ELSEVIER

Blood 142 (2023) 1947–1948

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

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

A Functional Genomic Screen to Identify Novel Genes Involved in Multiple Myeloma Tumour Development

Hayley Parkinson, BSc[1,2], *James Breen, PhD*[3], *Nhi Hin, PhD*[3], *Vasilios Panagopoulos, PhD*[2,1], *Andrew Zannettino, PhD*[1,2], *Kate Vandyke, PhD*[1,2], *Duncan Hewett, PhD*[1,2]

[1] Myeloma Research Laboratory, Faculty of Health and Medical Sciences, School of Biomedicine, The University of Adelaide, Adelaide, Australia
[2] Precision Cancer Medicine Theme, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia
[3] South Australian Genomics Center (SAGC), South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia

Multiple myeloma (MM) is an incurable malignancy characterised by uncontrolled proliferation of plasma cells (PCs) in the bone marrow (BM). MM is a genetically heterogeneous disease with each patient's PCs harbouring unique genetic mutations, however the development of MM tumours is not only dependent on the underlying genetics but also on the selective pressures applied by the BM microenvironment. Hence, we hypothesise that identifying the dependencies which promote MM cell outgrowth in the BM microenvironment will allow for the identification of new drug targets. This project aims to use an *in vivo* functional screen, combining CRISPR-Cas9 gene editing, using a single guide RNA (sgRNA) library targeting thousands of genes, with a murine model of MM, to identify novel genes involved in MM tumour development.
The Bassik human apoptosis and cancer CRISPR knockout library (Addgene #101926) was used to transduce MM cells with 31,324 unique sgRNAs targeting 3,015 genes and 1,500 control regions. The human MM cell line OPM2 was transduced with the Cas9 transgene (FuCas9GFP), followed by the sgRNA expression vector (pMCB320) generating OPM2-Cas9-sgRNA cells. These cells were then injected ($5x10^5$ cells/mouse, n=8 mice + 1 tumour-naive control) into the tibiae of immunodeficient NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl}/SzJ (NSG) mice. Four weeks post-injection, the primary tumour within the injected tibia was isolated and sgRNA frequencies were assessed via next generation sequencing and compared with that of the initial library and the injected cells using the MAGeCK algorithm. To identify genes that play roles in MM tumour formation in patients, we assessed the expression of our identified genes in CD138-selected BM PCs from newly diagnosed MM patients (n=155) when compared with normal PCs from healthy donors (n=5) in microarray dataset E-MTAB-363 (ArrayExpress). Furthermore, to identify potential pathway involvement of our identified genes we performed gene set enrichment analysis (GSEA) using the University of California San Diego and Broad Institute developed GSEA software, comparing to Hallmark Gene Sets.
Comparison of the *in vitro* cultured cells with the initial library identified 34 genes with undetectable ($p<0.05$) sgRNAs post *in vitro* culture, suggesting these genes play key roles in the survival of OPM2 cells. This list contained a number of genes known to control MM cell survival, such as IRF4 and CCND2. Additionally, sgRNAs targeting a further 115 genes were significantly depleted ($p<0.05$) *in vitro* and were completely undetectable *in vivo*, suggesting that these are implicated in cell proliferation and tumour formation *in vivo*. This gene list contained some already known MM drivers such as MYC, KRAS and DIS3. Furthermore, we identified 28 genes where CRISPR knockout had no significant effect *in vitro* but were critical dependencies for tumour development, with sgRNAs being undetectable ($p<0.05$) *in vivo*. This list contained genes known to be important for MM and BM stromal cell interaction such as ALCAM and SPP1. Notably, 25% of the genes identified as critical dependencies for OPM2 cells *in vitro* and/or *in vivo* are significantly upregulated >2-fold ($p<0.05$; limma) in MM patient PCs when compared with normal PCs (E-MTAB-363), suggesting these may play a role in tumour development in patients. Gene set enrichment analysis revealed that the genes that were essential *in vitro* were predominantly involved in cell survival related pathways such as cell cycle regulation (G2M checkpoint) and DNA replication/repair; however, the uniquely *in vivo* genes are predominantly involved in cell metabolism pathways such as MTORC1 signalling, oxidative phosphorylation and glycolysis.
Overall, this study has successfully used a functional genetic screen to identify novel gene dependencies required for *in vitro* MM cell growth and *in vivo* MM tumour development. A secondary genetic screen will be performed *in vivo* to validate and refine this list of candidate genes. These validated genes will be further investigated using patient derived data sets to determine if they represent common vulnerabilities in patients and in multiple MM cell lines to investigate their specific mechanistic and pathway involvement. Identification and understanding the driving dependencies which promote MM cell

outgrowth in the BM microenvironment will allow for the identification and development of new drug targets to improve patient outcomes.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-174601