Evaluation of bone marrow stromal cells in view of their role in hematopoietic reconstitution.

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Introduction

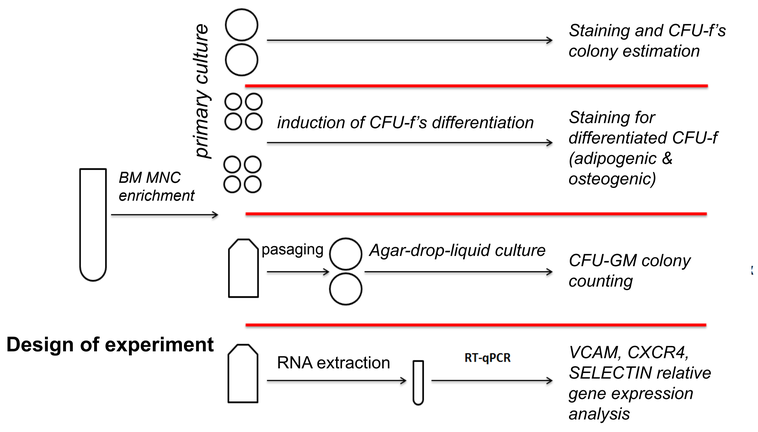
The role of stromal microenvironment in hematopoietic stem cell transplantation (HSCT) is based on its nonlineage-specific effects upon proliferation and differentiation of HSCs. Deficient graft functioning observed in some cases is a pre-requisite for predictive functional testing of stromal cells *in vitro*, in order to prove clinical indications for co-transplanting of hematopoietic and stromal cells (SC), and, probably, introduction of alternative therapeutic approaches.

Objectives

The aim of this study was to investigate the role of bone marrow stromal cells (BMSC) during engraftment of donor marrow cells, and its possible significance for post-transplant complications.

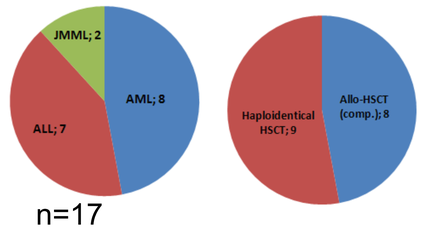
Methods

The study included clinical observations of post-transplant course in 17 patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), Juvenile myelomonocytic leukemia (JMML) and 13 healthy donors. Bone marrow nucleated cells were selectively harvested two weeks prior to BMT, followed by monolayer culture in alpha-MEM culture medium with 20% fetal bovine serum. Upon growth of fibroblast-like cell colonies (CFU-F), their hematopoiesis-supporting activity was determined in agar-drop/liquid culture system, along with their differentiating ability towards adipogenic and osteogenic pathways. We have also measured the relative expression of *VCAM, SELECTIN* and *CXCR4* genes in these populations.



Design of experiment

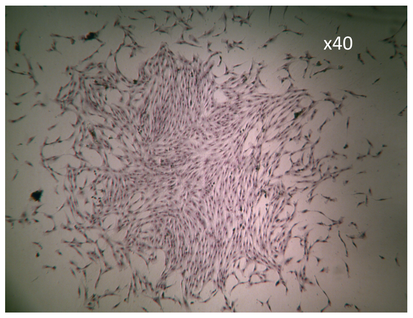
Patients characteristics



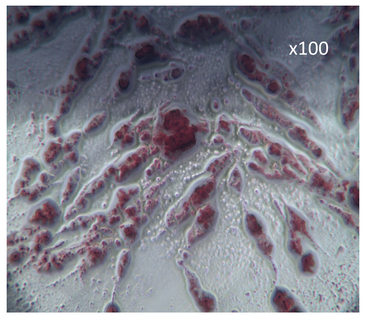
n=17
Average age 14 (0-45)

