Genotoxicity and functionality assessment of a bone marrow stromal cell line following chemotherapy in an *in vitro* model of multiple myeloma.

Simon William Andrews

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Faculty of Health and Applied Sciences, University of the West of England
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Abstract

Multiple myeloma (MM) is a haematological malignancy characterized by terminally differentiated plasma cells and their accumulation in the bone marrow (BM). Despite significant advances in therapeutic strategies it currently remains incurable. The interactions between the BM microenvironment and malignant plasma cells have been pivotal to understanding this disease. Previous reports have shown that patients with a haematological malignancy sustain “damage” to their BM, but how much of this is due to the disease and/or the treatment is currently unknown. Furthermore MM plasma cells have been documented to harness the BM microenvironment to their advantage, improving their growth and survival. However, little is known about the functionality of BM mesenchymal stem cells (MSC) in patients with MM disease which form an essential compartment of the BM microenvironment. It was hypothesised that MSC altruistically protect MM cells from therapy and consequently become phenotypically and genetically compromised.

To facilitate the study of the effects of chemotherapeutic agents and MM cells on MSC, a non-contact co-culture model was developed that allowed the investigation of functional and genetic damage. In line with previous studies, the MM cell line, U266B1 were found to be protected from drug-induced cell death when in co-culture with the stromal cell line HS5. However, the promoting effects of the BM appear to be at the detriment to their own survival. HS5 cells were found to have lower viability, altered morphology and disrupted differentiation when in a non-contact co-culture with U266B1 cells.

Results from this study have revealed that interactions of MSC with MM cells lead to an altruistic protection of MM cells by the BM. This work demonstrates that U266B1 cells have an improved viability following exposure to chemotherapy when in a non-contact co-culture with MSC/HS5. Furthermore, genotoxic assays also revealed that HS5/MSC interactions with U266B1 cells protect U266B1 from the genotoxic effects of melphalan in co-culture, whilst for the first time HS5 morphology was shown to be severely altered following exposure to chemotherapy and when in co-culture with U266B1 cells.
This work has demonstrated, for the first time, the cytotoxic effects of novel agents bortezomib and carfilzomib on HS5 cells when in co-culture with U266B1 cells. Results from this study also demonstrate that melphalan severely effects the ability of HS5 cells to differentiate in an osteogenic lineage with a further deficiency in differentiation when in co-culture with U266B1. Adipogenic differentiation of HS5 was unable to take place when in co-culture with MM cells and was again further impaired by chemotherapy. This is the first study to reveal that primary MSC secrete significantly high concentrations of IL-6 compared to the stromal cell line HS5. A further increase in expression of IL-6 was also shown when in co-culture with U266B1 cells.

Increased multi-nucleation was also identified in both HS5 and U266B1 cells when exposed to either thalidomide, lenalidomide and bortezomib with abnormalities providing possible explanations for the therapy related malignancies and neurotoxicity that is seen in some patients. Genotoxicity to the MSC/HS5 compartment of the co-culture measured by the micronucleus assay was also found to be reduced suggesting that the BM is protected from the DNA damaging effects of some agents when in co-culture with MM cells.

Combined work on the functionality and genotoxicity of the interactions between the BM and MM reveal a tropism of MSC and HS5 towards the MM cell line U266B1. With this research being conducted in a non-contact co-culture, it has indicated that cell-cell contact is not essential to provide protection of both the BM and MM cells against chemotherapy. This research provides further understanding of the MSC and MM interactions’ impact on the functionality of the BM and their protection from genotoxic damage. Elucidating the consequence of cytotoxic and genotoxic damage to MSC via chemotherapy treatment and/or through haematological disease may allow for the development of effective therapies and improve the quality of life for patients with MM.
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Abbreviations

