

because of its immune responsiveness. Yet, the reason(s) remain(s) unclear. We have previously suggested that a promising strategy for the understanding of melanoma immune responsiveness could consist of the study of tumor/host interactions *ex vivo* through genetic profiling of serial fine needle aspirate biopsies that allow direct correlation between experimental results and clinical outcome.<sup>1</sup> By prospectively studying the transcriptional profile of melanoma metastases during immunotherapy we observed that immune responsiveness is pre-determined by an immune reactive micro-environment.<sup>2</sup> Interestingly, the addition of systemic interleukin-2 therapy to active specific immunization seems to increase the frequency of immune rejections of cancer. Functional profiling of the effect of interleukin-2 in tumors suggested that this cytokine induces or enhances the effector function of immunization-induced T cells by causing an acute inflammatory process at the tumor site that can in turn recruit and activate T cells.<sup>3</sup> Thus, we hypothesize that effective immune responses occur when a pro-inflammatory inflammatory threshold is reached at tumor site capable of maintaining active immunization induced-T cells.<sup>4</sup> To search the reason for the erratic behavior of metastatic melanoma, we analyzed 62 melanoma metastases to identify functional signatures possibly responsible for immune responsiveness. Melanoma metastases were biopsied with a 23 gauge needle and anti-sense RNA was amplified to produce single stranded cDNA for hybridization to custom-made cDNA arrays.<sup>5</sup> Genes specific for the tumor microenvironment were sorted (Wilcoxon test  $p$ -value < 0.001). Eisen's hierarchical clustering was applied to the resulting gene pool and two subsets of melanomas were identified. A smaller cluster including 15 samples (24%) was characterized by significantly higher expression of the inflammatory cytokines GRO- $\alpha$ , MIP-1 $\alpha$  and  $\beta$ , MPC-1, -3 and -4, IL-1 $\beta$ , IL-8, RANTES, Lymphotactin and Lymphotoxin. This signature strongly correlated with up-regulation of IFN-responsive elements. The same cluster displayed a higher expression of MMP-9, 11 and 15 (cytokine-dependent metalloproteinases), genes encoding growth and angio-regulatory factors and cell cycle regulatory sequences. These findings suggested that some melanoma metastases display a very heterogeneous immune environment that could variably modulate T cell function at the receiving end of the immune response against cancer and could co-operate with the pro-inflammatory effects of the systemic administration of interleukin-2. Although these studies need to be confirmed in larger patient populations this report suggests that strategies are presently available for the efficient screening of biological principles and related biomarkers using high-throughput technology. **References:** 1. Wang E, Marincola FM. A natural history of melanoma: serial gene expression analysis. *Immunol Today* 2000; 21:619-23. 2. Wang E, Miller LD, Ohnmacht GA, Mocellin S, Petersen D, Zhao Y, et al. Prospective molecular profiling of subcutaneous melanoma metastases suggests classifiers of immune responsiveness. *Cancer Res* 2002;62: 3581-6. 3. Panelli MC, Wang E, Phan G, Puhlman M, Miller

L, Ohnmacht GA, et al. Genetic profiling of peripheral mononuclear cells and melanoma metastases in response to systemic interleukin-2 administration. *Genome Biol* 2002;3: RESEARCH0035. 4. Marincola FM, Wang E, Herlyn M, Seliger B, Ferrone S. Tumors as elusive targets of T cell-directed immunotherapy. *Trends Immunol* 2003;24:334-41. 5. Wang E, Miller L, Ohnmacht GA, Liu E, Marincola FM. High fidelity mRNA amplification for gene profiling using cDNA microarrays. *Nature Biotech* 2000;17:457-9.

**Key Words:** Melanoma, immunotherapy, functional genomics, cancer vaccines, T cell immunology

## Cell Transplantation to Improve Heart Function: Cell or Matrix

Ren-Ke Li

*Toronto General Hospital, Toronto, ON, Canada.*

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*Reprint address: requests to Ren-Ke Li, M.D., Ph.D., Toronto General Hospital, 200 Elizabeth St., Toronto, ON, Canada. Tel: 1-416-340-3361, Fax: 1-416-340-4806, E-mail: RenKe.Li@uhn.on.ca*

Current attempts to regenerate the damaged myocardium after myocardial infarction have primarily focused on therapies directed at increasing regional perfusion and reducing cell loss. Accumulating evidence suggests that implantation of healthy muscle cells into the damaged myocardium replaces the fibrotic tissue. In addition to muscle cells, stem cells in circulation, from bone marrow or in the myocardium, have recently been documented to have great potential to differentiate into myogenic cells. These neo-myogenic cells in the myocardial scar tissue prevented ventricular dilatation and delayed cardiac dysfunction. Early clinical trials show encouraging data for cellular cardiomyoplasty.

Although the beneficial effects of cell therapy for myocardial regeneration after an infarction have led to phase I clinical trials, the mechanism of the novel therapy is often questioned. Replacing the scar tissue with muscle cells and stimulating neo-vessel formation in the implanted area have been proposed. However, a number of studies recently demonstrated that the survival rate of implanted cells was too low and that number of implanted cells decreased with time after transplantation. The number of surviving cells may not be enough to form adequate new muscle tissue to repair the damaged myocardium.

