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Center for Cell-Based Therapy

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CENTER FOR CELL-BASED THERAPY

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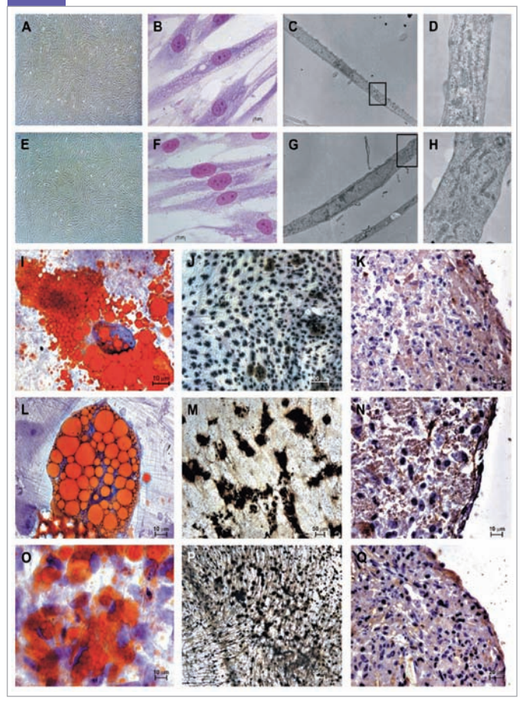


Figure 1. Morphology of human bone marrow-derived mesenchymal stromal cells (MSC) (A-D) and human foreskin-derived fibroblast (E-H). Phase contrast microscopy (A, E; magnification x40); Leishman staining (B, F); ultrastrucutre showing the nucleus with a spindle-shape fibroblastic morphology (C, G); higher magnification of the perinuclear region, showing the rough endoplasmic reticulum (D, H). Differentiation capacity of bone marrow MSC (I-K), pericytes (L-N) and skin fibroblast (O-Q) into adipocyte (I, L, O) (stained with Sudan II and scarlet), osteocytes (J, M, P) (von Kossa staining), and condrocytes (K, N, Q) (immunohistochemical demonstration of type II collagen)

The Center for Cell-Based Therapy was conceived based on the broad concept of using cells for therapy, under different conditions and from several sources. Our research has focused on areas considered essential for the understanding of the cellular mechanisms involved in regulation of stem cells activity, establishment of methods for stem cells isolation and *ex vivo* manipulation, and on the development of treatment strategies based on the use of stem cells to treat autoimmune diseases.

MAIN RESEARCH TOPICS

The project Stem cells for the treatment of neoplastic and inflammatory diseases aims to understand various aspects of Mesenchymal Stem Cells (MSC) biology that may be relevant for their use in medicine, including basic aspects and therapeutic applications in pre-clinical and clinical studies, and the pathways that control hematopoietic stem cell differentiation.

We are also focusing on separating the most primitive MSC from the remaining differentiated cellular population, by using specific markers such as STRO1, CD146, CD106, CD73 and CD63, by flow sorting or by purification with magnetic beads labeled with the specific antibodies. Animal models suitable for MSC transplant, to perform *in vivo* studies are under development. We are currently studying the behavior of MSC *in vivo*, by using animal models of various diseases, including post bone marrow transplantation graft versus host disease (GVHD), acute liver injury induced by carbon tetrachloride, chronic cardiac insufficiency induced by adriamycin, and in acute radiation disease.

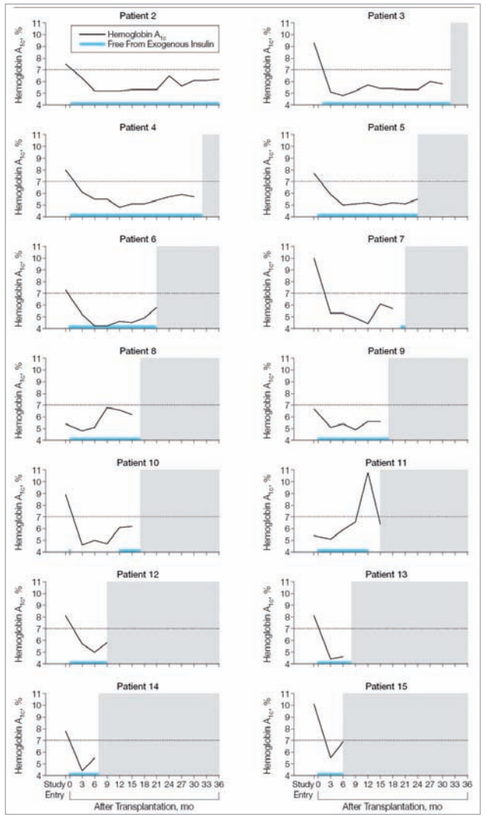
One of our projects uses an experimental model in rats to evaluate the potential impact of human mesenchymal bone marrow stem cells (hMSC) transplantation in chronic heart failure.