°C  Degree Celsius
µg  Microgram
µl  Microlitre
µM  Micro molar
µm  Micrometre
nM  Nanomolar
g/l  Grams per litre
g/dl  Grams per decilitre
pg/ml  Picograms per millilitre
µg/ml  Micrograms per millilitre
cm  Centimetre
I.U  International unit
U/ml  International unit per millilitre
V/cm  Volt per centimetre
mA  Milliamperes
mmol/L  Millimoles per litre
2D  Two dimensional
3D  Three dimensional
Ab  Antibody
ALP  Alkaline phosphatase
AOC  Avon Orthopaedic Centre
ASCT  Autologous stem cell transplant
ATCC  American Type Culture Collection
β2M  Beta 2 microglobulin
BER  Base excision repair
BM  Bone marrow
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BM-MSC</td>
<td>Bone marrow mesenchymal stem cell</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>CAM-DR</td>
<td>Cell adhesion mediated drug resistance</td>
</tr>
<tr>
<td>CFU-F</td>
<td>Colony forming unit fibroblast</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
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<tr>
<td>LFA-1/CD18</td>
<td>Lymphocyte function associated antigen / CD marker 18</td>
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<td>VLA-4/CD49d</td>
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<tr>
<td>NCAM/CD56</td>
<td>Neural cell adhesion molecule / CD marker 56</td>
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<tr>
<td>VCAM-1/CD106</td>
<td>Vascular cell adhesion molecule 1 / CD marker 106</td>
</tr>
<tr>
<td>Dil/DiO</td>
<td>Long chain dialkylcarbocyanine lipophilic tracer</td>
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<tr>
<td>Dkk1</td>
<td>Dickkopf 1</td>
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<tr>
<td>DMEM/LG</td>
<td>Dulbecco’s Modified Eagle Medium low glucose</td>
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<tr>
<td>DMEM/HG</td>
<td>Dulbecco’s Modified Eagle Medium high glucose</td>
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<tr>
<td>DMEM/F12</td>
<td>Dulbecco’s Modified Eagle Medium and Ham's F-12 Nutrient Mixture</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DSB</td>
<td>Double strand break</td>
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<td>ECACC</td>
<td>European Collection of Cell Culture</td>
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<td>EBV</td>
<td>Epstein Barr virus</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
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<td>FBS</td>
<td>Foetal bovine serum</td>
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<td>H2O2</td>
<td>Hydrogen peroxide</td>
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<td>HDT</td>
<td>High dose therapy</td>
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<td>Hsp90</td>
<td>Heat shock protein 90</td>
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<td>Acronym</td>
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<tr>
<td>HQC</td>
<td>High quality control</td>
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<td>HMDS</td>
<td>Hexadimethylsilazane</td>
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<tr>
<td>Hr</td>
<td>Hour (s)</td>
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<tr>
<td>HR</td>
<td>Homologous repair</td>
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<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<td>ISCT</td>
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<td>JAK</td>
<td>Janus kinase</td>
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<td>LFA-1</td>
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<td>LMA</td>
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<td>LQC</td>
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<td>M-CSF</td>
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<tr>
<td>MDR</td>
<td>Multidrug-resistant</td>
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<td>MGUS</td>
<td>Monoclonal gammopathy of undetermined significance</td>
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<tr>
<td>MNC</td>
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<tr>
<td>MSC</td>
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<td>MM</td>
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<td>MIP1-α</td>
<td>Macrophage inflammatory protein 1-alpha</td>
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<td>Min</td>
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<td>NaCl</td>
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<td>NF-κB</td>
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<td>NHEJ</td>
<td>Non-homologous end-joining</td>
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<td>NRES</td>
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<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
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<td>Abbreviation</td>
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<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>PE</td>
<td>Phycoerythrin</td>
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<td>PI</td>
<td>Propidium iodide</td>
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<tr>
<td>PN</td>
<td>Peripheral neuropathy</td>
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<tr>
<td>RANK</td>
<td>Receptor activator of nuclear factor kappa-B</td>
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<td>RANKL</td>
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<tr>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<td>SE</td>
<td>Standard error</td>
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<td>Smouldering multiple myeloma</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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