Tissue Extracts From Infarcted Myocardium of Rats in Promoting the Differentiation of Bone Marrow Stromal Cells Into Cardiomyocyte-like Cells[1]

XIAO-NING LIU[#,*], QI YIN[#,+,*], HAO ZHANG[#], HONG ZHANG[#], SHEN-JUN ZHU[#], YING-JIE WEI[#,2], AND SHENG-SHOU HU[#,2]

[#]Key Laboratory of Cardiovascular Regenerative Medicine, Ministry of Health, and Department of Cardiovascular Surgery, Cardiovascular Institute of Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100037, China; [+]Department of Thoracic and Cardiovascular Surgery, First Affiliated Hospital, Wenzhou Medical College, Wenzhou 325000, Zhejiang, China

Objective To investigate whether cardiac tissue extracts from rats could mimic the cardiac microenvironment and act as a natural inducer in promoting the differentiation of bone marrow stromal cells (BMSCs) into cardiomyocytes. **Methods** Three kinds of tissue extract or cell lysate [infarcted myocardial tissue extract (IMTE), normal myocardial tissue extract (NMTE) and cultured neonatal myocardial lysate (NML)] were employed to induce BMSCs into cardiomyocyte-like cells. The cells were harvested at each time point for reverse transcription-polymerase chain reaction (RT-PCR) detection, immunocytochemical analysis, and transmission electron microscopy. **Results** After a 7-day induction, BMSCs were enlarged and polygonal in morphology. Myofilaments, striated sarcomeres, Z-lines, and more mitochondia were observed under transmission electron microscope. Elevated expression levels of cardiac-specific genes and proteins were also confirmed by RT-PCR and immunocytochemistry. Moreover, IMTE showed a greater capacity of differentiating BMSCs into cardiomyocyte-like cells. **Conclusions** Cardiac tissue extracts, especially IMTE, can effectively differentiate BMSCs into cardiomyocyte-like cells.

Key words: Bone marrow stromal cells; Cell differentiation; Cardiac tissue extracts; Myocardial infarction

REFERENCES

1. Kocher A A, Schuster M D, Szabolcs M J, *et al*. (2001). Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* **7**(4), 430-436.
2. Orlic D, Kajstura J, Chimenti S, *et al*. (2001). Bone marrow cells regenerate infarcted myocardium. *Nature* **410**(6829), 701-715.
3. Scorsin M, Hagege A, Vilquin J T, *et al*. (2000). Comparison of the effects of fetal cardiomyocyte and skeletal myoblast transplantation on postinfarction left ventricular function. *J Thorac Cardiovasc Surg* **119**(6), 1169-1175.
4. Tomita S, Li R K, Weisel R D, *et al*. (1999). Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* **100**, II247-256.
5. Wollert K C, Meyer G P, Lotz J, *et al*. (2004). Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* **364**(9429), 141-148.
6. Pittenger M F, Martin B J. (2004). Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* **95**(1), 9-20.
7. Nagaya N, Fujii T, Iwase T, *et al*. (2004). Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. *Am J Physiol Heart Circ* **287**(6), H2670-2676.
8. Toma C, Pittenger M F, Cahill K S, *et al*. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* **105**(1), 93-98.
9. Gojo S, Gojo N, Takeda Y, *et al*. (2003). *In vivo* cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells. *Exp Cell Res* **288**(1), 51-59.
10. Zhang M, Methot D, Poppa V, *et al*. (2001). Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol* **33**(5), 907-921.
11. Zhu W, Chen J, Cong X, *et al*. (2006). Hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells. *Stem Cells* **24**(2), 416-425.
12. Makino S, Fukuda K, Miyoshi S, *et al*. (1999). Cardiomyocytes

[1]This work was supported by the National Natural Science Foundation of China (No. 30570722).

[2]Correspondence should be addressed to Ying-Jie WEI and Sheng-Shou HU. Tel: 86-10-88398494. Fax: 86-10-88396050. E-mail: weiyingjie@yahoo.com and shengshouhu@yahoo.com

Biographical note of the first authors: Xiao-Ning LIU, majoring in molecular technology, Tel: 86-10-88398847. Qi YIN, master, majoring in thoracic and cardiovascular surgery, Tel: 86-10-88398847, E-mail: yinqiscott@sohu.com

[*]Equal contributors to this article.

