ABSTRACT

Introduction: Each disease stage in myeloma (MM) is associated with parallel changes in both the MM clone and the bone marrow (BM) microenvironment. Mesenchymal cell lineages derived from mesenchymal stem cells (MSCs), including osteoblasts, adipocytes and pericytes play an important role in MM cell growth mediated by the modification of the MM niche in the BM. The overall goal of the study was to test and identify changes induced in MSCs by high-risk (HR) MM cells that impact MSC function and promote oncogenic pathways capable of supporting low-risk (LR) MM cells.

Methods: Normal MSCs were either cultured alone (“unconditioned”) or co-cultured with MM cells for 5 days. The cultured and co-cultured cells were trypsinized, replated for 40 min, followed by serial washing to remove MM cells from the adherent MSCs. More than 95% of the remaining adherent cells after culture were MSCs (“unconditioned”). The preconditioned and unconditioned MSCs or their 24 hrs conditioned media (CM; 50%) were tested for their ability to support the 5-days growth of MM cells from LR (p<0.0004) and HR (p<0.005) patients. To identify factors altered in MSCs by HR MM cells, the unconditioned and preconditioned MSCs and their serum-free conditioned media (n=4) underwent gene expression profiling and proteomic analysis. Whole bone biopsy gene expression profiles from newly diagnosed patients with MM enrolled in Total Therapy clinical trials were used to correlate the altered expression profiling and proteomic analysis. Novel gene expression profiles from newly diagnosed patients with MM enrolled in Total Therapy clinical trials were used to correlate the altered expression profiling and proteomic analysis. Whole bone biopsy gene expression profiles from newly diagnosed patients with MM enrolled in Total Therapy clinical trials were used to correlate the altered expression profiling and proteomic analysis.

Results: Growth of all MM cells tested was increased by inclusion of MSCs preconditioned with HR MM cells by 2.2±0.2 (p<0.0004) and by CM from these MSCs by 9.6±2.0 (p<0.004), compared to culture of MM cells in fresh media. In contrast, CM from unconditioned MSCs increased growth of HR MM cells by 2.6±0.6 (p<0.01) fold but had minor effect on growth of LR MM cells. CM from MSCs preconditioned with HR MM cells increased growth of LR and HR MM cells to 5.7±1.1 (p<0.0002) and 2.6±1.2 (p<0.04), compared to culture of MM cells in CM from unconditioned MSCs, respectively. Growth of LR MM cells was higher by 2.9±0.3 fold using CM from MSCs preconditioned with HR MM cells than by using CM from MSCs preconditioned with LR MM cells (p<0.005). To determine the role of cell-cell contact, we compared the effect of the preconditioned MSCs and their CM on growth of LR and HR MM cells. Growth of LR MM cells (p<0.003) and HR MM cells (p<0.005) was higher when cultured in CM than in co-culture with MSCs. These data imply that soluble factors from preconditioned MSCs support MM cell proliferation and that adhesion of MM cells to MSCs may restrain proliferation. Genes overexpressed in preconditioned MSCs included growth factors (e.g. IL6) and receptors (e.g. EDNRA); genes underexpressed included those that adhesion of MM cells to MSCs may restrain proliferation. Genes overexpressed in preconditioned MSCs included growth factors (e.g. IL6) and receptors (e.g. EDNRA); genes underexpressed included those that adhesion of MM cells to MSCs may restrain proliferation. Genes overexpressed in preconditioned MSCs included growth factors (e.g. IL6) and receptors (e.g. EDNRA); genes underexpressed included those that adhesion of MM cells to MSCs may restrain proliferation.

Conclusion: Preconditioning of the MSCs with HR MM cells significantly promotes the growth of LR MM cells. Soluble factors present in the primed conditioned medium supports MM cell proliferation, whereas adhesion of MM cells to MSCs may restrain proliferation.

AIMS OF STUDY

Aim 1: Test whether MM cells from high-risk (HR) patients can prime normal MSCs to support growth of MM cells from low-risk (LR) patients.

Aim 2: Identify and study MSC factors that mediate MM cell growth.

RESULTS

Figure 1. Preparation and molecular properties of primed MSCs and their conditioned media.

Figure 2. MSCs primed with high-risk MM cells promote MM cell growth.

Figure 3. Conditioned medium from MSCs primed with HR MM blocks MM cell growth.

Figure 4. Reconstituent IGFBP2 blocks IGFI-induced MM cell growth.

SUMMARY & CONCLUSIONS

- Preconditioning of the MSCs with HR MM cells significantly promotes the growth of LR MM cells.
- Soluble factors present in the primed conditioned medium supports MM cell proliferation, whereas adhesion of MM cells to MSCs may restrain proliferation.
- Priming MSCs with HR MM cells and their conditioned medium have higher stimulatory effect on LR MM cells than MSCs primed with LR MM cells.
- EDNRA is upregulated whereas IGFBP2 expression is downregulated in BM from HR MM patients.
- IGFBP2 blocks IGFI-induced MM cell growth.

DISCLOSURES

All authors have no relevant conflicts of interest to disclose.