left atrium derived pluripotent cells (LA-PCs). Biochem Biophys Res Commun. 2010;396:615–25.
10. Hasan MK, Komoike Y, Tsunesumi S, et al. Myogenic differentiation in atrium-derived adult cardiac pluripotent cells and the transcriptional regulation of GATA4 and myogenin on ANP promoter. Genes Cells. 2010;15:439–53.

11. Hosseinkhani H, Hosseinkhani M, Hattori S, et al. Micro and nano-scale *in vitro* 3D culture system for cardiac stem cells. J Biomed Mater Res A. 2010;94A:1–8.
12. Miyamoto S, Kawaguchi N, Ellison G, et al. Characterization of long-term cultured cardiac stem cells (CSCs) derived from adult rat hearts. Stem Cells Dev. 2010;19:105–16.