0895-3988/2008
CN 11-2816/Q

can be generated from marrow stromal cells *in vitro*. *J Clin Invest* **103**(5), 697-705.
13. Shi J H, Hu X Y, Niu Y H, *et al*. (2004). Genes expression of bone marrow stromal cells to myocardial differentiation induced by DMSO. *Fudan Univ J Med Sci* **31**(5), 454-457. (in Chinese)
14. Li X, Yu X, Lin Q, *et al*. (2007). Bone marrow mesenchymal stem cells differentiate into functional cardiac phenotypes by cardiac microenvironment. *J Mol Cell Cardiol* **42**(2), 295-303.
15. Rangappa S, Entwistle J W, Wechsler A S, *et al*. (2003). Cardiomyocyte-mediated contact programs human mesenchymal stem cells to express cardiogenic phenotype. *J Thorac Cardiovasc Surg* **126**(1), 124-132.
16. Behfar A, Terzic A (2007). Optimizing adult mesenchymal stem cells for heart repair. *J Mol Cell Cardiol* **42**(2), 283-284.
17. Caplan AI (1991). Mesenchymal stem cells. *J Orthop Res* **9**, 641-650.
18. Yang L Y, Huang T H, Ma L (2006). Bone marrow stromal cells express neural phenotypes *in vitro* and migrate in brain after transplantation *in vivo*. *Biomed Environ Sci* **19**(5), 329-335.
19. Su W, Zhang H, Jia Z, *et al*. (2006). Cartilage-derived stromal cells: is it a novel cell resource for cell therapy to regenerate infarcted myocardium? *Stem Cells* **24**(2), 349-356.
20. Ferrari G, Cusella-De Angelis G, Coletta M, *et al*. (1998). Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* **279**(5356), 1528-1530.
21. Jiang Y, Jahagirdar B N, Reinhardt R L, *et al*. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418**(6893), 41-49.
22. Pittenger M F, Mackay A M, Beck S C, *et al*. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* **284**(5411), 143-147.
23. Fukuhara S, Tomita S, Yamashiro S, *et al*. (2003). Direct cell-cell interaction of cardiomyocytes is key for bone marrow stromal cells to go into cardiac lineage *in vitro*. *J Thorac Cardiovasc Surg* **125**(6), 1470-1480.
24. Kodama H, Hirotani T, Suzuki Y, *et al*. (2002). Cardiomyogenic differentiation in cardiac myxoma expressing lineage-specific transcription factors. *Am J Pathol* **161**(2), 381-389.
25. Skerjanc I S, Petropoulos H, Ridgeway A G, *et al*. (1998). Myocyte enhancer factor 2C and Nkx2-5 up-regulate each other's expression and initiate cardiomyogenesis in P19 cells. *J Biol Chem* **273**(52), 34904-34910.
26. Monzen K, Zhu W, Kasai H, *et al*. (2002). Dual effects of the homeobox transcription factor Csx/Nkx2-5 on cardiomyocytes. *Biochem Biophys Res Commun* **298**(4), 493-500.
27. Sepulveda J L, Vlahopoulos S, Iyer D, *et al*. (2002). Combinatorial expression of GATA4, Nkx2-5, and serum response factor directs early cardiac gene activity. *J Biol Chem* **277**(28), 25775-25782.
28. Xu M, Wani M, Dai Y S, *et al*. (2004). Differentiation of bone marrow stromal cells into the cardiac phenotype requires intercellular communication with myocytes. *Circulation* **110**(17), 2658-2665.
29. Yoon J, Shim W J, Ro Y M, *et al*. (2005). Transdifferentiation of mesenchymal stem cells into cardiomyocytes by direct cell-to-cell contact with neonatal cardiomyocyte but not adult cardiomyocytes. *Ann Hematol* **84**(11), 715-721.
30. Hackney J A, Charbord P, Brunk B P, *et al*. (2002). A molecular profile of a hematopoietic stem cell niche. *Proc Natl Acad Sci USA* **99**(20), 13061-13066.
31. Tosh D, Slack J M (2003). How cells change their phenotype. *Nat Rev Mol Cell Biol* **3**(3), 187-194.
32. Dai W, Hale S L, Martin B J, *et al*. (2005). Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. *Circulation* **112**(2), 214-223.
33. Yoon Y S, Wecker A, Heyd L, *et al*. (2005). Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. *J Clin Invest* **115**(2), 326-338.
34. Zhang S, Ge J, Sun A, *et al*. (2006). Comparison of various kinds of bone marrow stem cells for the repair of infarcted myocardium: single clonally purified non-hematopoietic mesenchymal stem cells serve as a superior source. *J Cell Biochem* **99**(4), 1132-1147.

(*Received September* 20, 2007 *Accepted November* 21, 2007)