Single-Cell Analysis of Mesenchymal Stem Cells Reveals Their Molecular and Functional Heterogeneity in Myeloma

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ABSTRACT

Introduction: Bone marrow (BM) mesenchymal stem cells (MSCs) are rare but heterogeneous cell populations, and both MSCs and their lineages mediate BM niches, hematopoiesis, and myeloma (MM) growth. The aim of the study was to assess variation in gene expression of unexpanded single MSCs generated from the same source, and study the heterogeneous effect on MM cell growth of MSCs lines generated from single MSCs or normal MSCs primed by MM cells. We also studied changes in MSCs during MM progression and identified MSC factors that mediate MM growth.

Methods: Cells collected from bone pieces following enzymatic digestion were cultured for 2-4 days. The adherent cells were trypsinized, replated to separate MSCs from macrophages and allowed to adhere overnight. Cells were then trypsinized and single MSCs were sorted into 96-well plates. Validation of single cell sorting and viability was assessed microscopically by staining for CFSE. Single MSCs were generated from two normal samples and two MM patients. Sorted single cells were subjected to qRT-PCR using primers of 34 genes implicated in MSC biology (e.g. CD73), cell cycle (e.g. KI67, CDKN2A), differentiation (e.g. RUNX2, PPARG, CYR61), transcription (e.g. FOXC1), and specific MSC genes we identified to be to top overexpressed (e.g. POSTN) or underexpressed (e.g. IGFBP2, LEPR) in bone biopsy samples from high-risk MM patients. Also included were CD45 and CD34 to exclude hematopoietic cells, and GAPDH and ACTB as housekeeping genes. After excluding CD45+ cells and cells with low expression of housekeeping genes a total of 111 and 104 single MSCs from normal and MM subjects, respectively, were analyzed. Single MSCs expressed typical MSC markers such as CD73, CD90, FN1 and FAP. For generating MSC lines from the same source, single MSCs from each source were subjected to long-term culture supplemented with conditioned media (CM) from whole BM and MSC cultures. For preparing primed MSCs, normal MSCs generated by standard method were cultured alones (unprimed MSCs) or cocultured with MM cells for 5 days (primed MSCs).The cultured and co-cultured cells were trypsinized, replated for 40 min followed by serial washing that attain >95% purified adherent MSCs. Primed and unprimed MSCs were subjected to gene expression profiling (GEP) and their CM used for proteomics and growth assays.

Results: Single MSCs from the same source (i.e. normal and MM bones) heterogeneously expressed genes related to cell cycle, differentiation, and extracellular matrix. MM single MSCs had lower expression of markers of proliferation (e.g. KI67, p<0.005) and differentiation (e.g. RUNX2, p<0.03), and higher expression of the senescence marker CDKN2A (p<0.001). To further capture MSC heterogeneity, MSCs lines were generated from single MSCs and tested for their ability to support MM cells. Growth of individual MSCs varied in culture, most had a limited proliferation rate, and few were capable of generating MSC lines. MSC lines generated from the same source (4-10 lines from normal or MM subjects) had variable effects on growth of MM cells in co-cultures, or when their CM used on MM cell grown alone (p<0.0008 between lowest to highest growth stimulating lines). CM from primed MSCs stimulated the growth of primary MM cells compared to CM from unprimed MSCs in short-term culture (n=10, p<0.007) or 10-days co-couture with normal MSCs (n=6, p<0.002). Primed MM cells had reduced expression and secretion of factors related to the IGF1 pathways including IGFBP2, IGFBP3 and IGF2, whereas IGF1 was not impacted. Recombinant IGF1 promoted growth of BM-dependent MM cells in serum-free conditions, an effect that was significantly blocked by recombinant IGFBP2 (p<0.0003). IGFBP2 expression by GEP was lower in biopsy samples from MM patients (n=531) than normal subjects (n=68), lower in samples from high-risk than low-risk patients, and lower in focal lesion than random BM biopsies from the same patients (n=77). Immunohistochemistry showed high IGFBP2 protein expression by BM mesenchymal cells, particularly, small adipocytes, and changes in the proportion of IGFBP2+ cells correlated with the reduced gene expression in MM patients' biopsies, and with disease stage.

Conclusions: These findings suggest that MSCs from normal subjects and MM patients are heterogeneous population of cells, are deregulated as part of MM disease progression, and that production of factors such as IGFBP2 by these cells impacts bioavailability of IGF1.

AIMS OF STUDY

Aim 1: Study the variation in gene expression of unexpanded single MSCs generated from the same source.

Aim 2: Study the heterogeneous effect on MM cell growth of MSCs lines generated from single MSCs.

Aim 3: Study the effect of normal MSCs primed by MM cells on growth of primary MM cells.

Aim 4: Identify MSC factors that mediate MM growth.

RESULTS

Figure 1: Single MSCs from MM patients express lower level of the proliferation marker, MKI67 and higher level of the senescent marker, CDKN2A.

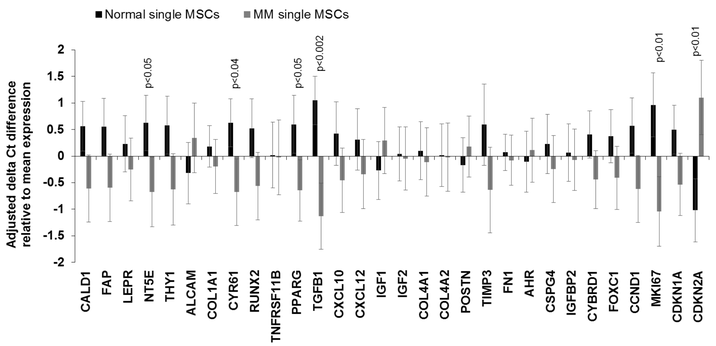


Figure 2: In vitro growth pattern single MSCs isolated from BM of MM patients and normal donors.

