J. Biol. Res. 2006 n. 1 - Vol. LXXXI - Rubbettino - Soveria Mannelli

In vitro models of migration of stem-cells in the presence of cardiac myocytes

R. Ramella, O. Boero, R. Levi, G. Alloatti, M. P. Gallo

Department of Animal and Human Biology, University of Turin

KEY WORDS: mesenchymal stem cells, cardiomyocytes, migration

Abstract

Myocardial infarction is a leading cause of heart failure. In the recent years transplantation of different types of stem cells into infarcted hearts has been reported in clinical trials and in animal studies, but many issues remain to be clarified on the process following stem cells transplantation in the myocardium. In particular, the molecular mechanisms involved in the migration and sprouting of implanted or infused stem cells remain to be clarified. Recent evidences suggest the role of several growth factors (HGF, IGF-1, TNF-alfa) in stem cells migration, but it remains undetermined the molecular mechanism occourred during stem cells homing. This study investigate whether cardiomyocytes were able to induce migration of bone marrow mesenchymal stem cells in an in vitro model. We used a Transwell assay in wich adult rat cardiomyocytes obtained from enzymatic dissociation were plated in the lower compartments and adult rat mesenchymal stem cells (MSCs) tagged with green fluorescent protein were added to the upper compartment (8 µm PET membrane). After 18h we evaluated MSCs migration through the membranes and MSCs diving in the cardiomyocytes plates. If diving occourred, MSCs and cardiomyocytes were cocultured for at least 72h and immunofluorescence experiments with anti-sarcomeric myosin control, antibody were performed. As a control MSCs migration was evaluated in the same in vitro model in the absence of cardiomyocytes. Preliminary experiments show no marked differences in the MSCs migration through the membrane but the appearance of diving MSCs only in the presence of cardiomyocytes on the bottom plates. Future experiments were aimed to investigate the molecular mechanisms involved in the MSCs chemoattraction toward cardiomyocytes and to charachterize with morphological and functional studies the migrating MSCs.

Mesenchimal stem cells (MSCs) can be found in bone marrow (0.001% to 0.01% of the nucleated cells), muscle, skin and adipose tissue and are characterized by the potential to differentiate into muscle, bone, tendon, ligament and adipose tissue (Caplan, 1991), neurons (Gussoni et al., 1999), hepatocytes (Lagasse et al., 2000), vascular cells and cardiomyocytes.
MSCs were dotained from vigorous washing of bone shaft with medium. Cells filtered supension was plated and cells were selected by different adhesion. This technique has been used to isolate MSCs from humans, rats and mice (Krebsbach et al., 1997; Colter et al., 2000; Barbash et al., 2003).
A feature that distinguishes MSC lineage from hematopoietic stem cell lineage is that differentiation pathways are not strictly delineated, since even apparently fully differentiated cells from a given lineage have the potential to convert into another lineage and intermediate cell phenotype are observed.
Unlike embryonic stem (ES) cells, MSCs do not spontaneously differentiate into cardiomyocytes in vitro, but there have been reports that upon drug or growth factor stimulation they might proceed toward a cardiomyocytic lineage (i.e.: 5-azacytidine, Makino et al., 1999, FGF-VEGF-PDGF, Xaymardan et al., 2004).
In coculture experiments the cardiac cells were always either neonatal/embryonic cells or cardiac cell lines. Several studies report cardiac differentiation of MSCs trasplanted to the heart in both non-injury and myocardial infarction models (Wang et al., 2000; Toma et al., 2002; Gojo et al., 2003), but further investigations are needed to fully characterize the morphological and functional aspects

of MSCs in the host myocardium, as several recent studies have challenged these results.
This study investigate whether cardiomyocytes were able to induce migration of bone marrow mesenchymal stem cells in an in vitro model. We used a Transwell assay in wich adult rat cardiomyocytes obtained from enzymatic dissociation were plated in the lower compartments and adult rat mesenchymal stem cells (MSCs) tagged with green fluorescent protein were added to the upper compartment (8 µm PET membrane).
After 18h we evaluated MSCs migration through the membranes and MSCs diving in the cardiomyocytes plates.

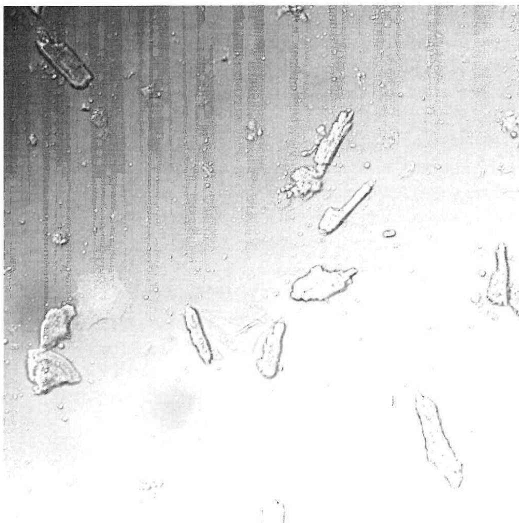


Fig. 1 - *Typical 'transwell assay' experiment with MSCs and cardiomyocytes. The images show green fluorescent MSCs dived in the cardiomyocytes compartment.*

