Cytogenetic Characterization of Bone Marrow Mesenchymal Stem Cells from Patients with Myelodysplastic Syndrome

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INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders. The establishment of a neoplastic clone is reflected by dysplastic features and impaired function which may affect all three hematopoietic cell lineages. The ineffective hematopoiesis which causes bone marrow failure is accompanied by peripheral blood cytopenias as a result from increased apoptosis at initial MDS stage. The MDS may progress into acute myeloid leukemia (AML). Chromosomal aberrations and genetic mutations play a pivotal role in the MDS pathogenesis. In addition, the hematopoietic microenvironment (HM) is also involved in the MDS pathophysiology. The central componentof HM are bone marrow (BM) mesenchymal stem cells (MSCs) (Figure 1). The involvement of the BM-MSCs in the pathogenetic/ pathophysiologic process of MDS has been recently studied, but existing data on MSCs cytogenetic are controversial.

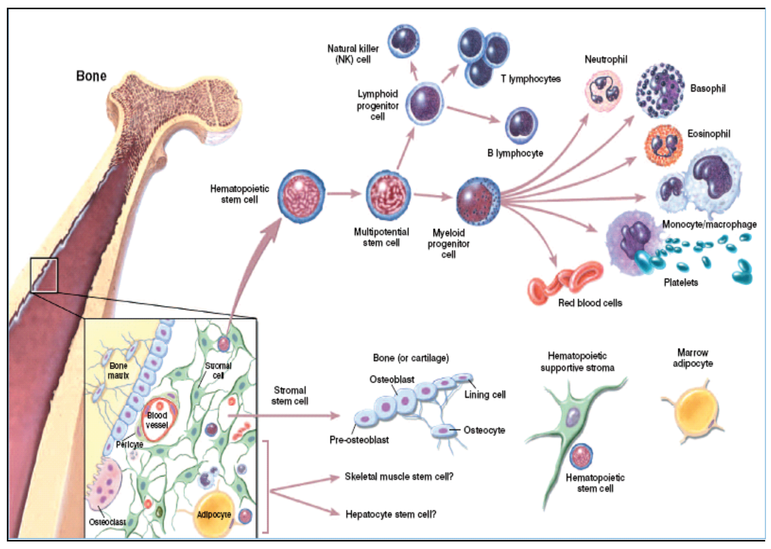

Figure 1: Hematopoietic stem cells and MSC stromal.

OBJECTIVE

The aim of this study was to analyze the chromosomal pattern of BM-MSCs and hematopoietic cells of patients with primary MDS and to compare with karyotypes from BM-MSCs of healthy individuals (donors for allogeneic hematopoietic stem cell transplantation) through classical and molecular cytogenetics (FISH), verifying the instability of these cells *in vitro*.

MATERIAL AND METHODS

Isolation and Culture of Bone Marrow Stromal Mesenchymal Stem Cells

Bone marrow (BM)-derived samples were obtained from 20 healthy individuals (donors for allogeneic hematopoietic stem cell transplantation) and 15 patients with MDS (11 RA; 2 RAEB and 2 RAEB-t) in accordance with guidelines of the INCA Ethics Committee. Mononuclear cells (MNC) were isolated from BM samples using density-gradient centrifugation (Ficoll). Cells were plated in non-coated 75-cm^2 polystyrene culture flasks at 500 000 cells/cm^2 density, in low-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine calf serum, antibiotics and glutamine. After 48h, non-adherent cells were removed and MSCs were cultured until 80% confluence. Subsequently, cells were removed from plates using 0.05% trypsin for 5 minutes at 37°C and replated at 2000 cells/cm^2 density (passage1) in a fresh culture flask. When 80% confluency was obtained, cells were trypsinized and replated in fresh culture flasks (passage 2). The cells were cultured until passage 3-10, when the BM-MSCs were used for cytogenetic analysis using G-banding and FISH.

Cytogenetic and Immunophenotypic Analysis

Prior to obtaining confluence, cells from MDS patients and from health donors were incubated at 37 °C with colcemid for 2 h. Then, they were trypsinized and processed using standard cytogenetic procedures. GTG banding was performed, and chromosomes were identified and analysed according to the International System of Human Cytogenetic Nomenclature (ISCN, 2013). The cytogenetic analyses were performed using the Ikaros-MetaSystems system (Zeiss, Germany). FISH analyses were performed in the cases of abnormal karyotypes detected in hematopoietic cells from MDS patients. The cultured plastic-adherent cells from MDS patients and health donors were analyzed by flow cytometry using CD73, CD90,CD105, CD14, CD19, CD34, CD45 and HLA-DR (BD Biosciences, San Jose, CA, USA).

RESULTS

The BM-MSCs in culture (passage 3) (Figure 2) were analyzed by flow cytometry using CD73, CD90, CD105, CD14, CD19, CD34, CD45 and HLA-DR (BD Biosciences, San Jose, CA, USA) from 20 donors and 15 MDS patients. Patients and donor MSCs showed similar phenotypes. Our results showed that BM-MSCs from 20 donors and 15 MDS patients remained cytogenetically stable at passage 3, showing a normal karyotype pattern. However, in cells with many passages we observed aneuploidy karyotypes, in BM-MSCs from donors and patients, showing a cytogenetic instability. The cytogenetic analyses of BM-MSCs and hematopoietic cells of patients with primary MDS showed different chromosomal pattern (Table 1).

