

## Levels of genetic and epigenetic instability in multiple myeloma appears to be associated with clinical outcomes

Katarzyna Ewa Sokolowska<sup>1\*</sup>, Jakub Rosik<sup>1\*</sup>, Karolina Łuczowska<sup>2</sup>, Marta Sobalska-Kwapis<sup>4</sup>, Dominik Strapagiel<sup>4</sup>, Bogusław Machaliński<sup>2,4</sup>, Tomasz K. Wojdacz<sup>1</sup>

### Affiliations:

<sup>1</sup> Independent Clinical Epigenetics Laboratory, Pomeranian Medical University, Unii Lubelskiej 1, 71-252 Szczecin, Poland.

<sup>2</sup> Department of General Pathology, Pomeranian Medical University, Powstańców Wielkopolskich 72, 70-111, Szczecin, Poland.

<sup>3</sup> Centre for Digital Biology and Biomedical Science - Biobank Lodz, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 139, 90-235 Lodz, Poland.

<sup>4</sup> Department of Hematology and Transplantology, Pomeranian Medical University, Unii Lubelskiej 1, 71-252, Szczecin, Poland.

*\*Contributed equally*

### Background:

Multiple myeloma (MM), the second most common hematologic malignancy, develops from precursor non-malignant stages monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). Clinical signs are unspecific, though hypercalcemia, renal failure, bone lesions, or anemia are common. Genetic aberrations in MAPK and DNA repair pathways, as well as MYC, are linked to MM and its progression from MGUS. However, so far, genome-wide genetic instability has not been comprehensively studied. Similarly, only a few studies have investigated genome-wide epigenetic changes crucial for MM pathology using former (450K) generations of microarray technology.

### Methods:

Genome-wide methylation profiling with Infinium MethylationEPIC BeadChip (Illumina) was performed on DNA extracted from enriched for plasmocytes (using CD138 MicroBeads) bone marrow aspirates from 37 patients with MM (43–78 years (mean 64); 24M/13F) recruited at the Pomeranian Medical University. MM patients were followed for 36 months and within follow up 12 of the patients died from MM. To provide a reference for copy number variations (CNVs) analysis, we used publicly available blood methylomes from 69 healthy individuals (EPIC).

Raw data were processed with SeSAME with default settings. To identify differentially methylated positions (DMPs) between patients who survived 3-years of follow-up and who did not, we applied a three-step CpG site selection. Using champ.DMP from ChAMP R package, we identified sites with  $p\text{-value} \leq 0.05$  and  $\Delta\beta \geq 0.1$ , then filtered them by Hedges'  $g > 0.5$  to account for variability. GREAT platform was used for GSEA and was based on four ontology databases. Transcription factor binding sites (TFBS) potentially affected by identified methylation changes were analyzed using HOMER v4.11. CNVs were calculated using the

conumee 2.0 R package. Statistical significance ( $p\text{-value} \leq 0.05$ ) was calculated using the Mann–Whitney U test.

### **Results:**

Comparison of methylation profiles between MM patients surviving 3-years follow-up and not surviving this period identified 2,439 DMPs. GSEA performed using the genomic coordinates (chr, start, end) of the identified DMPs resulted in 13 genes in the Ensembl Genes (EG), 11 terms in GO Molecular Function (GO MF), 20 in GO Biological Process (GO BP) and 3 in GO Cellular Component (GO CC) databases, all reaching a significance threshold of  $p\text{-value} \leq 1 \times 10^{-10}$ . The fourth strongest hit in the EG database was *IGF1R*, a gene previously shown to play a key role in MM progression and poor prognosis.

Interestingly, 5 out of 11 ontology terms identified in GO MF database and 1 out of 3 in GO CC database, were related to *IGF1R* function. In GO BP, only one term was directly associated with *IGF1R* function; however, it represented the strongest hit. The majority of ontology term identified in GSEA included also cancer-related processes for example immune or defense response to tumor cells, indicating that the methylation changes we identified may disrupt pathways relevant to tumor–host interactions and disease progression.

The analysis of TFBS showed that identified DMPs were enriched in motifs targeted by the PAX5 and TEAD TFs. Previous studies have shown that PAX5 may regulate B cell to plasma cell differentiation and induce MM cell apoptosis, while TEAD affects MM cell growth, interactions, and treatment resistance, with TAZ/TEAD1 knockdown partially sensitizing cells to proteasome inhibitors. Finally, the number of CNVs was more than twice as high in MM patients who did not survive 3 years after diagnosis (median 11 [IQR: 7.75] for patients who died within 3 years vs. median 5 [IQR: 5] for patients alive at 3 years;  $p\text{-value} = 0.033$ ).

### **Conclusions:**

Epigenetic aberrations identified in MM patients that died 3 years from the diagnosis appear to affect a number of pathways previously associated with MM particularly related to *IGF1R* gene and PAX5 and TEAD TF binding sites. Moreover, the genomes of patients that experienced outcome within follow up are characterized by increased genetic and epigenetic complexity when compared to genomes of patients that survived 3 years with this disease.