Second International Conference on Natural Products Genomics and Drug Discovery

"Basic and Translational Research on Cancer and Inflammation

Abstract

Molecular Characterization of Mesenchymal Stem Cells in Myelodysplastic Syndromes

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Myelodysplastic Syndromes (MDS) are a complex spectrum of bone marrow stem cell disorders, characterized by peripheral cytopenias, morphologic bone marrow (BM) dysplasia and cellular dysfunction resulting in an ineffective hematopoiesis. The disease originates from a clone of malignant cells in the hematopoietic compartment in BM and whether these genetic abnormalities extend to the stromal compartment is debatable. Mesenchymal stem cells are considered as precursors of many cell types in marrow stroma that regulate hematopoiesis through direct cell to cell contacts and secretion of regulatory factors. Therefore it is worthwhile to assess the phenotypic and cytogenetic profiles of Mesenchymal Stromal Cells (MSCs) in MDS to better understand the nature of MSCs in MDS and to expand the existing knowledge of MDS biology. Here, we present phenotypic and cytogenetic characteristics of BM resident MSCs derived from MDS patients.

MSCs obtained from the BM of 17 primary MDS patients were culture-expanded by seeding mono nuclear cells at density of $1X10^6$ cells/ml in DMEM-KO supplemented with 10% FBS and 2mM glutamine. Immunophenotypes of MSCs were analyzed using passage 2 (P2) cells by flowcytometry. Differentiation studies of MSCs were performed by inducing P2-P4 MSCs towards osteogenic and adipogenic cell lineages. MSCs were karyotyped using G-banding technique and karyotypes were given according to the International System for Human Cytogenetic Nomenclature (ISCN) guidelines. Presence of an abnormal clone was ascertained by the observation of more than two metaphase spreads having the same numerical or structural abnormality. FISH was carried out on BM and MSCs of confirmed del(5q) patients (probe XL 5q31.2/5q33), 200 cells were counted and ≥95% was set as the cut-off.

Culture-expanded MDS-MSCs were fibroblast-like, thin spindle-shaped cells which showed similar morphology to that of control MSCs. Both control and MDS derived MSCs were able to differentiate towards adipogenic and osteogenic tissues. All the MSCs were positive for CD73, CD90 and CD105 and were negative for CD34 and CD45. Normal karyotypes were present in 61% of the patient MSCs. 31% of the patient MSCs showed abnormal karyotypes. Distinct chromosomal aberrations were observed in MSCs whose BM karyotypes were normal. None of the patients who had abnormal BM karyotypes showed aberrations in their MSCs. The karyotype of MSCs of a confirmed del(5q) patient (by karyotype and FISH on BM cells) was normal (46,XX) and the finding was confirmed by FISH. Two clones of MSCs (normal and aberrant) were identified in all the patients with genetically abnormal MSC karyotypes.

MDS-MSCs are comparable with control MSCs with respect to cell morphology, positivity for CD73, CD90, CD105, CD34, CD45 and differentiation ability towards adipogenic and osteogenic tissues. MSC clones with distinct genetic abnormalities are present in MDS. Presence and the nature of the genetic abnormality in BM-MSCs in MDS could be regarded as an independent

event from that of their hematopoietic counterparts. The exact role of these abnormalities in pathogenesis of MDS needs to be elucidated in further studies.

Keywords: Myelodysplastic syndromes, mesenchymal stem cells, cytogenetic features