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

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This thesis is submitted in partial fulfilment of the requirements of the University of the West of England for the degree of Doctor of Philosophy in Biomedical Science

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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.

Acknowledgements

This thesis could not have been accomplished without the support and encouragement of numerous people including my family, friends and colleagues. It is my great pleasure to acknowledge all those people who made this thesis possible and an unforgettable experience for me.

Firstly I must acknowledge the invaluable guidance, understanding, and unceasing assistance that I have received from my director of studies, Dr Ruth Morse, and from which I have benefitted throughout my time at UWE. I am so grateful for her having constantly made time to discuss extensively my research, giving valuable suggestions and constructive criticism, whilst always providing encouragement. I also express my sincere gratitude to my supervisors Dr Craig Donaldson and Dr Jennifer May for all the help and guidance that they have afforded throughout my study.

In addition I would like to express my appreciation for the assistance and technical advice provided by Mr David Corry and Dr Jeff Davey. I am grateful to Dr David Patton, for his assistance with scanning electron microscopy, Mr Scott Lake for his involvement in the development of the ELISA for IL-6 and Ms Jennifer Razik for her contribution and help with the study of immunomodulatory agents on TK6 cells. My research would not have been possible without their help.

I am most grateful to UWE for providing the funding of this research. My time here was made enjoyable in large part by members of the CRIB lab and in particular the PhD students who were a source not only of friendship, but also sound advice and encouragement. You have all made the lab and the university a great place to work.

This PhD would not have been possible without the unfailing support of my family. Foremost, an enormous thank you to my parents for their love, unwavering support and immeasurable sacrifice. I am grateful for their having allowed me to move back home these last two years to complete my thesis and for their financial support. Thank you to my sister Kate and brother in law Steve, who have also encouraged me through the ups and downs of these last

few years. I am deeply indebted to my late grandparents, for their everlasting love, care and encouragement. Together they had always been enthusiastic supporters of my education and will always be remembered. Finally but definitely not least, I express my deep gratitude to my girlfriend Abbie. I am so appreciative of all her love and patience over the last few years and who has always been there to cheer me up and stood by me through the good times and bad. I would not have been able to do this without you.

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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

BM-MSC	Bone marrow mesenchymal stem cell
BSA	Bovine serum albumin
bFGF	Basic fibroblast growth factor
CAM-DR	Cell adhesion mediated drug resistance
CFU-F	Colony forming unit fibroblast
CD	Cluster of differentiation
LFA-1/CD18	Lymphocyte function associated antigen / CD marker 18
VLA-4/CD49d	Very late antigen 4 / CD marker 49d
NCAM/CD56	Neural cell adhesion molecule / CD marker 56
VCAM-1/CD106	Vascular cell adhesion molecule 1 / CD marker 106
Dil/DiO	Long chain dialkylcarbocyanine lipophilic tracer
Dkk1	Dickkopf 1
DMEM/LG	Dulbecco's Modified Eagle Medium low glucose
DMEM/HG	Dulbecco's Modified Eagle Medium high glucose
DMEM/F12	Dulbecco's Modified Eagle Medium and Ham's F-12 Nutrient Mixture
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DSB	Double strand break
ECACC	European Collection of Cell Culture
EBV	Epstein Barr virus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FITC	Fluorescein isothiocyanate
FBS	Foetal bovine serum
H2O2	Hydrogen peroxide
HDT	High dose therapy
Hsp90	Heat shock protein 90

HQC	High quality control
HMDS	Hexadimethylsilazane
Hr	Hour (s)
HR	Homologous repair
HRP	Horseradish peroxidase
IL	Interleukin
Ig	Immunoglobulin
ISCT	International Society for Cellular Therapy
JAK	Janus kinase
LFA-1	Lymphocyte function associated antigen-1
LMA	Low melt agarose
LQC	Low quality control
M-CSF	Macrophage colony stimulating factor
MDR	Multidrug-resistant
MGUS	Monoclonal gammopathy of undetermined significance
MNC	Mononuclear cells
MSC	Mesenchymal stem cell
MM	Multiple myeloma
MIP1-α	Macrophage inflammatory protein 1-alpha
Min	Min (s)
NaCl	Sodium chloride
NCAM	Neural cell adhesion molecule
NER	Nucleotide excision repair
NF-κB	Nuclear factor κB
NHEJ	Non-homologous end-joining
NRES	National Research Ethics Service
OECD	Organisation for Economic Co-operation and Development

OPG	Osteoprotegerin
PBS	Phosphate buffered saline
PE	Phycoerythrin
PI	Propidium iodide
PN	Peripheral neuropathy
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
RPMI	Roswell Park Memorial Institute
ROS	Reactive oxygen species
SE	Standard error
SEM	Scanning electron microscopy
SSB	Single strand break
STAT3	Signal transducer of transcription 3
SMM	Smouldering multiple myeloma
TGFβ	Transforming Growth Factor-Beta
TNF-α	Tumour necrosis factor alpha
UPS	Ubiquitin-proteasome system
UREC	University of the West of England Ethics Committee
VEGF	Vascular endothelial growth factor
ZOL	Zoledronic acid