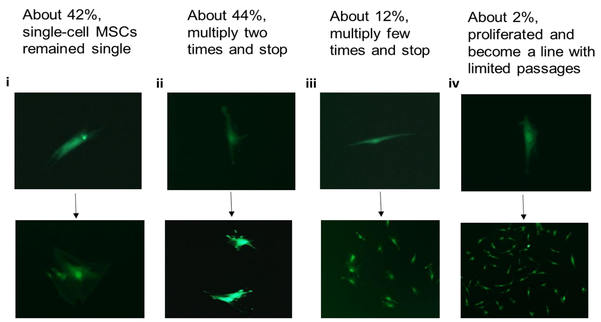
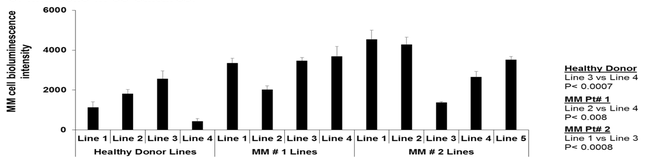
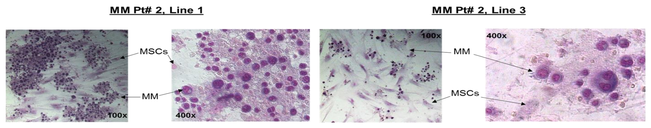


Figure 3: MSC lines generated from single-cell MSCs from the same source variably support growth of MM cells in co-cultures.



* Luciferase-expressing MM cells were co-cultured with MSC lines generated 5 days.



* Co-cultures of lines 1 and 3 from MM patient #2 stained with Geimsa.

SUMMARY & CONCLUSIONS

- Single cell gene expression analysis revealed that MSCs from the same source are heterogeneous.
- MSC lines generated from single cell MSC from the same source showed variable effect on MM cell growth.
- Protein concentration of IGF-1 related factors except IGF-1 is lower in conditioned medium from primed MSCs than from unprimed MSCs.
- Conditioned medium from MSCs primed with MM cells supports growth of primary MM cells cultured alone and in long-term co-culture with normal MSCs.
- IGFBP2+ mesenchymal cells are reduced in MM BM.
- IGFBP2 effectively blocks IGF1-induced MM cell growth.

Figure 4: Preparation of primed MSCs and their conditioned media.

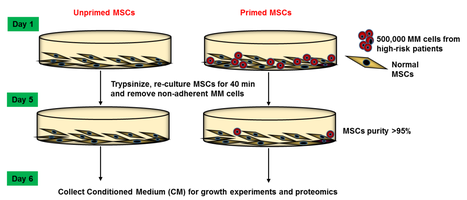


Figure 5: Conditioned medium from primed MSCs promotes growth of primary MM cells cultured alone or co-cultured with normal MSCs.

Primary MM cells were cultured with fresh media (Control) or conditioned media (50%) from unprimed and primed MSCs for 5 days.

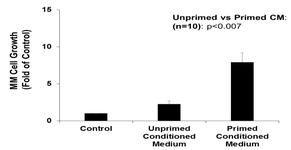


Figure 6: Conditioned medium (n=7) from primed MSCs has reduced levels of factors related to IGF-1 pathways.

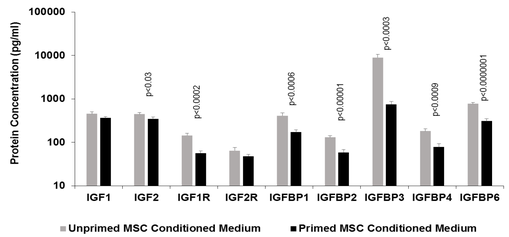
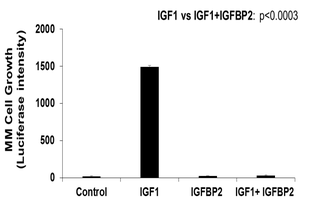


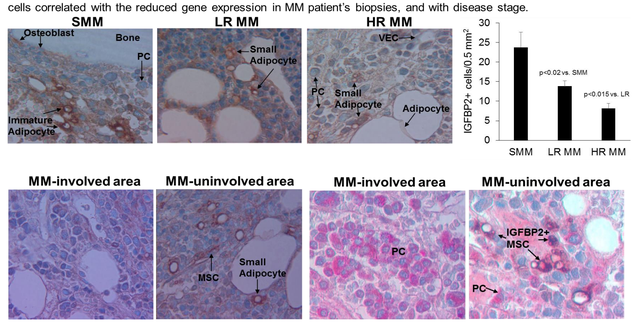
Figure 7: Recombinant IGFBP2 blocks IGF-induced MM cell growth.



* Luciferase-expressing MM cells were cultured in for 48 hrs in the absence and presence of IGF1 (40 ng/ml) and IGFBP2 (5 ug/ml).

Figure 8: IGFBP2+ cells are depleted in High Risk MM BM.

IGFBP2+ expressing mesenchymal cells exhibit MSCs and small adipocytes morphology. Immunohistochemical staining of the biopsy bone sections from patients with SMM and LR and HR MM shows lower proportion of IGFBP2+ cells correlated with the reduced gene expression in MM patient's biopsies, and with disease stage.



DISCLOSURES

All authors have no relevant conflicts of interest to disclose

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