

Osteoblast–myeloma crosstalk drives trophic interferon pathway signaling and chemokines in multiple myeloma

Background: Multiple myeloma (MM) progression and therapeutic resistance are influenced by interactions within the bone marrow tumor microenvironment (TME). However, the mechanisms underlying osteoblast–MM cross-talk remain poorly understood.

Methods: MM cell lines (MM.1S, U266, NCI-H929) were co-cultured with hFOB osteoblasts or HUVEC endothelial cells. FACS-isolated cells underwent RNA-seq and gene set enrichment analysis (GSEA), with validation by western blotting, cytokine assays, ELISA, and qPCR. The cGAS–STING–interferon (IFN) axis was investigated using the cGAS inhibitor RU.521, exogenous IFN- α , and 5-aza-dC (DAC). Clinical relevance was assessed using primary MM gene expression, survival, and DNA methylation data.

Results: Osteoblast co-culture enhanced MM.1S proliferation and partially protected against bortezomib, accompanied by marked activation of Type I IFN signaling, including a 7.5-fold increase in IRF7 expression ($p < 0.001$). In contrast, HUVEC co-culture promoted proliferation but elicited an approximately eight-fold weaker IFN response, preferentially activating translation, proteasomal degradation, and non-canonical NF- κ B pathways. MM cells reciprocally induced osteoblast secretion of pro-inflammatory chemokines CXCL1 and CXCL8. Exogenous IFN- α revealed a biphasic response: low-to-intermediate signaling supported MM.1S survival and proliferation, whereas high doses induced cytotoxicity in MM.1S and U266 cells. cGAS inhibition abolished osteoblast-mediated protection, while DAC combined with IFN- α produced greater-than-additive cytotoxicity. U266 cells exhibited minimal osteoblast-induced IFN activation despite sensitivity to exogenous IFN- α , indicating cell-line-dependent responses. In primary MM, an IFN-target gene signature correlated with adverse survival. DNA methylation profiling of 44 cases revealed preferential hypermethylation of Polycomb-regulated and growth-suppressive genes, while IFN pathway promoters remained relatively spared, particularly in ultra-high-risk disease.

Conclusions: Osteoblast–MM cross-talk promotes proliferation and drug resistance through trophic activation of the cGAS–STING–IFN axis, alongside reciprocal inflammatory programming of osteoblasts. The biphasic IFN response suggests a therapeutic opportunity to overcome microenvironment-mediated protection through pathway hyperactivation, potentially enhanced by DAC. Clinical and epigenomic findings further implicate sustained IFN pathway accessibility and activation in aggressive MM.

Keywords: multiple myeloma, osteoblasts, bone stromal cells, tumor-stroma interactions, gene expression profiling, co-culture, interferon signaling, cGAS-STING, 5-aza-dC, DNA methylation