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HUMAN MESENCHYMAL STROMAL CELLS ENHANCE METASTASIS OF NEUROBLASTOMA VIA SDF-1/CXCR4 AND SDF-1/CXCR7 AXES

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Background: Bone marrow is a frequent metastatic site for neuroblastoma, a common form of childhood cancer with poor prognosis. The SDF-1/CXCR4 axis has been proposed as an important pathway during metastasis of neuroblastoma. But the role of mesenchymal stromal cells (MSCs) and CXCR7, the other known receptor for SDF-1, in metastatic neuroblastoma is yet to be clarified.

Materials & Methods: In this study, we investigated the chemotactic effects of human bone marrow derived MSCs on the migration of neuroblastma cells, with specific emphasis on the SDF-1/CXCR4 and SDF-1/CXCR7 axes. To determine the contribution of SDF-1 released by MSCs, we collected serum poor conditioned media from three different MSC cultures and estimated their SDF-1 levels by ELISA. We then examined the cell surface expression of CXCR4 and CXCR7 on five known neuroblastoma cell lines (BE2C, BE2M17, IMR32, SK-N-LP and SY5Y) with metastatic potential by flow cytometer. The metastatic functional assessment was performed by transwell migration and invasion assay.

Results: SDF-1 can be identified from the MSCs culture medium. The migration and invasion of neuroblastoma was enhanced by either MSCs co-culture or SDF-1 under transwell migration and invasion assay. Flow cytometry analysis revealed that all of the five cell lines expressed CXCR7, and four of them expressed CXCR4. The migration efficiency of neuroblastoma cells in response to either MSCs conditioned media or SDF-1 treatments was considerably higher than that to control medium ($n = 3$, $p < 0.01$), and such effect could be significantly blocked by AMD3100, an inhibitor of CXCR4.

Conclusion: Our preliminary data suggested that MSCs enhanced neuroblastoma cells migration and invasion and this is probably acting through the SDF-1/CXCR4 and SDF-1/CXCR7 axes.

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STEM CELL DERIVED HEPATOCYTES AFTER CO-TRANSPLANTATION OF MESENCHYMAL AND HEMATOPOIETIC STEM CELLS IN CLASS III BETA THALASSEMIA MAJOR PATIENTS

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Background: Bone marrow derived mesenchymal cells and stem cells are potential to differentiate into mature cells of various organs. Here we describe the initial results of study which suggests that these cells transformed to hepatocytes.

Methods: In this prospective, single-center study during 18 months, liver biopsy were obtained from 9 patients who had undergone Co-Transplantation of Mesenchymal and Hematopoietic Stem Cell for beta thalassemia major class III. Five female patients and 4 male patients had received transplant from their sex mismatch donors. The biopsies were studied for the presence of donor-derived hepatocytes with the use of fluorescence in situ hybridization (FISH) and immunohistochemical staining (IHC) for CD45 (leukocyte common antigen), and a hepatocyte-specific antigen.

Results: According to sex mismatch transplantation, mixed donor-recipient XY-positive hepatocytes in liver specimen means that new chimer stem cells were originated from donor cells. These cells accounted for 8 to 70 percent of the cells on FISH slides and their hepatocyte properities were shown by IHC methods.

Conclusions: Co-transplantation of Mesenchymal and Hematopoietic Stem Cell can enhance regeneration of mature hepatocytes in liver tissue.

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PHENOTYPICAL AND FUNCTIONAL CHARACTERIZATION OF MESENCHYMAL STEM CELLS DERIVED FROM PATIENTS AFFECTED BY SCHWACHMAN-DIAMOND SYNDROME

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Shwachman-Diamond Syndrome (SDS) is an inherited marrow failure disorder characterized by varying cytopenias, pancreatic dysfunction, and metaphyseal dysostosis. Neutropenia plays a crucial role in the occurrence of recurrent and severe infectious complications representing one of the major causes of death in SDS patients. The aim of our study is to better comprehend the marrow dysfunction occurring in SDS patients, by analyzing the functional properties of bone marrow (BM)-derived mesenchymal stem cells (MSCs). After informed consent, BM cells obtained from 16 SDS patients were plated in sterile tissue culture flasks. At the third passage of the culture, cells were tested for the expression of specific surface markers, their ability to differentiate into mesengenic lineages, their capability to abrogate T cell proliferation and their capacity to prevent neutrophils apoptosis. MSCs derived from SDS patients (SDS-MSCs) displayed typical fibroblastoid morphology; they were consistently devoid of contaminating hematopoietic cells, being negative for CD34, CD45, HLA-DR, CD11b, CD19, and CD14, but expressed common MSC markers including CD90, CD73, CD105 and HLA-ABC. Similarly to MSCs obtained from healthy donors (HD-MSCs), these cells were able to differentiate into adipocytes and osteoblasts. In addition, SDS-MSCs drastically decreased the mitogen-induced lymphocyte proliferation, in a dose dependent manner. We also cultured neutrophils obtained from HDs in presence or absence of MSCs at different time points. We demonstrated that SDS-MSCs were comparable to HD-MSCs in supporting the viability of neutrophils. Importantly, SDS-MSC were able to produce high amount of IL-6 (mean = 2658 pg/ml, range = 2086-3229 pg/ml), a crucial cytokine involved in the protection of neutrophils from apoptosis.In conclusion, we successfully isolated and characterized MSCs from SDS patients. These cells did not show any significant differences from HD-MSCs. Further studies are needed to better comprehend the functional and molecular features of SDS-MSCs, which are potentially involved in the hematological abnormalities typical of SDS patients.

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STROMAL STEM CELL THERAPY FOR PROPHYLAXIS OF ACUTE GVHD: PRELIMINARY RESULTS FROM A PHASE I TRIAL

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Background: Stromal stem cells are actively studied as a novel adjunct therapy after HSCT. Safety of mesenchymal stromal cells was shown in early phase clinical trials with benefit for engraftment and treatment of acute or steroid refractory GVHD (Lazarus 2005, LeBlanc 2008, Kebriaei 2009). Phase III trials have reported mixed outcomes for MSC therapy for acute or steroid refractory GVHD (Martin 2010, Prasad 2010). Multipotent adult progenitor cells (MultiStem®) is a stromal stem cell source with similar immunemodulatory activity as MSC and can be manufactured to clinical scale allowing all patients on an individual study to be treated with uniform allogeneic cell product from a universal donor. Pre-clinical studies have demonstrated safety for iv infusion of MultiStem and survival benefit in a haploidentical acute GVHD rat model when used in a prophylactic manner (Kovacsovics 2008, 2009).

Methods: The primary goal of this open label Phase I clinical dose escalation study is to assess safety of MultiStem in adult hematological malignancy patients when administered shortly after allogeneic HSCT. Patients with AML, ALL, CML, or MDS are enrolled in either one of two study arms for MultiStem infusion at 1, 5 or 10