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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Melphalan-Induced Multiple Myeloma Cells Exhibit a Senescent-like Dormant Phenotype

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Multiple myeloma (MM) is an incurable plasma cell malignancy. Despite therapeutic advances that have vastly improved survival, all patients are expected to relapse. One mechanism that may contribute to MM relapse is tumor dormancy. Dormant MM cells may persist in the bone marrow, where they exhibit reduced proliferative capacity and resistance to treatment. These cells may then exit dormancy following treatment to drive disease relapse. Dormancy shares many features with cellular senescence, a state of stable growth arrest induced by cell stress. Chemotherapies that produce genotoxic stress have been associated with therapy-induced senescence (TIS). One such genotoxic agent, melphalan (Mel), is routinely administered to MM patients for myeloablation prior to autologous bone marrow transplant. We hypothesized that High Dose Mel (HDM) induces a senescent-like dormant phenotype in surviving MM cells.

To test our hypothesis, we developed an *in vitro* model for HDM. We identified 10μM Mel as a concentration to induce >90% cell toxicity (HDM) in 5TGM1 mouse MM cells over 10 days. 5TGM1-GFP MM cells were cultured with HDM or vehicle (Veh) for 6 hours; cells were then cultured in normal growth medium in the presence of primary mouse (C57BL/6) bone marrow stromal cells (BMSCs). Co-cultures were imaged by fluorescent microscopy every other day to quantify cumulative population doublings (CPD) and cell morphology over the following 10 days. The effect of HDM on CPDs was also evaluated in the human RPMI-8226 MM cell line. 5TGM1-GFP cells were isolated by FACS on day 10 for evaluation of senescence histological characteristics and gene expression. All studies represent n=3 independent experiments unless otherwise indicated.

HDM-5TGM1 cells exhibited a significant reduction in mean CPDs over 10 days compared to Veh (HDM 0.32±0.31, Veh 6.72±0.69, $p<0.0001$), indicating stable growth arrest in HDM treated cells consistent with senescence. HDM-5TGM1 cells were also significantly larger versus Veh (median size in pixels units: HDM 413.2±99.98, Veh 207±8.88, $p<0.05$), consistent with classical enlarged morphology in senescent cells. Preliminary assessment (n=1) of HDM-RPMI-8226 cells showed similar growth arrest (mean cumulative doublings: HDM 0.058±0.101, Veh 4.185±0.358) and enlargement (mean size in pixel units: HDM 804.1±6.048, Veh 526.9±2.115), suggesting a translatable mechanism in humans.

FACS-sorted 5TGM1-GFP cells were stained for telomere associated foci (TAF), a marker of persistent DNA damage characteristic of senescent cells. HDM-5TGM1 cells showed a higher percentage of TAF+ cells (Percent TAF+ cells: HDM 39.7%±9.1%, Veh 10.6%±0.8%, n=2). Sorted HDM-5TGM1 cells also exhibited significant increases over Veh ($p<0.001$) in senescence markers (*Cdkn1a, Cdkn1c, Glb1)*, senescence cell anti-apoptosis pathway genes (SCAPs, *Bcl2l1)*, and senescence associated secretory phenotype (SASP) genes (*Ccl5, Icam1, Mmp13*). In addition, HDM-5TGM1 cells showed significant increases in myeloid markers that have previously been shown to be increased in dormant 5TGM1 (*Axl, Fcer1g, Mpeg1*).

Single cell RNA-sequencing was used to further evaluate senescence gene expression changes in growth arrested HDM-5TGM1 cells. Gene set enrichment analysis (GSEA) of genes differentially expressed in HDM-5TGM1 versus Veh cells was performed for custom senescence phenotyping gene sets (Senescence Up, SenUp; Senescence Growth Arrest, SenGA; Inflammatory SASP, iSASP; SCAPs; and Plasma Cell Senescence; PCSen) as well as mouse ortholog versions of a published senescence and biological aging gene set (SenMayo) and KEGG curated gene sets. GSEA of HDM-5TGM1 versus Veh cells confirmed significant (FDR<0.25) upregulation of iSASP, MayoSen, PCSen, SenUp, SCAPs, and SenGA gene sets. Concurrently, GSEA revealed significant downregulation of KEGG DNA replication, cell cycle, and nucleotide excision repair gene sets, supporting our observations of proliferative arrest and persistent DNA damage TAF.

Our findings demonstrate that HDM-MM cells exhibit multiple features of senescence, suggesting a potential mechanistic role for TIS in MM dormancy and highlight a novel therapeutic target that may be present in patients post-HDM. Thus, targeting senescence may be a novel approach to eliminate dormant MM cells and prevent relapse.

Disclosures No relevant conflicts of interest to declare.

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