Donors characteristics

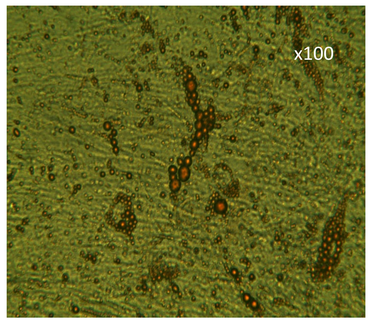
n=13
Average age 34 (20-45)



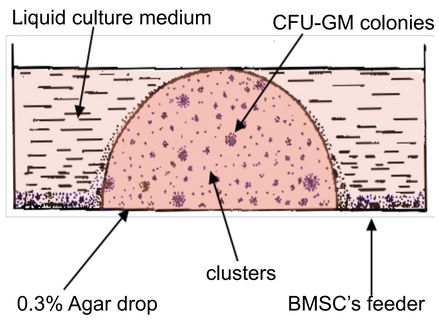
BMSC's proliferative capacity analysis by CFU-F colony evaluation

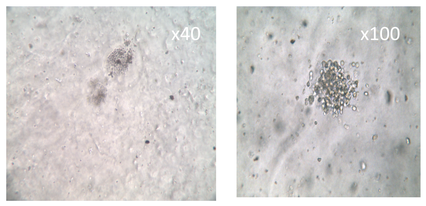


Evaluation of BMSC CFU-F's to osteogenic differentiation *in vitro*



Evaluation of BMSC CFU-F's to adipogenic differentiation *in vitro*

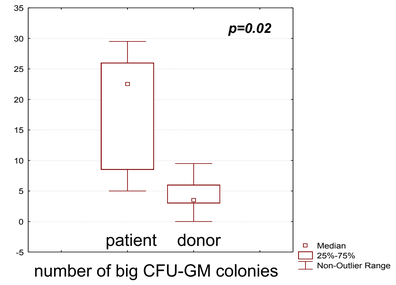




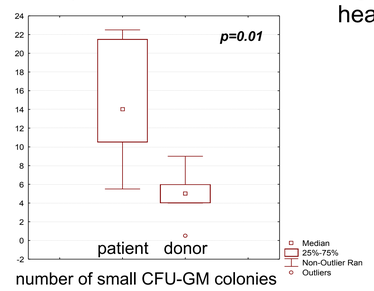
BMSC's hemostimulation ability evaluation in Agar drop-Liquid culture system

Results

When comparing functional characteristics of BMSCs from healthy donors and patients, an increased hematopoiesis-supporting activity of leukemia BMSCs was noted, as shown by increased numbers of large and small CFU-GM (p <0.02).

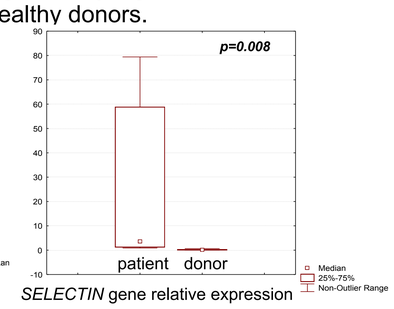


number of big CFU-GM colonies



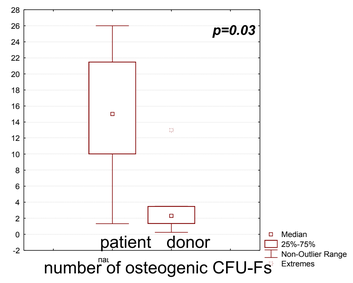
number of small CFU-GM colonies

It was also found that higher *SELECTIN* and *VECAM* gene expression in BMSC population of AML patients was significantly higher than in the cells from healthy donors.

