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ABSTRACT BOOK

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Example: RITUXIMAB CONSOLIDATION AND MAINTENANCE THERAPY PROLONG RESPONSE DURATION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA
G. Del Poeta, M.I. Del Principe, A. Siniscalchi, L. Maurillo, F. Buccisano, A. Venditti, F. Luciano, P. Niscola, A. Zucchetto, V. Gattei, A.P. Perrotti, P. De Fabritiis, S. Amadori
Haematologica 2008; 93(s1):34. abstract n. 0085

experiments including RT-PCR ,colony forming unit (CFUs), flow cytometer (FACs) and magnetic active cell sorting (MACS). **Results.** Fat hyperplasia appeared in the control group since one week after HD chemotherapy which can be restrained in the BADGE-treated group. After 2 weeks BADGE treatment, peripheral white blood cells increase 30%~40% over the control group while the neutrophils were 1.5~2 times higher. CFUs of BM cells increased by 25%~30%. Meanwhile, compared with the control group, though HSCs (ckit+Lin-Sca1+) seemed no obvious difference between the two groups, percentage of hematopoietic progenitor cells (HPCs, ckit+Lin-Sca1-)sorted by MACs were 2 times higher. Further more, the proportion of Ki67 (+) CD45 (+)BM cells and Ki67 (+) LSK HSCs analyzed by FACs were significantly increased by 1.5~2 times. **Conclusions.** Fat hyperplasia within BM induced by HD chemotherapy can be restrained by PPAR γ inhibitors, resulting an improvement of hematopoietic recovery after the hematopoietic stress.

0708

PHYSIOLOGICAL AND PROTEOMIC ASPECTS OF FUNCTIONAL CORD BLOOD CD34+ CELLS MAINTENANCE AT 4°C UNDER HYPOXIA AND HYPERCAPNIA

M Vlaski[1], L Negroni[2], M Kovacevic-Filipovic[3], S Berets[4], G Le Reverend[4], M Hammoud[5], J Chevaleyre[6], P Duchez[6], P Brunet de la Grange[6], X Lafarge[6], JM Boiron[6], Z Ivanovic[6]
[1]1Etablissement Français du Sang Aquitaine-Limousin, Bordeaux, France
[2]University Bordeaux Segalen, Bordeaux, France
[3]Faculty of Veterinary Medicine, Belgrade, Serbia
[4]ESTBB, Bordeaux, France
[5]Etablissement Français du Sang Aquitaine-Limousin,, Bordeaux, France
[6]Etablissement Français du Sang Aquitaine-Limousin, Bordeaux, France

Background. The short-term conservation of hematopoietic stem and progenitors cells is actually performed in hypothermia (+4°C). In nature, long-term survival of some animals in hypothermia is enabled by regulated metabolic depression which can be induced by their exposure to low O_2 (hypoxia) and increased CO_2 (hypercapnia) environment. Similarly, in physiological conditions, the primitive hematopoietic cells are maintained in a hypometabolic state in the poorly oxygenated bone marrow niche. Recently, we demonstrated that the same protective mechanism is operational in mobilized peripheral blood primitive hematopoietic progenitors and stem cells during their storage at4°C (Jeanne et al, Transfusion 2009). **Aims.** The aim of this study was to test if exposure to hypoxic/hypercapnic gas mixture could be also beneficial for the preservation of functional cord blood $CD34^+$ hematopoetic progenitors in hypothermia and, if so, to analyse physiological and proteomic aspects of this phenomenon. **Methods.** Cord blood $CD34^+$ cells were incubated in conservation medium Stem-α S3 for 10 days at +4°C under hypoxic (1 and 5% O2) and hypercapnic (2,5 and 9% CO2) concentration or air (20% O_2 and 0,05% CO_2). The functional maintenance of committed hematopoietic progenitors (CFC assay) and stem cells (Scid-Repopulating Cells (SRCs) assay estimated by the presence of human markers [CD45, CD19, CD33] and human CFC in NOG/Scid mice femur 6 weeks after transplantation) in parallel with a flow-cytometry analysis of phenotype and vital functions (Aldefluor, AnnexinV/PI, ATP bioluminescent assay, DiBAC4, TMRM, H2DCFDA staining). Proteomic analysis was performed using iTRAQ labelling followed by peptide fractionation (SXC chromatography) and mass spectrometry (LC/MS/MS). **Results.** Incubation in hypoxia and hypercapnia doubled the survival $CD34^+$ cells (60%) comparing to air (30%). Similar ratio is obtained for CFC (34±9% vs 18±6% in air). $5\%O_2/9\%CO_2$ ratio appeared to be the optimal hypoxic/hypercapnic combination. Better cell maintenance in this condition was associated with a higher frequency of primitive a*ldehyde dehydrogenase* (ALDH) expressing cells and SRCs. These cell-protective effects seem to concern a better preservation of the plasma and mitochondrial membrane potential. Hypoxia/hypercapnia also ensures maintenance of viable cells and induces less apoptosis, in spite of a production of deleterious ROS and a drop of ATP amount/per viable cell which were equivalent to that obtained in air. These effects seem to be principally mediated via hypercapnia. Proteomic study revealed that overall protein's content was better preserved in hypoxia/hypercapnia. In addition, this analysis enabled to identify and to distinguish proteins sensitive and insensitive to hypothermia irrespective to the gas phase, as well as the proteins contributing specifically to the hypoxia/hypercapnia cell protective effect. Among them are some protein families known to be implicated in the long time survival of hibernating animals in hypothermia. **Conclusions.** Present work demonstrates the critical physiological effects and indicates protein candidates implicated in hypoxia/hypercapnia-mediated functional maintenance of hematopoietic progenitors in hypothermia. These results suggest a way to optimise cell conservation without freezing and to design a new generation of conservation media.