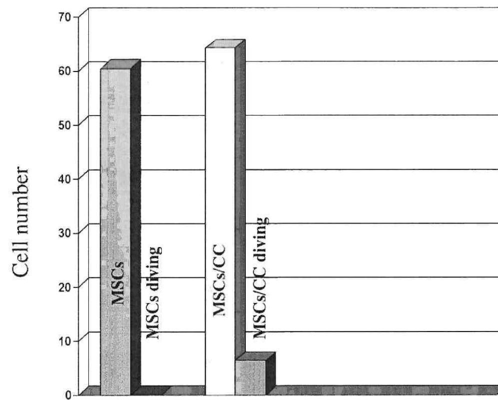
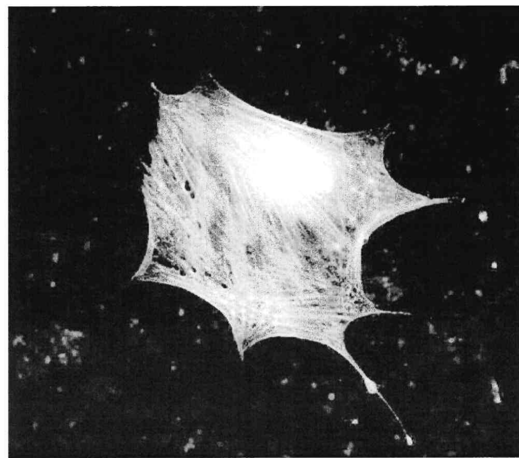


Fig. 2 - *Bar grap representing the number of MSCs migrated under the PET membrane or dived in the lower compartment, in the absence or in the presence of cardiomyocytes.*



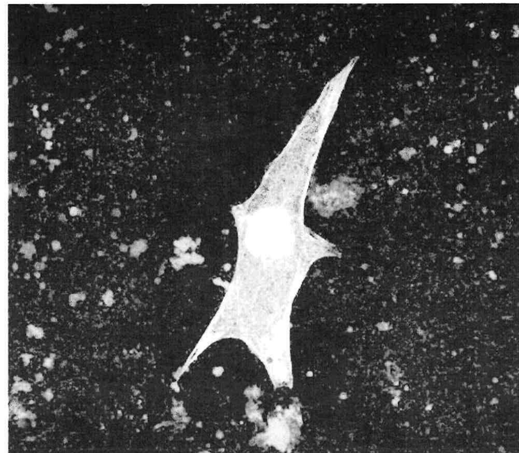


Fig. 3 - *Images from a typical immunofluorescence experiment with anti-sarcomeric myosin antibody (red) in 72h cocultured cardiomyocytes and dived MSCs.*

If diving occourred, MSCs and cardiomyocytes were cocultured for at least 72h and immunofluorescence experiments with anti-sarcomeric myosin antibody were control, performed. As a control MSCs migration was evaluated in the same in vitro model in the absence of cardiomyocytes.
Preliminary experiments show no marked differences in the MSCs migration through the membrane but the appearance of dived MSCs only in the presence of cardiomyocytes on the bottom plates (bar graph in Fig. 2). Immunofluorescence experiments revealed in some dived MSCs positivity for sarcomeric myosin (Fig. 3, right panel). Future experiments were aimed to investigate the molecular mechanisms involved in the MSCs chemoattraction toward cardiomyocytes and to charachterize with morphological and functional studies the migrating MSCs.

References

Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. 2003. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation. Aug 19; 108 (7): 863-8.

Caplan, I. 1991. Mesenchymal stem cells. J Orthop Res. Sep 9 (5): 641-50.

Colter DC, Class R, Di Girolamo CM, Prockop DJ. 2000. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. Proc Natl Acad Sci U S A. Mar 28; 97 (7): 3213-8.

Gojo S, Gojo N, Takeda Y, Mori T, Abe H, Kyo S, Hata J, Umezawa A. 2003. In vivo cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells. Exp Cell Res. Aug 1; 288 (1): 51-9.

Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC. 1999. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature. Sep 23; 401 (6751): 390-4.

Krebsbach PH, Kuznetsov SA, Bianco P, Robey PG. 1999. Bone marrow stromal cells: characterization and clinical application. Crit Rev Oral Biol Med.; 10 (2): 165-81.

Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M. 2000. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo Nat Med. Nov; 6 (11): 1229-34.

Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S. 1999. Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest. Mar; 103 (5): 697-705.

Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. 2002. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation. Jan 1; 105 (1): 93-8.

Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC. 2000. Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg. Nov; 120 (5): 999-1005.

Xaymardan M, Tang L, Zagreda L, Pallante B, Zheng J, Chazen JL, Chin A, Duignan I, Nahirney P, Rafii S, Mikawa T, Edelberg JM. 2004. Platelet-derived growth factor-AB promotes the generation of adult bone marrow-derived cardiac myocytes. Circ Res. Mar 19; 94 (5): E39-45.