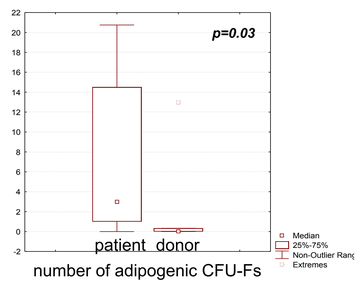


SELECTIN gene relative expression

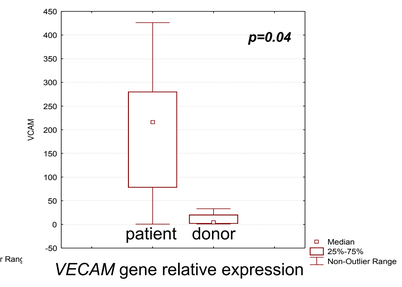
In addition, an increased ability for conventional differentiation was observed in leukemia BMSC (p = 0.03).



number of osteogenic CFU-Fs

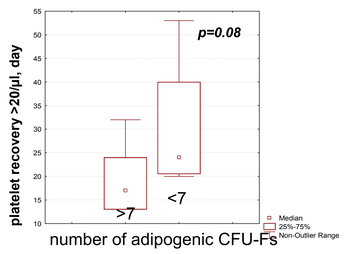


number of adipogenic CFU-Fs

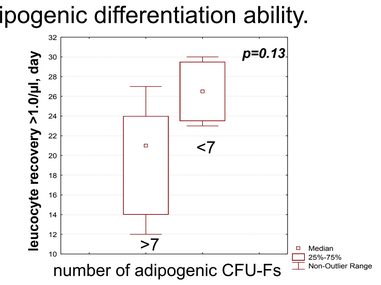


VECAM gene relative expression

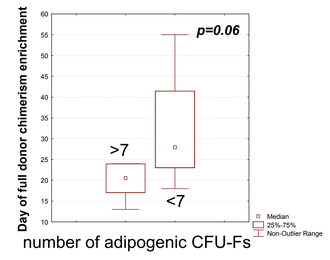
Moreover, the number of CFU-Fs, capable for adipogenic differentiation showed inverse correlation with platelet and leucocyte recovery terms. Besides the term of full donor chimerism enrichment was lower in case of BMSCs higher adipogenic differentiation ability.



number of adipogenic CFU-Fs

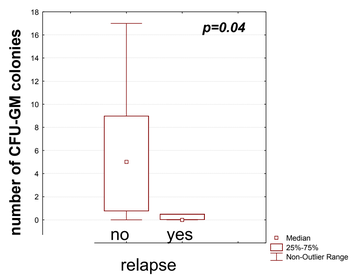


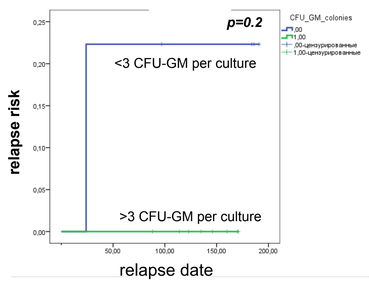
number of adipogenic CFU-Fs



number of adipogenic CFU-Fs

By the contrast, higher numbers of osteogenic colonies in culture were associated with delayed leukocyte engraftment (p = 0.05, data not shown). By the analysis of in vitro hemostimulation activity we have not observed any clinical-laboratory correlations – neither with engraftment nor full donor chimerism enrichment terms (data not shown). Nevertheless we observed lower relapse incidence in case of higher hemostimulation in culture (p=0.04).





We have not observed any statistically significant correlations between BMSCs functional characteristics and aGVHD development (data not shown).

Conclusion

Stromal cells derived from the bone marrow of AML patients exhibit higher proliferative activity and marked expression of molecules mediating HSC homing, as compared with a group of healthy donors. This finding could be explained by affection of stromal cells due to previous chemotherapy and myelosuppression. BMSC from leukemia patients taken before bone marrow transplantation possess a more pronounced capacity for osteogenic and adipogenic differentiation than those from healthy donors. Increased adipogenic differentiation ability of the BMSCs and high VCAM gene expression are associated with a more rapid recovery of hematopoiesis.

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