0709

CYTOKINE PROFILE AND EFFECT OF ALLOGENIC BONE MARROW MESENCHYMAL STEM CELLS ON PROLIFERATION OF NORMAL HEMATOPOIETIC PROGENITORS AND LEUKEMIC CELLS

T Shamanskaya[1], E Osipova[1], O Tatarinova[1], S Plyasynova[1], O Kozlitina[1], Z Dishlevaya[2], E Skorobogatova[2], S Roumiantsev[1]
[1]Federal Clinical Research Centre of Pediatric Hematology, Oncology, Immunology, Moscow, Russian Federation
[2]Russian Children's Clinical Hospital, Moscow, Russian Federation

Background. Currently, immunological properties of MSCs have found a greater therapeutic use in transplantation to improve engraftment, treatment of GVHD and in regenerative medicine. MSCs have the capacity to maintain hematopoiesis (through the production of G-CSF, GM-CSF), immunomodulation properties (through the production of IFN-γ, IL-2, IL-6, IL-1β, TNF-α and chemokines - IL-8, MCP-1, MIP-1β) and stimulation of reparative processes (through the production of VEGF, FGF-basic, IGF-1, IL-6, TIMP-1/MMP-9). MSC has impact to leukemic cells present in bone marrow of the recipient in the form of minimal residual disease. **Aims.** to investigate the mechanism of the effect of allogeneic bone marrow MSCs on the ability of tumor cells to spontaneous and induced apoptosis. We also assessed the cytokine profile of MSCs according to the time of cultivation. **Methods.** Leukemic cells from bone marrow samples of patients (age 6 months - 17 years) with newly diagnosed acute leukemia (AL): B-ALL-45, T-ALL-13, AML-19. MSC (from 54 healthy donors) were cultured in DMEM with 20% FCS. Clonogenic study of granulocyte-macrophage bone marrow progenitors (from 15healthy donors) were performed in semisolid agar medium. The sensitivity of blast cells to cytotoxic drugs was studied by the MTT-assay. To determine the levels of spontaneous and induced apoptosis in leukemia cells we use Apoptosis Detection Kit II (BD). Cytokines produced by MSCs from the bone marrow was determined by flow Cytofluorometer using reagents BD Cytometric Bead Array early and late passages. **Results.** MSCs stimulate colony formation of granulocyte-macrophage precursors, surpassing the effectiveness of phytohemagglutinin-leukocyte conditioned medium as a source of colony stimulating factors. Efficiency of cloning was 27.9 (SD 1.5) and 22.9 (SD 2.4), respectively. When leukemic cells were cultured on MSC for 4 days the proportion of viable cells was higher at 47.2% in ALL, and 63.1% in AML compared with controls. Incubation of leukemic cells with MSCs resulted in a decrease of sensitivity to cytarabine in 2 times in B-ALL, in 1.5 times in AML and 6 times in T-ALL. Under the influence of MSCs sensitivity of leukemic cells to daunorubicin decreased in all groups. MSC increased the sensitivity of blast cells from ALL patients to MP, but did not influence the sensitivity of AML blast cells to MP. MSCs inhibit apoptosis of leukemic cells induced by cytarabine. The study of MSCs cultures with CBA showed that MSCs produce a wide range of cytokines (IL-2, 4, 6, 10; FGF, VEGF), which may mediate the effect of MSCs on the viability of hematopoietic progenitors and leukemic cells. Some changes in production of various cytokins depending on time of cultivation have been observed. **Conclusions.** On the basis of the research is clear that the important aspect is the safety of MSCs in terms of their influence on the sensitivity of leukemic cells to chemotherapy in patients with hematologic malignancies. The functional capacity of MSCs, which is realized through the production of a wide variety of cytokines, may play an important role in the regulation of immunological, reparative and other processes.

0710

THE NUCLEAR FACTOR NF-YA IN HUMAN BONE MARROW MESENCHYMAL STEM CELLS: AN EMERGING ROLE IN ADIPOGENESIS

A Anthi[1], K Pavlaki[1], M Kastrinaki[1], E Simantirakis[1], J Papamatheakis[2], C Pontikoglou[1], H Papadaki[1]
[1]University of Crete School of Medicine, Heraklion, Greece
[2]Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

Background. The nuclear transcription factor Y (NF-Y) is a heterotrimeric CCAAT-binding transcription factor consisting of the subunits A, B, and C that appears to have a complex role in hematopoietic stem cell (HSC) self-renewal and differentiation along different hematopoietic cell lineages. No available data, however, exist on the role of NF-Y in cells of the non-hematopoietic bone marrow (BM) stroma, namely the mesenchymal stem cells (MSCs) and their progeny. **Aims.** To evaluate mRNA expression and protein levels of the main regulatory NF-Y subunit, namely the NF-YA, throughout long-term BM-MSC cultures and probe its possible involvement in BM-MSC differentiation process towards the adipogenic and osteogenic lineages *in vitro*. **Methods.** MSCs were isolated from BM aspirates of hematologically healthy subjects undergoing orthopedic surgeries after informed consent. MSCs were *in vitro* expanded until passage (P)-10. P3 BM-MSCs were *in vitro* induced to adipogenic or osteogenic differentiation according to standard protocols. Differentiation were