The Hematopoietic progenitors study compares the functional properties and gene expression profiles of pure populations of bone marrow, peripheral blood, and umbilical cord blood CD133+, CD34+, CD34+KDR+, CD133+KDR+, CD34-CD133+, CD34+CD133+ cells to study their capacity to form hematopoietic colonies in long term-culture (LTC-IC), and endothelial colonies in matrigel plaques.

We are starting to study biological characteristics of donated Embryonic Stem Cells (ES) lines to: 1. evaluate the ES gene expression profiles by quantitative and qualitative methods; 2. compare the ES gene expression profile with the gene expression profile of adult stem cells including hematopoietic stem cells (CD34+, CD133+) and mesenchymal stem cells from bone marrow and umbilical cord blood; and 3. understand the genetic and molecular mechanisms involved in the initial phase of ES differentiation.

We are currently in the phase I/II trial of hematopoietic stem cell transplantation (HSCT) for early-onset type I diabetes mellitus.

In searching for new therapeutic targets in cancer, two groups of diseases were selected for further analysis: lymphoproliferative disorders and acute myelogenous leukemia.



Hemoglobin A_{1c} Levels and Periods Free From Exogenous Insulin Requirement

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We have characterized the transcriptome of mesenchymal stem cells (MSCs) and hematopoetic stem cells (HSCs), and determined whether their source affected gene expression profile. In addition, we have succeeded in isolating MSCs from several tissues. Recently, we have compared three cell types: MSCs, fibroblasts and pericytes. Our results showed that pericytes and fibroblasts could be induced to differentiate and presented morphologic and immunophenotypic features similar to MSCs. In agreement, the gene expression profile of these cells were similar. This was the first study to prove that human MSC and pericytes are similar cells located in the wall of the vasculature, where they are involved in tissue repair.

Animal models have been instrumental to our studies on leukemogenesis and HSC function. Regarding leukemogenesis, we have selected acute promyelocytic leukemia (APL) as a model. We have characterized three transgenic mouse models (TM) expressing distinct fusion genes associated with APL. All of them developed a form of leukemia after a long latency, but their leukemic cells displayed distinct morphologic features and response to treatment. Our results indicate that these fusion proteins are necessary but not sufficient to cause leukemia, and that they are relevant for leukemia phenotype. On a different line, our studies involving Dkc1m mice (mutants that express low levels of a pseudouridine synthase) were the first to prove that the impairment of ribosome biogenesis may affect stem cells.

CTC performed the first clinical trial evaluating the safety and metabolic effects of high-dose immunosuppression followed by autologous nonmyeloablative hematopoietic stem cell transplantation (AHST) in newly diagnosed type 1 diabetes mellitus (DM). This is a phase 1/2 prospective study involving 15 patients. During a 7-36-month follow-up, 14 patients became insulin-free and the C-peptide response curve was significantly greater than the pretreatment values. There was no mortality. We concluded that AHST has acceptable toxicity and has benefited patients with newly diagnosed type 1 DM.

Regarding technological innovation, our main project is focused on the development and production of recombinant factor VIII to be used in the treatment of type A hemophiliacs. We have generated 41 transgenic cell lines expressing more than 100 IU/mL of FVIII, of which two were chosen for industrial process escalation.

Finally, the CTC activities of diffusion are focused on the improvement of science teaching in public schools. These activities have the participation of teachers, and students can attend at two facilities created by CTC: House of Sciences and in the Museum and Laboratory for Science Teaching (MuLEC). Finally, the book "Células-tronco: a Nova Fronteira da Medicina" published by CTC investigators won the 2007 Jaboti Award.