We recently found that extracellular matrix in the myocardium plays an important role in maintaining the ventricular chamber size, and disruption of the matrix network may contribute to the apoptosis of cardiomyocytes leading to dilated cardiomyopathy. We implanted smooth muscle cells into the heart with dilated cardiomyopathy prior to ventricular dilata-

tion. We found that implanted cells survived in the implanted area and altered myocardial matrix metabolism both within and remote from the region of implantation. Matrix metalloproteinase activity decreased in the transplanted group as compared with control group. The matrix structure was maintained and ventricular dilatation was prevented. These data suggest that implanted cells prevented ventricular dilatation through the alteration of matrix metabolism, which is a possible mechanism for implanted cells to improve heart function.

**Key Words:** Cell transplantation, myocardial infarction, extracellular matrix, myocardial regeneration, heart function

## Autologous Bone Marrow Cell Transplantation Combined with Off-Pump Coronary Artery Bypass Grafting in Human Ischemic Myocardium

Kyung-Jong Yoo<sup>1</sup>, Hyun-Ok Kim<sup>2</sup>,  
Young-Lan Kwak<sup>3</sup>, Seok-Min Kang<sup>4</sup>,  
Yang-Soo Jang<sup>4</sup>, Sang-Hyun Lim<sup>1</sup>,  
Ji-Young Ahn<sup>1</sup>, and Ren-Ke Li<sup>5</sup>

Departments of <sup>1</sup>Thoracic and Cardiovascular Surgery, Yonsei Cardiovascular Research Institute, <sup>2</sup>Laboratory Medicine, <sup>3</sup>Anesthesia and Pain Medicine, <sup>4</sup>Internal Medicine, Yonsei University College of Medicine, Seoul, Korea; <sup>5</sup>Department of Surgery, Division of Cardiovascular Surgery, University of Toronto, Toronto, ON, Canada.

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Reprint address: requests to Kyung-Jong Yoo, M.D., Department of Thoracic and Cardiovascular Surgery, Yonsei Cardiovascular Research Institute, Yonsei University College of Medicine, Seoul, Korea. Tel: 82-2-361-7286, Fax: 82-2-313-29920, E-mail: kjj@yumc.yonsei.ac.kr

Recently, autologous bone marrow cell transplantation (CTx) for angiogenesis and myogenesis in ischemic myocardium has been extensively investigated to improve heart function. This study was designed to evaluate the effects of CTx with off-pump coronary artery bypass grafting (OPCAB) in patients who were not feasible for complete revascularization. Seven male patients underwent CTx combined with OPCAB in 5, CTx only in 1, and mitral valve repair in 1 patient simultaneously. Bone marrow was aspirated from iliac bone. Mean  $1.5 \times 10^9$  mononuclear cells including mean  $7.3 \times 10^6$  CD34+ cells and  $2.4 \times 10^6$  AC133+ cells were obtained and concentrated with 10cc. These cells were transplanted into non-graftable ischemic myocardium. Heart function was

evaluated in all patients using MIBI scan, echocardiogram and heart magnetic resonance imaging (MRI) preoperatively. The effect of CTx was evaluated using MIBI scan, echocardiogram, and MRI postoperatively. An average of 2 grafts were bypassed. Other territories were transplanted with isolated mononuclear cell. All patients had an uncomplicated postoperative course. After 2 to 7 months follow-up, there was improvement in symptom, ejection fraction (from 43% to 47%) on echocardiogram and myocardial perfusion on MIBI scan and MRI in all patients. These preliminary data showed improvement of heart function and myocardial perfusion and also showed the feasibility and safety of combined therapy with OPCAB and CTx in ischemic myocardium. However, the effectiveness of CTx alone cannot be readily assessed. Further randomized, controlled studies are required to evaluate the effectiveness of CTx alone.

**Key Words:** Autologous bone marrow cell transplantation, off-pump coronary artery bypass grafting, ischemic myocardium

## Mid-term Clinical Results of Tissue-Engineered Vascular Autografts Seeded with Autologous Bone Marrow Cells

Toshiharu Shin'oka

Department of Cardiovascular Surgery, Tokyo Women's Medical University, Tokyo, Japan.

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Reprint address: requests to Toshiharu Shin'oka, M.D., Department of Cardiovascular Surgery, Tokyo Women's Medical University, Japan. Tel: 81-3-3353-8111, Fax: 81-3-3356-0441, E-mail: ssinoka@hij.twmu.ac.jp

**Objective:** Prosthetic and bioprosthetic materials currently in use lack growth potential and therefore must be repeatedly replaced in pediatric patients as they develop. Tissue engineering (TE) is a new discipline that offers the potential for creating replacement structures from autologous cells and biodegradable polymer scaffolds. In May 2000 we initiated clinical application of tissue-engineered vascular grafts seeded with cultured cells. However, cell culturing is time-consuming and xeno-serum must be used. To overcome these disadvantages, we started the usage of bone marrow cells (BMCs), readily available on the day of surgery, as a cell source. The aim of the study was to assess the safety and feasibility of this technique for creating pulmonary artery conduits. **Methods:** Since August 2000, TE grafts seeded with autologous BMCs have been implanted in thirty-five patients. The patients and/or their parents were fully informed and had given consent to the procedure. Five ml/kg of bone-marrow was aspirated under