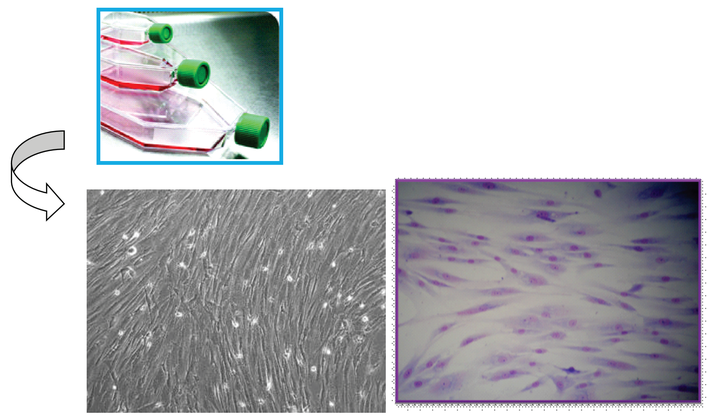

Figure 2: BM-MSCs in culture (passage 3).

Table 1: Cytogenetic Analysis of Mesenchymal Stem Cells of Patients withPrimary MDS.

Patients	MDS Subtypes	Karyotype of BM-Hematopoietic Cells	Karyotype of BM-MSCs
1	RA	46,XX,del(20)(q21)[4]/46,XX[18]	46,XX[18]
2	RA	46,XY[27]	46,XY[13]
3	RA	46,XX[25]	46,XX[16]
4	RA	46,XY[20]	46,XY[15]
5	RA	46,XY[28]	46,XY[20]
6	RA	46,XY[28]	46,XY[13]
7	RA	46,XY[22]	46,XY[15]
8	RA	46,XX,del(5)(q13q33)[4]/46,XX[18]	46,XX[20]
9	RA	46,XY[20]	46,XY[14]
10	RA	46,XY,del(17)(p12)[5]/46,XY[16]*	46,XY[20]
11	RA	46,XY,del(12)(p12)[6]/46,XY[15]	46,XY[20]
12	AREB	45,XY,-7 [9]/[46,XY[14]	46,XY[19]
13	AREB	46,XY,del(7)(q22)[5]/46,XX[17]	46,XY[16]
14	AREB-t	Hyperdiploid karyotype	46,XY[15]
15	AREB -t	47,XY,+8[13]/46,XY[7]	46,XY[10]

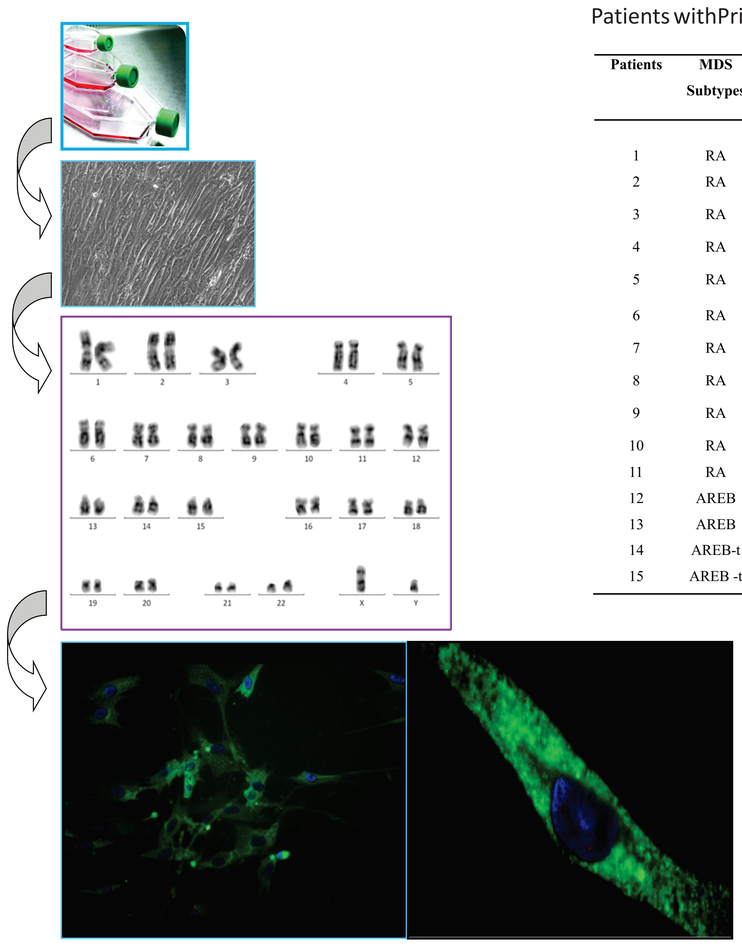

Figure 3: Cytogenetic analysis: RA patients, BM-hematopoietic cells showed the karyotype 46,XY,del(17)(p12)[5]/46,XY[16] and BM-MSCSs showed 46,XY[20].

CONCLUSIONS

The differences of chromosome abnormalities in the hematopoietic cells and MSCs point to the fact that the stromal microenvironment is not part of the abnormal clone in MDS, however, it may be important during the cross-talking in the development and evolution of disease. These results are important for the use of BM-MSCs in the cellular therapy and to characterize the chromosome pattern of BM-MSCs in patients with MDS.

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